The proportional contribution of disease stage and antiretroviral treatment to HIV transmission in men who have sex with men: an epidemiological phylogenetic approach incorporating the enhanced identification of recent infection.

This work is presented by

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Candidate's own contribution

I, David Shien-Phen Pao, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

In conjunction with my clinical supervisor (Martin Fisher) and principal supervisor (Deenan Pillay), I have been responsible for these studies from conception, design, ethical approval and implementation, through to conclusion and dissemination of results. Where work has been undertaken primarily by collaborators, this has been indicated in the acknowledgments.

For the majority of the time spent on this work I was in a full-time clinical position at Brighton & Sussex University Hospitals NHS Trust.

ABSTRACT

AIM:

This work identifies recent HIV infection (RHI) in men who have sex with men (MSM) and the specific phylogenetically-linked transmission events during which they were infected. The overall aim is to identify the clinical characteristics of each infection source individual and evaluate their proportional contribution to transmission.

OBJECTIVES:

1. Construct a dataset to capture cohort characteristics;
2. Identify RHI in subtype B infection using the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS);
3. Identify the most-likely infection source individual for each RHI by phylogenetic analysis;
4. Observe the clinical characteristics of each infection source individual at the time of infection and estimate the proportional contribution to transmission of unknown infection, disease stage and HAART status

METHODOLOGY:

The study population was a cohort of mainly MSM attending the single HIV clinic in Brighton, UK. The study involved phylogenetic analysis of HIV genotypic sequences obtained for routine clinical care. Clinical data were also collected. Written, informed consent was obtained and every study described in this thesis had formal ethical approval.

RESULTS:

1. STARHS confirmed RHI in 71/74 (96%) individuals identified as RHI by conventional methods;
2. *Pol* sequence data were obtained for 859/1144 (75%), of whom 159/859 (19%) were RHI at diagnosis;
3. For only 41/159 (26%) RHI could an infection source individual be identified;
4. RHI contributed 3% of follow-up time but 41/59 (27%) transmissions (RR 4.44, 95% CI 2.11-9.33, p=0.0001);
5. 39/41 (95%) transmissions were from untreated individuals or those interrupting antiretroviral treatment (ART).

CONCLUSIONS:

Three-quarters of infections came from undiagnosed infections or from outside Brighton. Of those from diagnosed infections, transmission was significantly associated with RHI, STI and viral load, and reduced by effectively-taken ART. These results demonstrate that effective behavioural interventions to increase HIV testing and improve ART uptake and adherence will reduce onward transmission.

LIST OF PEER-REVIEWED PAPERS

Fisher M, **Pao D**, Brown AE, Sudarshi D, *et al.* (2010). *Determinants of HIV transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach.* AIDS **24**:1739-47

Pao D, Fisher M and Pillay D (2009). *Potential impact of early antiretroviral therapy on transmission*. Curr Opin HIV AIDS **4(3)**:215-21

Fisher M, **Pao D**, Murphy G, Dean G, *et al.* (2007). *Serological Testing Algorithm for Recent HIV Seroconversion (STARHS): 10 years of clinical application*. AIDS **21(17)**:2309-14

Sudarshi D, **Pao D**, Murphy G, Parry JV, *et al.* (2007). *Missed opportunities for diagnosing primary HIV infection.* STI **84(1)**:14-6.

Pao D, Smit E, Imami N and Fisher M (2006). *A case of multi-drug resistant primary HIV infection with delayed CD4 T-cell count decline despite low VL, treated with interleukin-2.* AIDS **20(11)**:1564-5

Pao D, Fisher M, Hué S, Murphy G, *et al.* (2005). *Transmission of HIV-1 during primary infection: relationship to sexual risk and sexually transmitted infections.* AIDS **19**:85-90

Pao D, Andrady U, Clarke J, Dean G, *et al.* (2004). *Long-term persistence of primary genotypic resistance after HIV-1 seroconversion.* J Acquir Immune Defic Syndr **37(5)**:1570-3

LIST OF ABSTRACTS

Fisher M, **Pao D**, Brown A, Sudarshi D, Gill N, Cane P, Buckton A, Parry J, Johnson A, Sabin C, Pillay D. Determinants of HIV-1 transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach. *16th HIV Evolution and Dynamics Conference, Oxford, 2009*

Sudarshi D, Nambiar K, **Pao D**, Dean G, Homer G, Parry J and Fisher M. *Primary HIV infection is associated with a higher prevalence of sexually transmitted infections in men who have sex with men.* 13th BHIVA Spring Meeting, Edinburgh, 2007

Nambiar K, Fisher M, **Pao D**, Sudarshi D, Murphy G, Parry J, Reeves I, Dean G, Pillay D. *Acquisition of transmitted drug-resistant HIV-1 infection is associated with the presence of sexually transmitted infections*. 14th Conference on Retroviruses & Opportunistic Infections, Los Angeles, USA, 2007

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Bond K, **Pao D**, Fisher M, and Dean G. *HIV partner notification has limitations but could reduce transmission by identifying primary infection.* 12th BHIVA Spring Meeting, Brighton, 2006

Aderogba K, **Pao D**, Dean G, Pillay D, Kellar I, Cane P, Smit E and Fisher *M. Trends in Transmitted Genotypic Drug Resistance in Primary Versus Longstanding HIV Infection in a UK cohort*. 3rd International AIDS Society Conference, Rio de Janeiro, Brasil, 2005

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ACRONYMS

AHI	Acute HIV Infection
AIDS	Acquired Immune Deficiency Syndrome
BASHH	British Association for Sexual Health and HIV
BHIVA	British HIV Association
CDC	U.S. Centers for Disease Control and Prevention
CRF	Circulating Recombinant Form
DoH	Department of Health (England)
GUM	Genitourinary Medicine
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HPA	Health Protection Agency
IDU	Intravenous Drug User
MSM	Men who have Sex with Men
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Transcriptase Inhibitor
PI	Protease Inhibitor
PYFU	Person Years Follow Up
RHI	Recent HIV Infection
RITA	Recent Infection Testing Algorithm
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
SD	Standard Deviation
SOPHID	Survey of Prevalent HIV Infections Diagnosed
STARHS	Serological Testing Algorithm for Recent HIV Seroconversion
STI	Sexually Transmitted Infection
TDR	Transmitted Drug Resistance
TRI	Tests for Recent Infection
UPAI	Unprotected Anal Intercourse
UNAIDS	Joint United Nations Programme on HIV/AIDS
VL	Viral Load
WHO	World Health Organisation

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CHAPTER 1

Introduction and background

This first chapter introduces the justification for this thesis and describes its aim, objectives and structure. The clinical features of infection with Human Immunodeficiency Virus Type 1 (HIV-1, hereafter termed HIV) are then described and the epidemiology of HIV briefly discussed, with particular reference to men who have sex with men (MSM) in the UK. This is followed by a description of the pathogenesis of HIV infection and an introduction to highly active antiretroviral therapy (HAART).

This is followed by a review of the research literature concerning the determinants of HIV transmission (with a focus on the contribution of recent HIV infection, RHI), the effectiveness of interventions (in particular HAART) to reduce onward transmission and the use of mathematical models to bridge the knowledge gap between empirical data and intervention effectiveness.

Finally, research limitations of phylogenetic analysis and mathematical modelling in the context of sexual transmission of HIV are discussed.

1.1 Thesis introduction, aim, objectives and structure

1.1.1 Introduction

Since its introduction in 1996 and increasingly over calendar time, HAART has transformed HIV from an inexorably fatal infection into a treatable condition with a normal or near-normal lifespan and moreover, one in which the majority of patients can maintain a high quality of life [HIV-CAUSAL Collaboration, 2010; Boyd, 2009; Willig, 2008; Gazzard, 2005]. In quantifiable terms, HAART has improved the prognosis of the majority of people living with HIV such that life expectancy in developed countries is in the order of 40 or even 50 years after initiation of therapy [Nakagawa, 2012; Ray, 2011; Antiretroviral Therapy Cohort Collaboration, 2008; Lohse, 2007].

HAART also has huge potential to benefit public health because it can prevent transmission of HIV from mothers to their children [Siegfried, 2011; Townsend, 2008] and between sexual partners [Cohen MS, 2011a; Vernazza, 2008], representing an opportunity for epidemic control at a time when promises of a cure by eradicating latency or by vaccine are failing fully to deliver [Archin, 2012; Rerks-Ngarm, 2009; Buchbinder, 2008].

However, despite robust evidence for the potential of HAART to reduce onward transmission and even in the resource-rich world, where access to HAART is relatively unrestricted, surveillance data suggest that transmissions are increasing. Across all risk groups, *UK-acquired* new diagnoses have almost doubled over the past decade from an estimated 1,950 in 2001 to 3,640 in 2010 and are at the highest ever level in the MSM risk group. While some of this increase may be attributable to more HIV testing, data on CD4 cell count and age at HIV diagnosis as well as data from the recent infection test algorithm (RITA) suggest on-going transmission of HIV at an increasingly high rate [Aghaizu, 2012; HPA, 2011].

Certain key factors threaten the effectiveness of HAART to reduce transmissions on a population level - in particular RHI, high-risk sexual behaviour and concomitant sexually transmitted infections (STI). As will be explored in depth in this thesis, RHI is difficult to diagnose and a disproportionately infectious disease stage, despite its short duration. With regards to sexual risk-taking, whilst behavioural interventions have been shown to have some impact in reducing HIV transmission [Hart GJ, 2010; Johnson WD, 2008] they can be costly and their effects are typically transient (see section 1.8.1). Even though some non-ART methods can potentially reduce the risk of onward transmission, disappointingly, of 27 randomised controlled trials (RCT) of different non-ART biomedical interventions including vaccines, microbicides and herpes suppression trials, 22 failed to demonstrate significant efficacy [Celum, 2010; Cohen J, 2008; Padian, 2008; Weiss, 2008].

Treatment as prevention describes the potential public health benefit of controlling onward transmission with HAART [Mayer, 2010], through its effect of dramatically lowering blood and genital tract viral load (VL). Indeed, *Science* magazine chose the early use of HAART for prevention of HIV transmission as its "breakthrough of the year 2011" [Alberts, 2011]. In its most extreme form, a policy of universal HIV testing to enhance the identification of all HIV-positive individuals, followed by immediate treatment with HAART irrespective of CD4 cell count (*universal test and treat*, UTT), has been postulated as a tool potentially capable of reducing HIV prevalence to <1% in sub-Saharan Africa [Granich, 2009].

However, although it is clear that HAART reduces transmission between individuals, in order to predict whether treatment as prevention is effective at a population level it is essential to explore how the HIV-infected population generate new HIV infections and, specifically, to what extent individuals with undiagnosed and/or RHI fuel transmission. Only when we have a more accurate understanding of the characteristics of those transmitting HIV can we fully address the question of whether reducing the VL of the HIV-diagnosed population with HAART will substantially impact upon population-level transmission.

1.1.2 Aim

The overarching aim of this thesis is to identify phylogenetically-confirmed transmission events and to describe the clinical characteristics of the most-likely HIV infection source individual at the time of each transmission. By doing this, we can ascertain the proportional contribution of undiagnosed HIV infection, RHI and longstanding untreated infection to onward transmission, as well as determine the influence of HAART.

It is important to note from the outset that we have not set out to identify all possible transmissions but only those in which we can be confident about direction of transmission. Thus our focus rests on obtaining an accurate estimate of proportional contribution to onward infection rather than absolute numbers.

1.1.3 Objectives

1. To construct a dataset to capture cohort characteristics;
2. To test the reliability of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) in identifying subtype B RHI;
3. Phylogenetic analysis of HIV-1 *pol* sequences to determine viral relatedness;
4. Identification of the single most-likely infection source individual for any RHI individual in a cluster;
5. Characterisation of the clinical characteristics for each most-likely infection source individual identified in (4) during the calendar quarter of transmission.

1.1.4 Thesis structure

This thesis has two distinct sections. Limitations in the methodology of each study are made explicit in each individual chapter.

The first section (Chapters 2, 3 and 4) sets out to identify and understand RHI. **Chapter Two** describes the use of the STARHS technique over a ten-year period, providing evidence for the validity and reliability of this method in subtype B infections. **Chapter Three** and **Chapter Four** describe how this enhanced ascertainment of RHI has increased our understanding of the nature of RHI with regards to both transmitted drug resistance (TDR) and patient/clinician HIV testing behaviour.

The second section (Chapters 5 and 6) explores the relationship between RHI and other biological factors and their relative contribution to onward transmission. **Chapter Five** describes the pilot phylogenetic study, which looks at transmission risk factors amongst the cohort of MSM identified as recently-infected in chapter two, and introduces the methodology used to capture genotypic sequence and clinical data. **Chapter Six** describes the full phylogenetic study, extended to include MSM with longstanding as well as RHI, and HAART status, and aims to determine the proportional contribution of the major determinants of sexual transmission.

Chapter Seven concludes with a summary of the thesis and how it contributes to our understanding of HIV transmission amongst MSM, particularly in the context of treatment as prevention as a UK prevention strategy. Suggestions for future research are briefly discussed.

1.2 An overview of HIV infection

The first recorded cases of infection with HIV occurred in MSM from the USA and Western Europe in the early 1980s [Gottlieb, 1981], and the virus was first isolated in 1983 [Barré-Sinoussi, 1983; Gallo, 1983].

There are two genetically distinct Human Immunodeficiency Viruses: HIV-1 and HIV-2. Both are of primate origin; HIV-1 from the Simian Immunodeficiency Virus (SIV) of the central common subspecies of chimpanzee [Gao, 1999] and HIV-2 from SIV of the sooty mangabey (*Cercocebus atys*), a monkey from Guinea Bissau, Gabon and Cameroon [Hirsch V, 1989]. It has been theorised that SIV moved from chimpanzees and sooty mangabeys to humans - evolving into pathogenic HIV-1 and HIV-2 respectively - through exposure to primate blood, most likely as a result of the bushmeat trade. Of note, SIV is not an immunodeficiency virus; it does not cause immune deficiency in its natural host.

HIV infection in humans, however, is characterised by a progressive and profound deterioration of the cellular immune system that leads to the development of Acquired Immune Deficiency Syndrome (AIDS) - defined by the presence of a specific, defined opportunistic infection or malignancy in those individuals with detectable anti-HIV antibody (Ab) - and ultimately death. The degree of immunodeficiency associated with HIV infection is closely correlated to the depletion of both the quantity and proportion of plasma CD4+ T-lymphocytes (hereafter termed "CD4 cells"). In addition, the plasma level of HIV ribonucleic acid (RNA, also termed VL) reflects to some degree the rate at which immunosuppression develops such that the higher the VL, the greater the loss of circulating CD4 cells per year [Soriano, 1988].

If untreated, the mean time to AIDS is 7.7 years in those infected through sex or transfusion, and 1 year in infants infected perinatally. Untreated AIDS survival times average 9-13 months. Perinatally infected infants survive only 8-9 months. The percentage of long-term (>4 years) AIDS survivors is low, at around 2.5% [Anderson R, 1988]. Rarely, in <1% of cases, clinical progression is slow or absent with controlled VL – these individuals are known as *long-term non-progressors*. Much rarer still,

progression is slow or absent with an undetectable VL, despite not being on treatment. These individuals are termed *elite controllers* [Saksena, 2007].

HIV is transmitted sexually (anal, vaginal, oral), vertically (almost exclusively during labour) and by the sharing of HIV-infected blood products.

HAART became available towards the end of 1996, and widely prescribed in the developed world by the end of 1998. Initiating treatment early enough reduces the risk of developing not only the HIV-related infections and cancers that represent AIDS but potentially also other conditions (of possible inflammatory aetiology) affecting the brain, heart, kidney, liver and bone [Gutierrez, 2011; Smith C, 2010; Campbell, 2009; Guaraldi, 2009; Phillips 2008], which appear to be accelerated by HIV infection and which are an increasingly important cause of morbidity and early mortality in people living with HIV.

On an individual level, effectively-taken (*i.e.* virologically fully suppressive) HAART also significantly decreases vertical [Siegfried, 2011; Townsend, 2008] and sexual infectiousness [Cohen MS, 2011a; Vernazza, 2008], but controversially not to zero [Donnell, 2010; Jin, 2010; Wilson DP, 2009; Stürmer, 2008].

1.3 The clinical disease stages of HIV infection

1.3.1 Clinical Staging

HIV clinical staging and classification systems are useful for providing clinicians and patients with information about clinical management and for monitoring the HIV epidemic. Two major classification systems currently in use are the Centers for Disease Control and Prevention (CDC) classification system [CDC, 1993] and the World Health Organisation (WHO) Clinical Staging and Disease Classification System [WHO, 2007].

The CDC disease staging system (last revised in 1993) assesses the severity of HIV disease by symptoms, CD4 cell count and by the presence of specific HIV-related conditions. The definition of AIDS includes all HIV-infected individuals with CD4 counts of <200 cells/mm^3 (or CD4 percentage <14%) as well as those with specific HIV-related infections, cancers and symptoms. Although the finer points of the classification system are not often used in the routine clinical management of HIV-infected patients, the CDC system is widely used in clinical and epidemiologic research.

In contrast to the CDC system, the WHO Clinical Staging and Disease Classification System (revised in 2007) can be applied without access to CD4 cell count measurements or other diagnostic and laboratory testing technology. The WHO system classifies HIV disease on the basis of clinical events, clinical diagnoses or definitive diagnoses that can be recognised and treated by clinicians in resource-constrained settings, and by clinicians with varying levels of HIV expertise. The clinical stages have been shown to correlate with survival, prognosis and disease progression without antiretroviral therapy in adults and children.

Both the WHO and CDC classification systems have a four-stage clinical classification and both recognise primary HIV infection as a disease

stage (see later for definition). Figure 1.1 is a schematic diagram of the typical course of HIV infection without intervention. The four clinical stages are explained in detail below.

Figure 1.1: Typical course of HIV infection without intervention
[Adapted from Fauci, 1996]
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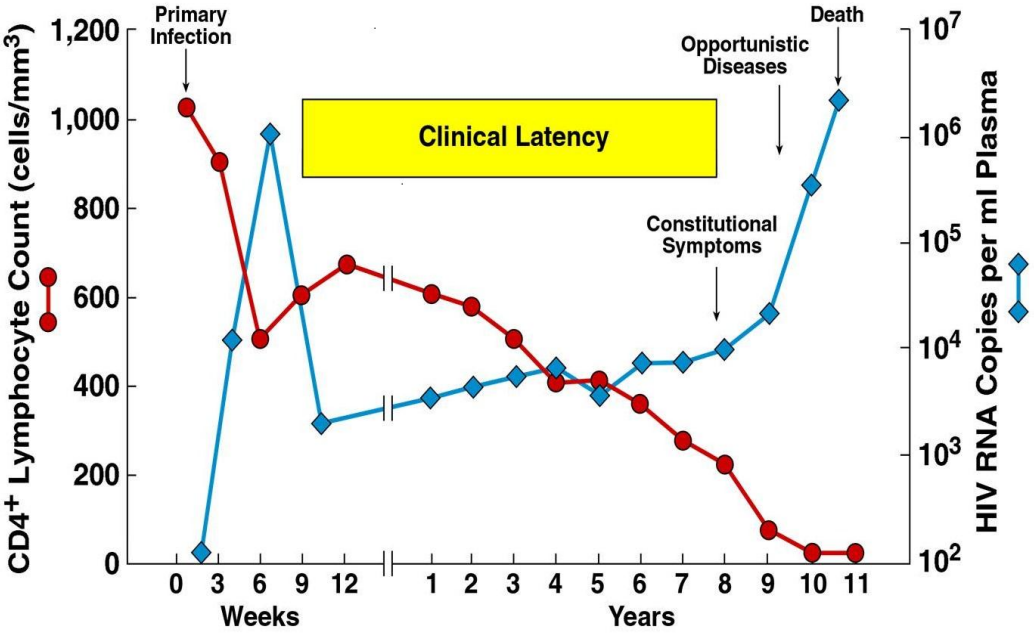


1.3.2 Recent HIV Infection

The term recent HIV infection (RHI) is commonly used to describe the first six months of infection and encompasses what has variously been referred to as acute HIV infection (AHI, the presence of HIV RNA or p24 Antigen (Ag) in the absence of Ab) and primary HIV infection (PHI, the influenza-like clinical syndrome associated with seroconversion).

In the 2 weeks following mucosal acquisition of HIV, active viral replication occurs at the site of transmission followed by rapid dissemination to local lymphoid tissues and subsequent trafficking of virus to gut-associated lymphoid tissue, whatever the route of infection [Veazey, 1988]. This is accompanied by a 2 to 4 week period of uncontrolled viral replication in mucosal sites, all lymphoid tissues and blood that results in high numbers of circulating HIV virions which rapidly disseminate to

relatively inaccessible sanctuary reservoirs and also lead to immunological damage [Richman, 2004].

This process is accompanied by a marked drop in the numbers of circulating CD4 cells and is associated in virtually all patients with the activation of CD8+ T-lymphocytes - which kill HIV-infected cells - and subsequent Ab production [Fiebig, 2003]. In addition to host-specific genetic factors that influence viraemia during AHI [Fellay, 2009], viral factors may also play a significant role [Alizon, 2010; Karlsson, 2007]. The high plasma VL is reflected in the genital tract secretions and confers a marked enhanced potential for onward transmission to sexual partners [Coombs, 2003].

Once an Ab response has been generated by the host, which occurs between 3 weeks and 6 months after infection, HIV replication is restrained and VL maintained at a lower set-point – which determines disease progression [Mellors, 2007; Mellors, 1996] - and the CD4 cell count returns to normal or near-normal levels (the normal laboratory range for an uninfected male is between 500 and 1200 cells/mm^3 and dependent on the laboratory used).

Clinically, during the acute period - which is usually two to four weeks after exposure - between 40 and 90% of individuals [Kahn, 1998] develop an influenza- or mononucleosis-like illness, termed PHI or symptomatic AHI. This is a non-specific and variable illness, usually lasting at least a week [Cohen MS, 2011b]. Whilst it is often the only clinical manifestation of HIV before the onset of AIDS several years later, PHI is easily overlooked [see Chapter 4].

There are currently neither national nor international HIV treatment guidelines recommending the use of HAART for asymptomatic AHI. SPARTAC was the largest randomised controlled treatment trial ever undertaken of recent HIV infection. The study ran between 2003 and 2011 across eight countries. The aim of SPARTAC was to examine whether treating people recently infected with HIV with HAART for a short period of time would slow down damage to the immune system and consequently delay the need to start long-term treatment. However, a 48-week course of

HAART started within six months of becoming infected only modestly delayed the need to initiate treatment as determined by surrogate markers [Fidler, 2011].

The treatment of recently HIV-infected individuals with HAART for the purpose of preventing transmission, rather than for clinical need, is not consensus practice anywhere in the world.

1.3.3 Asymptomatic HIV Infection

Six months or so after infection, an individual usually enters the latent or asymptomatic infection stage that can last several years, during which he or she will be free of clinical symptoms but remain infectious. Viral replication and CD4 cell turnover remain active but at lower levels, with the CD4 cell count being maintained and viral replication relatively restrained by the immune response. However, the regenerative capacity of the memory CD4 cell pool is limited, and after 5 to 7 years the CD4 cell count and percentage generally falls to 200 cells/mm^3 and 14% [Ho, 1995]. The risk of developing an AIDS-defining condition increases exponentially as the CD4 cell count falls, and is particularly high in those with a CD4 count below 200 cells/mm^3 [Mellors, 2007; Phillips 2004, Egger, 2002]. Recently, it has been suggested that untreated HIV may also accelerate the course of end-organ diseases such as brain, cardiovascular, renal, bone and liver disease, and may increase the risk of contracting non-AIDS-defining malignancies [Guiguet, 2009; Phillips, 2008].

The most recent guidelines from the European AIDS Clinical Society (EACS) [EACS, 2011], World Health Organisation (WHO) [WHO, 2010], International AIDS Society (IAS) [Thompson, 2010] and British HIV Association (BHIVA) [Gazzard, 2008] advocate HAART for all untreated persons with a CD4 cell count <350 cells/mm^3, and for certain patient groups with a higher CD4 cell count. The longer HAART is delayed when clinically indicated, the poorer the patient outcome [Sterne, 2009].

1.3.4 Symptomatic HIV Infection

As the untreated host's immunity falls and the CD4 cell count falls towards 200 cells/mm^3, symptoms can develop which include weight loss, tiredness, night sweats and some gastrointestinal and skin conditions. At the same time, susceptibility to common, non-opportunistic infections such as Human Papilloma Virus (HPV), Herpes Simplex (HSV) and *Candida albicans* increases (see Figure 1.1).

1.3.5 AIDS

AIDS is diagnosed when an HIV Ab-positive individual develops one or more conditions from a specific list of opportunistic infections or malignancies [Ancelle-Park, 1993; CDC, 1993]. The USA CDC criteria stipulate a CD4 cell count <200 cells/mm^3, whilst the European criteria do not. In the absence of HAART, mean survival time after diagnosis of AIDS is 9-13 months [Anderson R, 1988].

1.4 The epidemiology of HIV infection

1.4.1 Global epidemiology

Despite the huge reduction in morbidity and mortality as a result of effectively-taken HAART, that has transformed HIV into a chronic, treatable condition with a potentially normal life expectancy, onward transmissions show no evidence of decline. An estimated 34 million (31.6-25.2 million) adults globally were living with HIV at the end of 2010, with 1.8 million HIV-related deaths in that year alone. At that time, nearly half (about 6.6 million) of those in need of HAART in low- and middle- income countries were receiving it; an increase of 2.6 million people over 2008. However, also occurring in 2010 were an estimated 2.7 million new infections [UNAIDS, 2011].

In the developed world, against the background of low HIV prevalence in the general population, MSM continue to be disproportionately affected by HIV infection [HPA, 2011; UNAIDS, 2011]. Data on the increasing incidence of HIV in MSM are mirrored across Europe, Australia, Canada and the USA, confirming that the epidemic continues to grow across most settings [HPA, 2011; UNAIDS, 2011; Jansen, 2011; Wand, 2010; Hall, 2009; Van Griensven, 2009]. During the period 2003-2006, MSM accounted for more than half of the persons with estimated HIV incident infection in the USA [Van Griensven, 2009; Karon, 2008]. Therefore, even in the resource-rich developed world, with wide availability of prevention interventions as well relatively unrestricted access to HAART, surveillance data show that transmissions are increasing.

1.4.2 UK Epidemiology

New diagnoses

It is estimated that in 2010 there were 91,500 individuals living with the HIV (95% credible interval [CI] 85,400-99,000) and 6,660 new HIV diagnoses in the UK [HPA, 2011]. By 2012, it is likely that there will be more than 100,000 individuals living with HIV, a quarter of who are unaware of their infection.

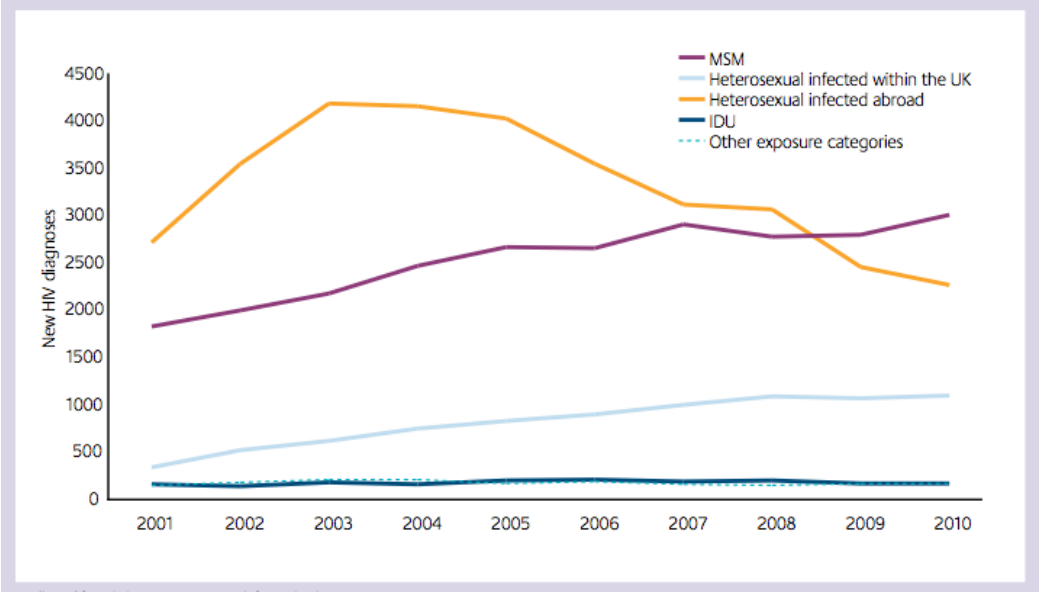
There are two main epidemics – in heterosexuals and MSM (Figure 1.2). In 2010, an estimated 50% (3,350) of newly diagnosed individuals acquired their infection heterosexually (1,350 men and 2,000 women) and 45% (3,000) through sex between men (Figure 1.2), with the remaining 5% of new diagnoses occurring in intravenous drug users (IDU), from mother-to-child, in blood tissue recipients or in unreported risk groups. In MSM, new diagnoses have increased by 70% between 2001 and 2010, whilst numbers of heterosexuals infected abroad have peaked and substantially declined.

Across all risk groups, *UK-acquired* new diagnoses have almost doubled over the past decade, from an estimated 1,950 in 2001 to 3,640 in 2010. This number now exceeds the number of infections acquired abroad. However, whilst the number of HIV diagnoses among people infected heterosexually in the UK has nearly stabilised, new diagnoses among MSM have reached an all-time high. Among the estimated 3,000 HIV-positive MSM newly diagnosed in 2010, 81% (2,440) probably acquired their infection in the UK. Indeed, 2010 saw the largest ever annual number of new HIV diagnoses in MSM (HPA, 2011].

Several factors may drive this increase in new diagnoses other than climbing incidence: increased HIV testing as a result of the National Sexual Health Strategy (2001) [Department of Health (England), 2001], the national HIV testing guidelines [BHIVA, 2008], travel and migration, and changes in data reporting. However, while some of this increase may be attributable to these factors, data on CD4 count and age at HIV diagnosis together with data from the recent infection test algorithm (RITA) suggest on-going transmission of HIV among MSM at a high rate.

Figure 1.2: New HIV diagnoses by exposure group: United Kingdom 2001-2010[1] [Source: HPA, 2011]

[1] adjusted for missing exposure group information in recent years

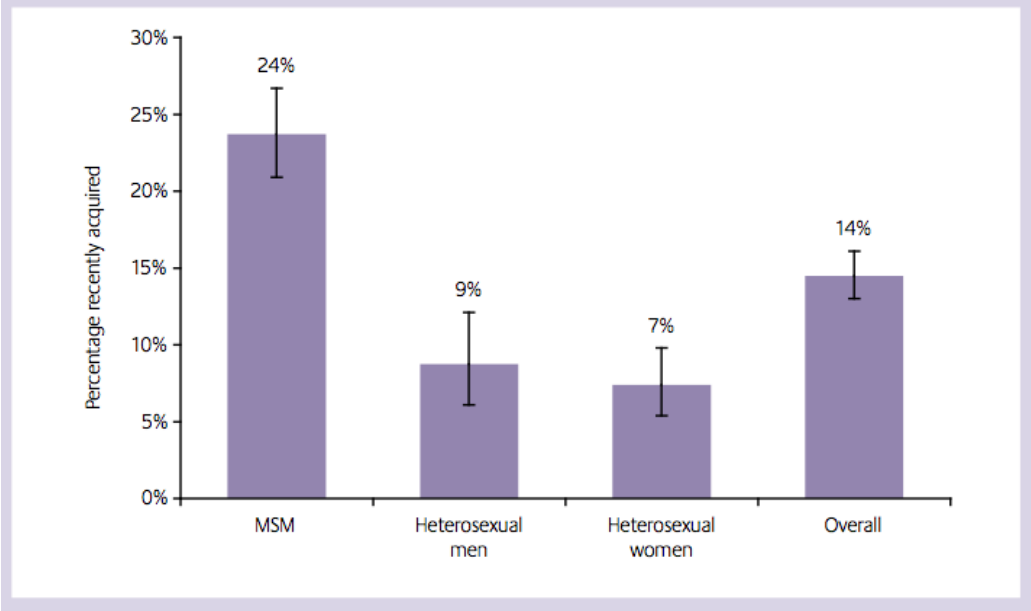


Recent infection

During 2010, 2,258 individuals were tested by the Recent Infection Testing Algorithm (RITA) as part of the national monitoring of recent HIV infections in England and Wales. In total, 327/2,258 (14%) of these HIV infections were classified as probably acquired within the previous 4-5 months. A higher proportion were in MSM (24%), compared to heterosexual women (7%) and heterosexual men (9%) (Figure 1.3).

Among MSM, younger men were more likely to be recently infected compared to older men, with 31% newly diagnosed MSM aged under 35 recently infected compared to 13% among MSM older than 50 years. Higher proportions of RHI were also seen among younger heterosexual women aged 15-24 (14%) and heterosexual men aged 25-34 (14%) versus <5% of newly diagnosed heterosexuals aged over 50 years of age [HPA, 2011]... High proportions of RHI among those newly diagnosed are indicative of high on-going HIV transmission rates, although those who test frequently are also more likely to be identified as recently infected

Figure 1.3: Recently-acquired infections among new HIV diagnoses by exposure group: England and Northern Ireland, 2010 [Source: HPA, 2011]



Late Diagnosis

Guidelines from the European AIDS Clinical Society (EACS) (EACS, 2011), World Health Organisation (WHO) [WHO, 2010], International AIDS Society (IAS) [Thompson, 2010] and British HIV Association (BHIVA) [Gazzard, 2008] advocate initiating HAART for all individuals with a CD4 count <350 cells/mm^3.

Across Europe, almost one-third of individuals infected with HIV do not enter health care until late in the course of their infection [Adler, 2009; Fisher, 2008]. These individuals presenting with late or advanced infection have a higher risk of HIV-related mortality [May M, 2011; Moreno, 2010]. Recent consensus in Europe defines "late presentation" as:

> *"persons presenting for care with a CD4 count below 350 cells/*mm^3 *or presenting with an AIDS-defining event, regardless of the CD4 cell count* and "presentation with advanced HIV disease" as *persons presenting for care with a CD4 count below 200 cells/*mm^3 *or presenting with an AIDS-defining event, regardless of the CD4 cell count."* [Antinori, 2011]

1.5 The pathogenesis of HIV infection

1.5.1 Types, groups and subtypes

HIV-1 (Type 1) is responsible for the majority of infections globally, whilst HIV-2 (Type 2) is more prevalent in West African countries (Kanki, 1997]. HIV-1 can be divided into four major groups, based on their genetic similarity: M (main), N (new), O (out-group) and P [Plantier, 2009; Sharp, 2001]. Group M is most widely distributed and responsible for the 90% of global HIV infections, and can be further subdivided into nine subtypes or clades (A-K) [Louwagie, 1993]. These subtypes are associated with geographic areas and specific risk groups. The most prevalent HIV-1 clades are subtypes C (47%), A (27.2%), B (12.3%), and D (5.3%) [Osmanov, 2002]. HIV infection is associated with subtype B in Western Europe and North America, and most frequently found amongst MSM and injecting drug users. There are also mosaics of subtypes, known as circulating recombinant forms (CRFs) and unique recombinant forms (URFs).

1.5.2 The HIV virion

HIV is an RNA virus that codes for the enzyme reverse transcriptase (RT), which is needed to transcribe the viral RNA into a DNA copy that is capable of integrating itself into the host CD4 cell genome. Within the retrovirus family, HIV is classified as a lentivirus.

With a diameter of about 120 nm (around 60 times smaller than a red blood cell), the HIV virus consists of two copies of positive single-stranded RNA that code for the virus' nine genes and are tightly bound to enzymes needed for the development of the virion such as RT, protease, ribonuclease and integrase. The RNA is enclosed by a conical capsid composed of 2000 copies of the viral protein p24. This capsid is surrounded

by a bilayered phospholipid envelope (taken from the human cell when a newly formed virus particle buds) and embedded in this viral envelope are proteins from the host cell and about 70 copies of *env*, a complex HIV protein that anchors the structure to the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle. See Figure 1.4.

Figure 1.4: The HIV virion

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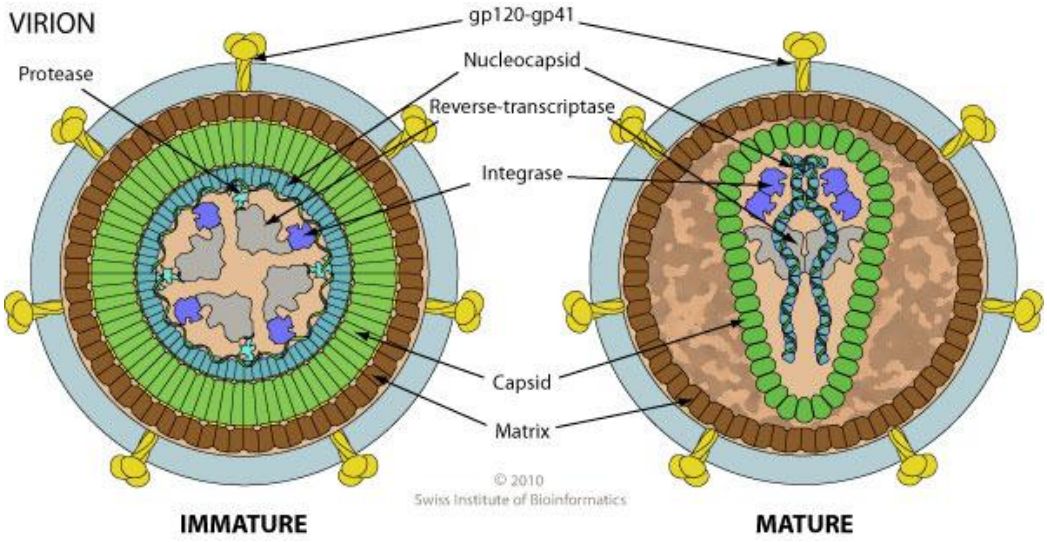
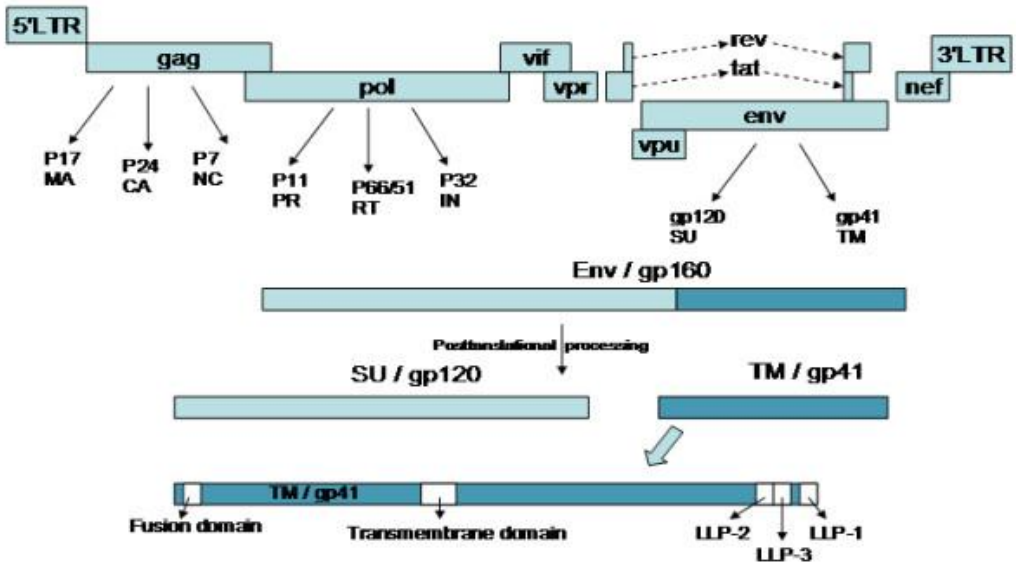


(Swiss Institute of Bioinformatics © 2010)

The HIV genome

The HIV genome consists of approximately 9200 nucleotides of RNA. The RNA consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS) and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu* and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins (Figure 1.5). Three of the genes are major: *gag* (encodes for internal structural proteins); *env* (encodes for transmembrane proteins); and *pol* (encodes enzymatic proteins e.g. RT, PR and integrase). *Pol*, which codes for the enzymatic proteins that are targeted by HAART, is routinely sequenced when testing for HAART resistance, and has relatively low genetic variability.

Figure 1.5: Organisation of the HIV genome [Costin, 2007]



1.5.3 HIV life cycle

HIV enters macrophages and CD4 cells by the adsorption of glycoproteins on its surface to receptors on the target cell, followed by fusion of the viral envelope with the cell which requires both a CD4 and chemokine receptor (either CCR5 or CXCR4) on the cell surface (Figure 1.6).

The first step in fusion involves the high-affinity attachment of gp120 to the CD4 protein. Once gp120 is bound, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor. This allows the N-terminal fusion peptide gp41 to penetrate the cell membrane. gp41 then collapses into a hairpin and it is this loop structure that brings the virus and cell membranes close together, allowing fusion and subsequent entry of the viral capsid [Chan, 1998].

After HIV has bound to the target cell, the HIV RNA and various enzymes (including RT, integrase, ribonuclease, and protease) are released into the cell. During the microtubule-based transport to the nucleus the viral single strand RNA genome is transcribed into double-stranded DNA. This process of reverse transcription is extremely error-prone and the

resulting mutations may cause drug resistance. This process can be inhibited by RT inhibitors. Dendritic cells (DC) are one of the first cells encountered by the virus during sexual transmission. HIV can infect DCs by this CD4-CCR5 route and also using mannose-specific C-type lectin receptors such as DC-SIGN [Pope, 2003].

Double-stranded viral DNA is then transported into the cell nucleus where its integration into the host cell's genome is carried out by integrase. The final step of the viral cycle occurs when HIV proteases cleave the polyproteins and the various structural components are assembled to produce a mature HIV virion, which is then able to infect another cell. The original cell dies. This cleavage step can be inhibited by protease inhibitors (PIs).

Figure 1.6: HIV life cycle [from Scherer, 2007]

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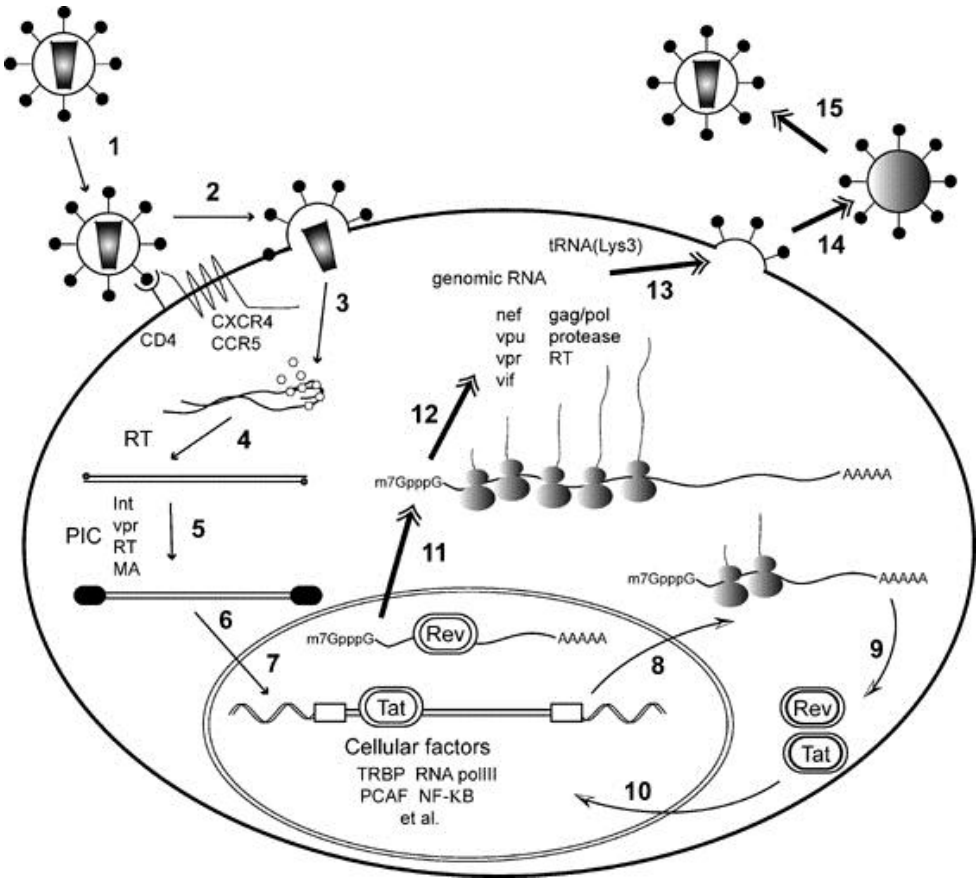

1.5.4 HIV in the genital tract

Although HIV RNA levels are generally a $\log_{10}$ or more lower in the genital tract than in plasma, studies [Powers, 2008; Coombs, 2003] have demonstrated close correlation between the two compartments. However, this correlation can be inconsistent and is particularly dependent on other factors such as concomitant STI - especially genital ulcer disease - and systemic co-infection [Anderson B, 2008; Johnson LF, 2008].

The heightened VL in blood during RHI is mirrored in both male and female genital secretions [Morrison, 2010; Pilcher, 2001] (see Figure 1.7). In men, peak VL is estimated to occur at 17 days in plasma and at 30 days in semen [Pilcher, 2007]. Female cervical VL is strongly correlated with plasma VL for the first 6 months of infection, and significantly higher than in longstanding infection, approximately 0.7 – 1.1 log copies/mL above set point [Morrison, 2010].

Note that these data on the interaction between VL and transmission have been derived from heterosexual HIV-discordant couples, and extrapolation to other risk groups such as MSM must be undertaken with caution.

Figure 1.7: HIV viraemia and shedding, measured over the natural course of HIV infection [from Pilcher, 2007]

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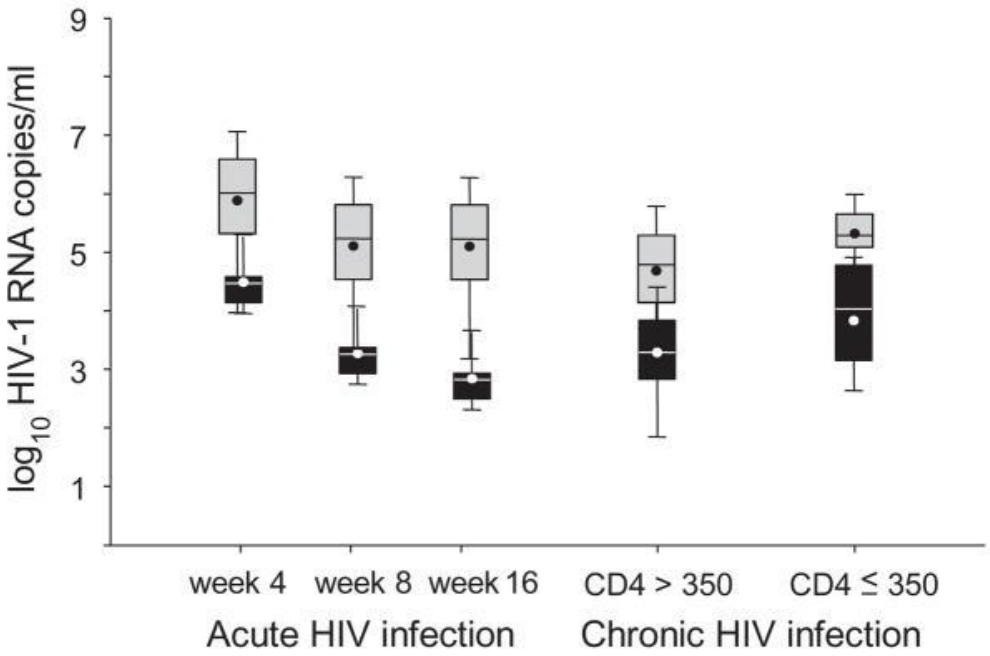

Box-and-whisker plots represent HIV-1 RNA levels in blood (grey) and semen (black), estimated for individual study subjects by disease stage. Numerical values at each time-point were estimated for each individual subject by empirical best linear unbiased prediction (eBLUP) making use of the final, unified best fit model including all observed data. Boxes and whiskers denote the 25th, 75th quartiles and total range of values. Internal circles and horizontal lines represent mean and median, respectively. Data for acutely infected subjects are shown at weeks 4, 8 and 16. The data for chronically HIV infected subjects represent the mean values of specimens collected over 16 weeks and are shown separately for high (>350 cells/mm^3) and low (≤350 cells/mm^3) CD4 cell count subjects.

1.5.5 Monitoring disease with surrogate markers

Once HIV has been diagnosed, two key clinical markers are used to monitor disease progression: the blood CD4 cell count and plasma VL. The CD4 cell count provides an indication of immune function and gradually declines over the course of infection. The VL measures how much HIV is in the blood and increases as immune function decreases**.** It is strongly associated with transmission risk: the higher the VL, the higher the risk of transmission [Fiore, 1997].

1.6 Antiretroviral therapy

1.6.1 Mechanism of action of antiretroviral therapy

Since 1998, in North America and Western Europe, the provision of HAART has been widespread. Patients treated with HAART have been shown to achieve reductions in VL to low or undetectable levels, over prolonged periods of time, provided they have excellent adherence to treatment [Montaner, 1998]. The advent of effective treatment has substantially reduced HIV-related morbidity and mortality in these settings [Bhaskaran, 2008; Mocroft, 2003; Palella, 1998]. However, treatment is only successful if followed indefinitely, and carries the risk of adverse side effects [Lucas, 1999] and the development of drug resistance [Beinker, 2001].

Antiretroviral drugs inhibit viral replication and reduce the damage to the host immune system, thus slowing the clinical progression of the infection. The drugs inhibit key stages of the HIV life cycle and are classified accordingly: fusion inhibitors; RT inhibitors; integrase inhibitors; and protease inhibitors. RT inhibitors include nucleoside (or nucleotide) and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs). Both work by blocking RT from transcribing the viral RNA into DNA. NRTIs compete with nucleoside analogues for incorporation into DNA and NNRTIs inhibit replication by binding to the active site of RT. PIs bind to the active site of the protease enzyme, preventing the production of viral proteins for the final assembly of new virions.

An undetectable plasma VL is the single most important marker of optimal HAART response.

1.6.2 Drug resistant HIV viruses

Drug resistant HIV viruses contain mutations, most obviously in *pol*, that reduce the susceptibility of the virus to certain antiretroviral drugs. Consequently, individuals infected with resistant virus are less likely to be treated successfully (Hirsch M, 2003) and have an increased risk of HIV-

related death (Beinker, 2001) compared to those individuals infected with a wild-type virus (*i.e.* virus that does not have drug resistance mutations).

It is important to distinguish between acquired resistance and TDR. Acquired drug resistance develops following antiretroviral therapy (ART), usually in patients taking a sub-optimal regimen or with poor adherence. TDR occurs after initial infection with a resistant strain. Both types of resistance have adverse consequences for the success of treatment, but present different challenges in the definition as well as the measurement of resistance [Bennett, 2009] – see Section 3.1.

1.7 Literature review 1/2: determinants of transmission

1.7.1 Sexual risk behaviour

1.7.2 Sexually Transmitted Infections

1.7.3 Blood and genital tract viral load

1.7.4 Recent HIV Infection

- **Biological plausibility**
- **Behavioural plausibility**
- **Empirical studies**
- **Phylogenetic studies**

Addressing the different approaches to prevention requires differentiation between HIV *acquisition*, which refers to an HIV-uninfected individual's risk of becoming infected, and *transmission*, which refers to an HIV-infected individual's risk of infecting an HIV-uninfected individual.

The risk of sexual acquisition of HIV by an uninfected individual reflects a composite of sexual behaviour [May R, 1989], male circumcision [Gray, 2007], co-infection with an STI (in particular genital ulcer disease [Sheffield, 2007, Galvin, 2004]), naturally occurring genetic [Lama, 2007] and immunological [Lehner, 2008] barriers to acquisition, as well as the characteristics of the exposing virus [Bar, 2010; Keele, 2009].

Sexual transmission of HIV reflects the quantification of the virus in the infected host (in particular the level of viral shedding from the genital tract), sexual behaviour, disease stage [Hollingsworth, 2008; Fraser, 2007] and STI co-infection [Fox J, 2009], as well as the characteristics of the exposing virus [Bar, 2010; Keele, 2009].

1.7.1 Sexual risk behaviour

The frequency, nature and duration of sexual exposure play a critical role in determining transmission of HIV [Siriwasin, 1998; Nicolosi, 1994; Saracco, 1993].

Transmission of HIV is known to be highest per coital act for receptive unprotected anal intercourse (UPAI) [Darrow, 1987; Padian, 1987] and is significantly more risky than unprotected vaginal sex (UPVI) [Lane, 2006]. Receptive sex is riskier than insertive sex [Macdonald, 2008; Gray, 2001; De Gruttola, 1988; Grant, 1987].

A systematic review and meta-analysis of transmission risk from UPAI assessed 16 studies; 4 studies reporting per-act transmission estimates and 12 assessing per-partner transmission risk [Baggaley, 2010]. The majority of these studies were from the pre-HAART era. The overall estimate of transmission risk per-act of UPAI was 1.4% (95% CI 0.2%–2.5%). There was no significant difference seen in per-act risk of receptive UPAI between heterosexuals and MSM.

Likewise, duration of relationship, number of sexual partners, rates of partner change, concurrency of partnerships and recreational drug use have all been associated with transmission [Elford 1999; Mastro, 1998; Giesecke, 1992; Grant, 1987]. However, estimates of per-contact risk of transmission for each sex act vary widely and the relative contribution of each of these factors governing seroconversion remains unclear.

1.7.2. Sexually Transmitted Infections

STI rates continue to rise in UK MSM. *Neisseria gonorrhoeae* diagnoses have increased by 61%, *Chlamydia trachomatis* by 48% and genital herpes by 32% in the single year 2011 [HPA, 2011]. These figures reflect what is observed globally [Chen XS, 2011; HPA, 2011, Rönn, 2011; Wolitski, 2011; van der Laar, 2010]. Not only can STI be a surrogate marker for risky sexual practices, but they also increase the risk of HIV transmission and acquisition. Biological findings support the mechanisms for STI increasing HIV acquisition and transmission through direct mucosal disruption, recruitment of HIV target cells to the genital tract, and increased HIV VL in

plasma and genital fluids. HIV can also alter the natural history of other STI [Fox R, 1987].

There is a strong epidemiological association between acute bacterial and viral STI and both the acquisition and transmission of HIV infection. This was first demonstrated in case series and retrospective studies that showed an association between previous STI and HIV [Piot, 1989; Weber, 1986]. Prospective studies strengthened this observation by showing a link between STI and incident HIV infection, with the strongest relative risks for genital ulcer disease [Cameron, 1989]. Such evidence has continued to accumulate over the decades, but has remained difficult to interpret because of confounding due to shared risk factors, particularly sexual behaviour, and difficulties in determining temporal relationships [Røttingen, 2002].

These observations have underpinned calls for STI management to be an essential part of HIV control programmes. Several large, well-conducted trials of enhanced STI treatment and care have failed to show a consistent impact on HIV incidence. The Mwanza study in Tanzania in the early 1990s showed a reduction in HIV incidence of 38% with enhanced syndromic management of STI [Grosskurth, 2000], but others did not [Kamali, 2003; Wawer, 1999]. Interpretation of these results probably rests on an understanding of different epidemic type and phase. Mwanza was conducted in a period of concentrated epidemic while the others were more generalised.

More recently, results of a major trial of the impact of HSV-2-suppressive therapy on HIV transmission were published. Over 3,000 HIV-discordant couples were recruited wherein the HIV-infected partner was co-infected with Herpes Simplex Type 2 (HSV-2). Aciclovir was given to the dually infected partner, and the outcome was transmission to the discordant partner. Aciclovir reduced genital ulcers by 73% and plasma HIV by 0.25 log, but it did not reduce HIV transmission [Celum, 2010].

Recently, injectable contraceptives have been associated with increased acquisition of HIV in females, an effect that remained even when adjusted for (male partner-reported) condom use. It is thought that the

synthetic progestogens in these compounds might alter the inflammatory response to STIs [Heffron, 2012].

In summary, STI are on the increase and although there is a plausible link between STI and HIV risk, intervention studies continue to be disappointing. This does not disprove a causal link, but mechanisms of action and the design and implementation of interventions need to be better understood.

1.7.3 Plasma and genital tract viral load

Plasma VL is a fundamental factor influencing the sexual transmission of HIV. A dose-response relationship between HIV plasma VL and risk of transmission in 15,127 heterosexual subjects was observed, with a rate ratio of 2.45 (95% CI 1.85–3.26) for each log increase in VL. There was no evidence of transmission for those with undetectable VL, or with those with plasma VL < 1,500 copies/mL [Quinn, 2000]. Similar results were seen in a study of 493 heterosexual couples in Thailand. In multivariate analysis, each log increment of HIV VL was associated with an odds ratio (OR) of 1.81 (95% CI 1.33–2.48) for transmission. No transmission events were documented for those with VL < 1094 copies/mL [Tovanabutra, 2002].

VL in the male and female genital tract is generally one $\log_{10}$ lower than that observed in the plasma [Coombs, 2003], and is influenced by ART [Zhang, 1998]. Whilst there is a strong correlation between HAART and decreased HIV RNA levels in semen, cervicovaginal fluid and anorectal mucosal tissue, it is not always predictable or persistent. Whilst Ghosn reported full seminal virological suppression with lopinavir/ritonavir at 48 weeks [Ghosn, 2008], Marcelin demonstrated that 7/145 (5%) of HIV-infected men harboured detectable HIV RNA in semen although they had no other STI and their plasma VL had been undetectable for at least 6 months under HAART [Marcelin, 2008]. Sheth also found seminal HIV shedding in men with undetectable plasma VL in 12/25 (48%) participants, with semen HIV RNA levels exceeding 5000 copies/mL in 4/25 (16%) [Sheth, 2009]. In another study of MSM, HIV-1 was detected in 18/101 (18%) blood and 30/101 (30%) semen samples. Of 83 men with undetectable HIV in blood

plasma, 25% had HIV in semen with copy numbers ranging from 80 - 2,560. Multivariate analysis identified STI/urethritis (p = 0.003), TNF-α (p=0.0003), and UPAI with an HIV-infected partner (p=0.007) as independent predictors of seminal HIV detection [Politch, 2012]. The situation is even less clear for HIV DNA levels in seminal cells [Cu-Uvin, 2006; Vernazza, 2005; Coombs, 2003].

A review of 19 studies with a total of 1,226 men found a mean Pearson correlation-coefficient between plasma and seminal VL of only 0.40 (range 0.07–0.64) [Kalichman, 2008a]; thus for any individual, achieving full suppression of plasma VL with HAART may not equate fully with genital suppression, with potential implications for ongoing risk of transmission.

1.7.4 Recent HIV Infection

Biological and behavioural plausibility, empirical and phylogenetic evidence, and mathematical modelling outputs strongly suggest that the phenomena that characterise RHI increase infectiousness, making it a potentially valuable disease stage for behavioural and biological intervention.

However, it has been difficult to make accurate observations because the diagnosis of RHI poses challenges for both the clinician and the laboratory, both contemporaneously and retrospectively. These difficulties are discussed further in section in 1.10.1 and explored in Chapter 2.

Biological Plausibility

RHI is thought to play a disproportionate role in HIV transmission, despite its short duration, mainly because of the high genital tract VL associated with high plasma VL [Pilcher, 2007, Pilcher 2001; Pedraza, 1999]. Among a cohort of heterosexual Malawian men with newly diagnosed HIV infection, the median plasma VL among those with AHI was $>10^6$ copies/mL compared with $10^{4.5}$ copies/mL observed among men with longstanding infection [Pilcher, 2004].

This heightened plasma VL during AHI is mirrored in genital secretions and leads to highly efficient sexual transmission [Wilson DP,

2008; Pilcher, 2007; Pilcher, 2001, Chakraborty, 2001; Quinn, 2000]. In men, the peak VL is estimated to occur at 17 days in plasma and at 30 days in semen, and remains elevated for approximately 10 weeks [Pilcher, 2007]. The entire period of increased infectiousness is thought to extend to 4.9 months [Cohen MS, 2010, *personal communication*] and therefore it is reasonable to use RHI (infection within 6 months) as opposed to AHI (p24 Ag or RNA positive and HIV Ab negative) as a suitable classification when studying transmission.

In most cases, MSM transmission appears to be mediated by a single infectious unit [Keele, 2008]. Transmission of multiple variants has also been observed, but is associated with factors that compromise the genital mucosa. Therefore, transmissibility is likely influenced by the viral subtype [Derdeyn, 2008]. During AHI, the level of infectiousness per potential transmission event is thought by some to be higher than would be predicted as function of the high plasma VL alone [Hollingsworth, 2008]. These calculations have suggested that viral factors beside the high VL immediately after HIV-1 acquisition are responsible for the increased transmissibility during AHI. Primate studies support this theory. In macaques, SIV virions isolated during AHI are more infectious than those from the longstanding phase of infection. Naïve macaques are productively infected with approximately one hundred-fold less AHI stage virions compared to the number of virions required from the longstanding stage of infection [Ma, 2009].

Potential explanations for this observation include a greater number of defective viruses circulating during the longstanding as compared to the acute stage of disease. Furthermore, host generated antibodies may coat the virus during the longstanding as compared to the acute phase of infection, retarding the ability of the viruses to infect target cells. In addition, modifications that occur over the course of infection may help the virus replicate within an individual host but may be counterproductive during transmission. Although the exact biological mechanism for the greater infectiousness observed among AHI variants is uncertain, these studies

suggest that acute stage circulating viruses possess unique properties that confer fitness for transmission.

Behavioural Plausibility

While most individuals diagnosed with RHI reduce their risk-taking sexual practices there is a significant minority, represented in both heterosexual and MSM groups, who do not [Pettifor, 2011; Seng, 2011; Fox J, 2010]. However, individuals with RHI are usually unaware of their infection. In a study by Rothenberg, individuals with RHI named 2.5 times (95% CI 2.1–3.0) as many current sexual partners as did individuals diagnosed with longstanding HIV infection [Rothenberg, 2009].

In contexts of high concurrency and/or rate of partner change, this contribution of RHI increases considerably. Concurrency operates not only as an individual factor for increased transmission but also by connecting multiple sexual dyads and clusters to one another at the population level [Marks, 2005]. Eaton and Garnett argue that the combination of long-term concurrent sexual partnerships and high infectiousness in RHI is a key driver of HIV transmission. They used a mathematical model to simulate HIV spreading in sexual networks with different amounts of concurrency. The models show that if HIV infectiousness is constant over the duration of infection, the amount of concurrency has much less influence on HIV spread compared to when infectiousness varies over three stages of infection with high infectiousness in the first few months. The proportion of transmissions during RHI is sensitive to the amount of concurrency and, in this model, is estimated to be between 16 and 28% in spreading epidemics with increasing concurrency [Eaton, 2011].

Empirical evidence

Despite the clear biological and behavioural plausibility outlined above, identifying transmissions from individuals with RHI has been difficult, which has hindered our ability to determine the proportion of transmission events attributable to RHI. The relative paucity of empirical data linking RHI to increased transmission reflects the dual challenges of identifying

individuals during or just after the brief disease stage and linking them to HIV-uninfected sexual partners who subsequently seroconvert. Therefore, the contribution of RHI to the spread of HIV remains to be quantified accurately.

In 2005, Wawer *et al.* published extensive empirical data (with phylogenetic support of transmission events, see Sections 1.7.4 and 1.10.3) from a cohort in Rakai, Uganda, that quantified how heterosexual transmission within stable discordant partnerships varies by disease stage. Notwithstanding the limitations of the study, it presents the best data available from which to directly estimate both the relative transmissibility of HIV during each stage of infection *and* the duration of periods of high infectiousness. They estimated that during the first 5 months of infection the probability of transmission per coital act was 8-10 times higher than during longstanding infection, and that the probability of transmission also increased by 4–8-fold during the 2 years before death. For a person newly-infected outside of a partnership, the probability of infecting their long-term partner within 2.5 months was 43% [Wawer, 2005].

These data have led Hayes to conclude that RHI may be responsible for 23% of transmissions [Hayes, 2006]. Hollingsworth and Fraser, who reanalysed data from this cohort with modified assumptions about sexual behaviour and transmission timing, estimated the hazard rate of transmission in acute HIV infection to be 26 times the rate compared to longstanding HIV infection [Hollingsworth, 2008].

Phylogenetic evidence

Phylogenetic sequence analysis has been commonly used [Blick, 2007; Wawer, 2005; Zhu, 1996] and is considered sufficient for the reconstruction of transmission events [Hué, 2004]. Such studies have correlated phylogenetic clustering with recent infection [Brenner, 2007; Yerly, 2001], viral factors [Lindström, 2006], risk behaviours and geography [Frost, 2007]. These data collectively suggest that chains or groups of transmission associated with RHI play an important role in transmission within these cohorts, which are predominately made up of MSM.

Phylogenetic studies have estimated that between 24 and 49% of RHI (in MSM and heterosexuals) are linked to other recent infections [Bezemer, 2010; Brenner, 2007; Yerly, 2001]. For example, in Quebec, likely transmission clusters among persons who seroconverted in the previous 6 months were identified. Individuals with treated or untreated longstanding infection were also included. Approximately half of the individuals who seroconverted in the previous 6 months fell into 75 transmission clusters whereas the remaining individuals had unique sequences, suggesting that RHI was responsible for approximately half of HIV transmission events [Brenner, 2007].

Lewis *et al.* focused on the internal architecture of the sequence clusters and used a combination of epidemiology, immunodynamics and evolutionary biology to infer the dynamics of HIV transmission in an MSM population in London. They applied a viral genetic relatedness cut-off to filter the data down to a computationally manageable subset of 402 HIV-infected individuals that exhibited at least one other close sequence relative in the study population. Nine large putative transmission clusters were identified within this subset of protease and RT sequence data on the basis of genetic (Hamming) distance. They then used a "relaxed clock" approach to generate time-scaled phylogenies of these data to infer the timing and distribution of transmission events within the 88 sequences contained in the six clusters that were large enough for analysis. In more than a quarter of cases, transmission events appear to have occurred fewer than six months after infection. Also, their results suggest that the sexual transmission of HIV in London over the previous decade may have occurred not as a slow and steady process, but rather via discrete outbreaks fuelled in part by efficient transmission during RHI [Lewis, 2008].

In contrast, Ambrose's recent work at UCL argues against RHI as a predominant factor in transmission. He took all individuals downstream of a particular infection (infection source A), within the same cluster as potential infectees, and used a date cut-off (at 0.18% nucleotide ambiguity, 125 days) to determine if these potential infections were during source A's recent phase of infection, or if A's infection had moved into the longstanding

phase. He simplified the methodology by defining the date of infection as *actually* being 125 days prior to the date the blood sample was taken. He used nucleotide ambiguity to identify RHI, and applied it to a national database of 43,002 samples, of which 40,627 clustered. Of these, 23.9% were classified as RHI at sample date, and these accounted for a median of only 7.3% of potential onward transmissions in clusters [Ambrose, 2011; Leigh Brown, 2011].

Mathematical models of transmission

Mathematical models provide an explicit framework within which to develop and communicate an understanding of transmission dynamics. They provide a tool to translate biological or behavioural interventions into quantifiable outcomes in terms of HIV incidence and prevalence. Several studies based on the concept of HIV transmission rates have recently appeared in the literature. The transmission rate for a particular group of HIV-infected persons is defined as the mean number of secondary infections per member of the group per unit time.

The degree of contribution from RHI will be influenced by the stage of the epidemic as well as the characteristics of the underlying population.

Models used to describe the proportional contribution of RHI have used a variety of model structures in varying populations and settings with differing parameter estimates [Prabhu, 2009; Abu-Raddad, 2008; Hollingsworth, 2008; Pinkerton, 2008; Pinkerton, 2007; Rapatski, 2005; Kretzschmar, 1998; Koopman, 1997; Pinkerton, 1996; Jacquez, 1994]. Most support the role of RHI as a critical driving force in the epidemic phase, but in some models of the endemic phase however, the impact of RHI has varied considerably, due in part to the varying approaches used and different populations studied [Prabhu, 2009; Abu-Raddad, 2008; Pinkerton, 2007].

The outputs from these modelling studies are interpreted and summarised in Figure 1.8, reproduced with permission from the author [Cohen MS, 2011b]. The methodology used to derive the ranges of estimates of infectiousness for each model in Figure 1.8 exemplifies the effect of studying different populations using widely varying assumptions. The

estimates in the graph reflect the proportion of all transmissions during an individual patient's entire infectious period, which depends on the epidemic phase and patterns of sexual behaviour [Abu-Raddad, 2008; Pinkerton, 2008; Kretzschmar, 1998; Koopman, 1997]. For this interpretation, transmission probabilities were drawn from the population category shown (sub-Saharan Africa heterosexual, USA heterosexual and MSM, USA MSM, Europe MSM) but the reported estimates result from a range of hypothetical sexual behaviour variables that do not necessarily reflect a specific population.

Figure 1.8: Role of Acute and Early HIV Infection on the spread of HIV, according to population studies in Sub-Saharan Africa, the United States and Europe [Cohen MS, 2011b]

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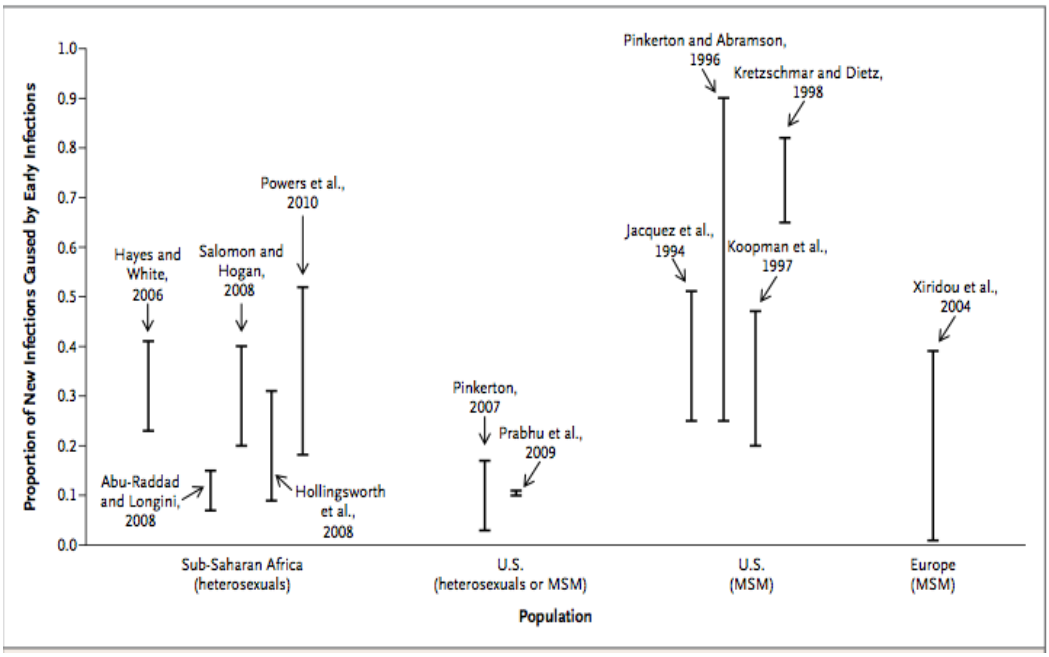

1.8 Literature review 2/2: evaluating prevention strategies

1.8.1 Behaviour change interventions

1.8.2 Non-ART methods: microbicides, vaccines and circumcision

1.8.3 Pre- and post- exposure prophylaxis

1.8.4 Studies of treatment as prevention

1.8.5 Mathematical models of treatment as prevention

Figure 1.9: The efficacy of prevention strategies [©AVAC, 2011]

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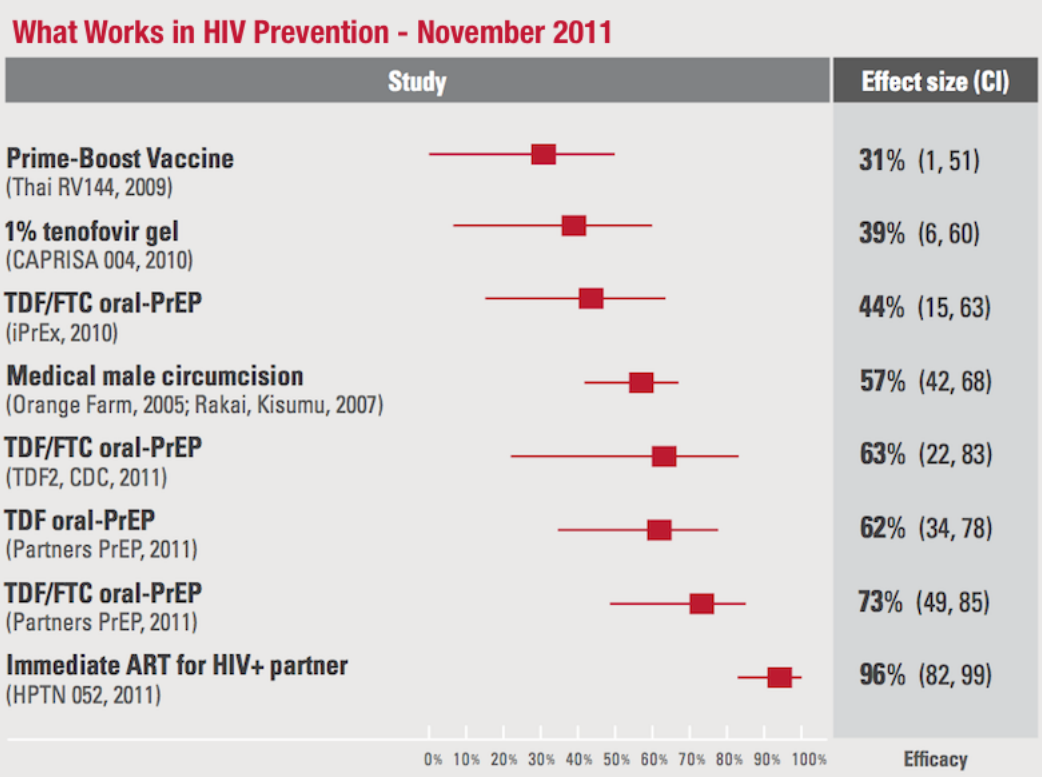


1.8.1 Behaviour change interventions

Condoms protect against STI including HIV (Malamba, 2005; Ryder, 2000; Pinkerton, 1998; Royce, 1997; Hanenberg, 1994). Prevention initiatives initially aimed to reduce HIV transmission through promoting safer sex. This means reducing unprotected sex (especially UPAI), number of sexual partners and concurrent partnerships. Early efforts in the USA to reduce HIV transmission through behaviour change centred on the "ABC of AIDS prevention": *Abstinence, Being Faithful and Condomizing.*

Evidence of the potential effectiveness of behavioural HIV interventions is growing, with a number of other systematic reviews and meta-analyses published. A 2008 Cochrane review [Johnson WD, 2008] demonstrated the effectiveness of individual, group and community level interventions in reducing high-risk sexual behaviour among MSM and found greater effectiveness for interventions that included the promotion of personal skills (*e.g.* having condoms available and avoiding excess alcohol or drug use) and the self-regulation of behaviour change.

Behavioural strategies for prevention in MSM have shifted from generic strategies to ones that are tailored toward the serostatus of both partners. Although patient-initiated risk reduction strategies, such as serosorting and strategic positioning, are being widely reported, these appear to offer limited protection to HIV-negative MSM. Fourteen to 44% of HIV-positive MSM, and 25–38% of those who are HIV-negative, report restricting UPAI to seroconcordant partners and 14–35% and 6–15% of MSM who are HIV-positive or negative, respectively, report selecting insertive or receptive sex on the basis of HIV status [McDaid, 2010].

Even though serosorting appears intuitively sensible, Pinkerton calculates that, taking the increased risk of transmission during often-unrecognised AHI into account, it remains a high-risk practice. He offers an example that in a population of MSM with a 1% annual incidence of HIV infection and an average 14 month time between HIV tests, the prevalence of undiagnosed, longstanding HIV infection would be expected to exceed 1%, and the prevalence of AHI would equal 0.134%, assuming only a 49-day period of AHI (not the 4.9 months suggested by Cohen [Cohen MS, 2010].

Therefore, assuming AHI to increase the likelihood of HIV transmission during UPAI by as much as a factor of 22 compared to longstanding HIV infection [Rapatski, 2005], the risk of acquiring HIV through UPAI with a presumably HIV-negative partner (who might be chronically or acutely infected) is equivalent to the risk associated with having UPAI with a partner drawn - without regard to his purported serostatus - from a population in which 1% + (22 x 0.134)% = 3.95% of MSM are living with longstanding HIV infection (a fairly low prevalence). Of course, those chosen partners with longstanding HIV infection are more likely to be on HAART and therefore minimally infectious whilst those with AHI are likely unaware of their status and will not be on HAART [Pinkerton, 2008].

Evidence is also available that MSM use partner plasma VL as another determinant of behaviour in an attempt to reduce risk of transmission [McDaid, 2010]. This became an increasingly popular strategy after the "Swiss statement" that HIV transmission in the context of fully suppressed VL and absence of STI was unlikely [Vernazza, 2008] which is further discussed in Section 1.10.

1.8.2 Non-ART methods: microbicides, vaccines and circumcision

Microbicides are compounds that can be applied inside the vagina or rectum to protect against HIV. They can be formulated as gels, creams or suppositories. There are different ways in which microbicides act. Some (*e.g.* Carraguard®, Cyanoviran®, cellulose sulphate, PRO 2000®) provide a physical barrier that keeps HIV and other pathogens from reaching the target cells. Another class of microbicides (e.g. Acidform®, BufferGel® and *Lactobacillus crispatus*) act by enhancing the natural vaginal defence mechanisms by maintaining an acidic pH. Some are topical antiretroviral agents. A key benefit of topical microbicides is that their use can be controlled by the receptive partner and as such they represent a useful strategy within relationships where the power balance is not level. At present however, there is no available microbicide that effectively prevents HIV transmission [AVAC, 2012].

Although no topical ART studies in humans have shown significant

protective effect, their use is complex and perhaps may be beneficial in those at particularly high risk. Topical Tenofovir® has been trialled and proven to confer protection from establishment of infection in primate models [García-Lerma, 2008]. Although not statistically significant, analysis of individual use subsets in the CAPRISA 004 study (Tenofovir®) suggested that high gel use/adherence (~50% to 80% of time) was associated with potentially greater efficacy (54%) [Abdool Karim, 2010].

There is no licensed vaccine against HIV infection, and no candidates currently in phase III trial [Barouch, 2008]. RV 144 is a vaccine trial combining two vaccines that failed on their own, vaccinating in Thailand over the course of 24 weeks in October 2003 then testing for HIV until July 2006, publicly releasing efficacy findings in September 2009. The initial report shows that the rate of HIV infection among volunteers who received the experimental vaccine was 31% lower than the rate of HIV infection in volunteers who received the placebo. The trial collaborators have stated that results of this trial give the first supporting evidence of any vaccine being effective in lowering the risk of contracting HIV [Rerks-Ngarm, 2009].

There is compelling evidence that male circumcision reduces the risk of heterosexually acquired HIV infection in men by around 50-60%. Observational studies indicate that circumcised men have lower levels of HIV infection than uncircumcised men and throughout the world, HIV prevalence is generally lower in populations that traditionally practise male circumcision than in populations where most men are not circumcised.

Three RCTs in South Africa [Auvert, 2005], Kenya [Bailey, 2007] and Uganda [Gray, 2007] have confirmed that male circumcision provided by well-trained health professionals in properly equipped settings is safe and effective at preventing acquisition of HIV. A systematic review and meta-analysis of 28 published studies found that circumcised men are two- to three-fold less likely to be infected by HIV than uncircumcised men, with differences most pronounced in men highly exposed to HIV infection [Siegfried, 2010]. A sub-analysis of 10 African studies found a 3.4-fold lower

incidence of HIV infection among men considered to be at high risk of becoming infected.

Pooled analyses of available observational studies of MSM, however, reveal insufficient evidence that male circumcision protects against HIV infection or other STIs. However, the comparable protective effect of male circumcision in MSM studies conducted before the era of HAART, as in the circumcision trials of heterosexual African men, supports further investigation of male circumcision for HIV prevention among MSM [Millett, 2008].

Unfortunately, although circumcision prevents female to male transmission, there is no evidence to support a protective effect for the receptive sexual partner. There is an argument, therefore, that circumcision disempowers the receptive partner and that there is an increased risk of sexual violence against these individuals as a result. Furthermore, circumcision is only protective in MSM who practice insertive sex exclusively.

1.8.3 Pre- and post- exposure prophylaxis

The use of HAART to prevent HIV acquisition in the HIV-uninfected after potential exposure to HIV - Post-Exposure Prophylaxis (PEP) - has a solid evidence base and, although an unlicensed indication for ART, is used widely in clinical practice [Benn, 2011]. The advantage of PEP is that it is a targeted approach to those most at risk, short-term, and is supported by data from primate and prevention of mother-to-child transmission (PMTCT) studies. Disadvantages are that it requires HIV testing prior to use, has high medical personnel costs and that it is difficult to assess cost-effectiveness.

Pre-Exposure Prophylaxis (PrEP) is the use of ART either by single oral agent (Tenofovir®, Maraviroc®, Raltegravir® or Darunavir®), dual agent (Truvada®) or topical gel (Tenofovir®) *before* exposure in the uninfected individual. Primate studies and the early trials (iPrex - the only PrEP study devoted to MSM, TDF2 and Partners PrEP) showed a significant reduction in transmissions with oral agents (up to 44%) [Thigpen, 2011; Baeten,

2011; Grant, 2010] but some subsequent trials (in African women) failed to show beneficial effect [FEM-PrEP and VOICE] and were modified early by an independent data and safety monitoring board (DSMB) for futility.

The majority of incident infections in the study drug arm of iPrex had pharmacokinetic data available (34/36), and only 9% of these individuals had detectable study drug-levels compared to 51% of those who did not get infected - suggesting that adherence to medication was the critical factor in non-protection. The most likely explanation for non-protection in the FEM-PrEP and VOICE is similarly high reported but low actual medication adherence, possibly resulting from methodological issues such as the use of financial incentivisation for participating in the trial.

There is also uncertainty about Tenofovir® concentration in the female genital tract or rectal mucosa after oral administrations, which are many fold lower than after topical application [Karim, 2011]. This can lead to an inability to completely and consistently suppress replication of HIV in the genital tract, suggesting that these compartments do not get full penetration by ART or that ART metabolism is different in lymphoid tissues [Cohen J., 2011].

There are currently 10 on-going PrEP trials worldwide that are expected to report between 2011 and 2013 [AVAC, 2011c]. Novel methods for delivering PrEP are also being investigated, including vaginal ring Dapivirine® (TMC 120) [Nel, 2011] or depot injection Rilpivirine® (TMC278) [Jackson, 2012].

Two new studies (HPTN 067 and HPTN 069) sponsored by the HIV Prevention Trials Network (HPTN), one study sponsored by ANRS (IPERGAY) and an extension of the iPrEx study (iPrEx OLE) are addressing some of the limitations of the previous PrEP studies. Addressing the adherence issues with daily PrEP dosing, the behavioural study HPTN 067 is designed to test the hypothesis that recommending intermittent usage of oral Tenofovir®/Emtricitabine®, compared with recommending daily usage, will be associated with equivalent coverage of sex events with pre- and post-exposure dosing, lower number of pills needed for coverage, and

decreased severity and frequency of self-reported side effects [HPTN 067, 2012].

PrEP effectiveness will depend on many factors and the choice of intervention must take into account the epidemiological context in a given setting. It is possible that the benefits of PrEP outweigh risks, but the challenges of poor adherence among HIV negative individuals and possible increase in sexual risk behaviour remain of concern. The other main danger is that of inadvertent administration of sub-optimal ART to an already HIV-infected individual and the subsequent risk of acquiring drug resistance [Abbas, 2011], which could potentially compromise their response to later therapy and outweigh reduced population HIV acquisition. Other questions concern length of treatment, benefit over time, toxicity (in particular renal toxicity with Tenofovir®), choosing whom to treat and how to monitor them. Again, similarly to PEP, an HIV test is required prior to treatment and it is not easy to assess cost-effectiveness.

1.8.4 Studies of treatment as prevention

Treatment for prevention describes the public health or community benefits from the use of HAART to decrease onward transmission of HIV [Mayer, 2010]. PMTCT offers proof of concept that HAART effectively interrupts HIV transmission, which is not observed if maternal plasma VL < 1,000 copies/mL [Garcia, 1999]. In the UK, it has been shown that perinatal HIV infections cases can be virtually eliminated with the implementation of guidelines for opt-out HIV testing and HAART for pregnant women and newborn infants [Townsend, 2008; Fowler, 2007].

Cohort studies of treatment as prevention

Plasma VL predicts the risk of sexual transmission of HIV, which is rare in heterosexuals with levels < 1,500 copies/mL [Quinn, 2000]. HAART dramatically lowers VL, and numerous heterosexual observational studies have demonstrated its potential for prevention of HIV transmission [Anglemyer, 2011; Reynolds, 2011; Donnell, 2010; Linpappa, 2010; Castilla, 2005; Wawer, 2005; Tovanabutra, 2002].

A 2009 meta-analysis that included 11 cohorts (5,021 heterosexual couples) found zero risk of sexual transmission while on HAART for plasma VL < 400 copies/mL (upper confidence limit of 1.27 per 100 years). Although there is a predictable relationship between HIV viraemia and transmission rates for VL > 400 copies per mL, in the range of VL relevant to the clinical use of HAART there is still uncertainty regarding transmission rates. Mathematical curve fits of the data diverge at low viral levels due to insufficient data, and extrapolation of standard regression models (linear relationships on the log scale) is inappropriate for estimating transmission risk for low VL. Available data on transmission events for VL < 400 copies/mL suggest a transmission rate estimate of 0.16 (95% confidence interval [CI] 0.02-1.13) per 100 person-years [Attia, 2009].

Nonetheless, the relationship between VL, HAART and transmission is so strong that a 2008 report controversially suggested that HIV serodiscordant heterosexual couples may have unprotected sex, provided the HIV-infected partner is successfully treated with plasma VL suppressed to < 40 copies/mL (Vernazza, 2008), with no risk to the uninfected partner. This "Swiss Statement" has been criticised as loosely-evidenced by the Joint United Nations Programme on HIV/AIDS (UNAIDS, 2008) and the USA's Centers for Disease Control and Prevention [CDC, 2008].

There are no data on risk of transmission for UPAI in MSM when the index partner is on HAART. Evidence is also not available for distinguishing transmission rates under HAART according to condom use, presence or absence of other STI or direction of sexual intercourse (insertive or receptive).

Randomised controlled studies of treatment as prevention

The Partners in Prevention HSV/HIV Transmission Study, which investigated the risks of HIV transmission from heterosexual individuals with suppressed viraemia in HIV serodiscordant relationships, reported a 92% reduction of HIV transmission between long-term, HIV-serodiscordant heterosexual couples in Africa if the HIV-positive partner was on HAART.

However, 39/103 (38%) transmissions were not phylogenetically linked to their partners [Donnell, 2010].

The most robust evidence of HAART drastically reducing heterosexual transmission came from the HPTN 052 study [Cohen MS, 2011a]. This study took place in Malawi, Brazil, India, Thailand and Zimbabwe and was a phase III, two-arm, multi-site, randomised trial to determine the effectiveness of two treatment strategies – one of which was early treatment – in preventing the sexual transmission in 1,750 HIV-serodiscordant couples. After just 39 phylogenetically confirmed transmissions, an independent DSMB intervened. So strong was the evidence that the protocol modified to offer HAART to all subjects a full 3 years before the study was due to end, when it became apparent that early HAART reduced transmission in discordant heterosexual couples by 96%.

A key question from both these studies is the generalisability to other contexts: heterosexual couples with CD4 counts lower and higher than those studied in HPTN 052, high-risk heterosexual subjects (*e.g.* sex workers and their clients), MSM and IDU. There are no data that address this issue directly.

Finally, the Partners of People on ART: a New Evaluation of the Risks (PARTNER) study will investigate the factors associated with condom use in HIV discordant partnerships (heterosexual and MSM) to estimate the rate of HIV transmission whilst on fully-suppressive ART. PARTNER is an international, observational multi-centre study in which HIV serodiscordant couples, where the infected partner has VL < 50 copies/mL and who report unprotected anal or vaginal sex, are followed over time. Primary outcome is HIV transmission [Rodger, 2012].

There are currently 8 on-going studies of HAART-based prevention in HIV-infected individuals [AVAC, 2011a].

Community viral load

In populations with high testing rates, a low proportion of undiagnosed HIV infection and very high levels of suppressive HAART coverage, the effect of reducing community VL can start to be seen. The best evidence comes from

San Francisco. Here, amongst MSM, two values of community VL were determined: the mean community VL and total community VL (the sum of the most recent VL of all reported HIV-infected persons for a particular year). HIV incidence was calculated using methodology based on a detuned assay system. There were statistically significant declines in mean and total community VL from 2004 to 2008, matched by increase in proportion in virological suppression (defined as a VL < 75 copies/mL). There was a statistically significant decline in new HIV diagnoses from 793 cases in 2004 to 434 cases in 2008 ($p < 0.005$), but association with HIV incidence was not statistically significant in meta-regression ($p>0.3$) [Das, 2010].

The concept of a community VL and relationship with risk of HIV transmission was also explored in a prospective cohort study of IDU in Vancouver, Canada [Montaner, 2010a]. Two cohorts, one HIV-uninfected (n=1,429), the other with known HIV infection (n=622), were followed between 1996 and 2007. The community VL, calculated as the median measure of all VL measurements for the HIV-infected cohort, was followed longitudinally and the impact of the community VL on HIV incidence in the uninfected cohort was evaluated, while adjusting for HIV risk behaviour. The multivariate model was adjusted for nature and frequency of drug use, sexual practice and needle sharing. Overall, the median community VL fell below 20,000 copies/mL after 1998, mirroring increasing uptake of HAART. The incidence density of new HIV infection was 2.49 (95% CI 2.09–2.88)/100 person years. Community VL remained independently associated with time to HIV seroconversion (HR 3.32; 95% CI 1.82–6.08), but this was no longer statistically significant after median VL declined below 20,000 copies/mL.

Additional population-based associations between increased HAART coverage and decreasing new HIV diagnoses have recently been presented [Montaner, 2010b]. Estimates of the annual median community VL were undertaken by using the highest VL value available for an individual in a given year. Poisson log-linear regression models were used to estimate the association between new HIV diagnoses (new positive HIV tests per 100 population) and covariates including HIV VL and number of individuals on

HAART. The overall correlation between numbers of individuals receiving HAART and new diagnoses per year was −0.89 ($p < 0.0001$). For every 100 additional individuals receiving HAART, the number of new HIV cases decreased by a factor of 0.97 (95% CI 0.96–0.98) and for each log decrease in community VL, the number of new cases decreased by a factor of 0.86 (95% CI 0.75–0.98). The rates of syphilis, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* also increased during this time period, indicating a protective effect despite continued high-risk sexual practices.

These studies suggest an association between increasing HAART uptake at the community level and a decrease in community VL, with a subsequent decrease in new diagnoses of HIV. However, it should be noted that no significant effect on HIV incidence could be confirmed.

1.8.5 Mathematical models of treatment as prevention

With limited empirical data, models have been a key tool for evaluating and predicting the effect of HAART on the evolution of HIV epidemics. However, they are susceptible to inaccuracies in the assumptions used where complete data are not available. The reliability of these models to forecast HIV epidemics under conditions of high HAART coverage will therefore depend on the accurate ascertainment of RHI and its infectiousness, as well as accurate data regarding (heterosexual and MSM) transmission risk in the presence of HAART with variable condom use.

Since the introduction of HAART, many papers have been published presenting results from HIV transmission models exploring the impact that HAART has had or will have on HIV incidence, prevalence and infections averted in various settings [Pinkerton 2011a,b; Wilson DP, 2011; Lima, 2010; Epstein, 2009; Granich, 2010; Bezemer, 2008; Salomon, 2008; Wilson DP, 2008; Baggaley, 2005; Law, 2002; Blower, 2001].

Bezemer *et al*. [Bezemer, 2008] conclude that a resurgent epidemic in the era of HAART is probable, most likely caused by increasing sexual risk behaviour. Other modelling that evaluates the potential impact of HAART on transmission concludes that treatment alone would not be expected to alter population-level HIV incidence in the absence of changes in behaviour

of individuals who are either uninfected or infected and not on treatment [Salomon, 2008].

Conversely, modelling by a Canadian group, using varying assumptions regarding drug resistance, adherence to HAART, therapeutic guidelines, degree of HAART coverage and the timing of HAART uptake concludes the opposite, that expansion of HAART would lead to substantial reductions in the growth of the HIV epidemic [Lima, 2010].

When taking into account both the undiagnosed pool of infection as well as the enhanced transmission potential of RHI, results from an extended transmission rate model indicate that approximately 50% of new infections in the USA result from transmission risk behaviours of those infected but unaware of their infection, including 10% from persons in the acute phase of HIV infection. Findings from this study suggest that significant reductions in HIV incidence likely will require a combination of increased Ab testing, enhanced early detection of RHI, HAART adherence counselling and behavioural risk reduction interventions [Pinkerton, 2011a, b].

Universal Test and Treat

It was hypothesised a decade ago that the enhanced identification of HIV-infected individuals followed by immediate access to HAART, irrespective of CD4 cell count or disease stage, would reduce infectiousness at a population level, thereby reducing HIV incidence [Stover, 2002; Velasco-Hernandez, 2002].

The concept of UTT represents a true paradigm shift in the use of HAART, targeting infectious individuals for prevention rather than protecting uninfected exposed populations, and is under active consideration as a strategy to reduce HIV incidence [Lima, 2010]. According to one modelling study, UTT in South Africa would result in a 95% reduction in HIV incidence in 10 years, with HIV prevalence becoming less than 1% by the year 2050 [Granich, 2009].

However, to achieve this outcome requires > 90% of HIV-infected individuals in a population to be identified and to agree to take lifelong

HAART. The assumptions underlying this model have provoked much debate, although similar models by other groups [Dodd, 2010; Garnett, 2009; Baggaley, 2005] have concurred on the potential population impact of this strategy, but even minor modifications to these highly optimistic assumptions could wipe out predicted impact.

More recent modelling work by Hallett suggests that even to achieve a 60% reduction in new infections through early treatment, testing would have to be so frequent that 60% of those infected were diagnosed within a year of infection, 90% of diagnosed people would have to be treated, 87% would need to be virally suppressed within six months of starting therapy with only a 1% drop-out rate from treatment programmes. Hallett has noted that although we know from the HPTN 052 trial that early initiation of treatment can reduce transmission to stable partners by 96%, this does not mean that changing treatment guidelines will bring about a 96% reduction in new infections [Hallett, 2011a].

One area of special interest is the potential balance between PrEP and HAART treatment, as well as the targeted use of other ART-based prevention strategies within discordant couples. Using available data to look at the management of HIV discordant couples, Hallett also reports that use of PrEP by the uninfected partner could be at least as cost effective as earlier HAART initiation by the infected partner, provided that the annual cost of PrEP is less than 40% the cost of HAART and the effectiveness of PrEP exceeds 70% [Hallett, 2011a].

There is also a lack of information on potential negative effects of a UTT policy, in particular the risk of increasing transmission of drug-resistant strains [Hoare, 2010; Smith R, 2010; Lange, 2004] induced by poor adherence to HAART (as HAART coverage widens, the absolute number of this subgroup will also increase), the risk of sexual disinhibition [Kalichman, 2003] and the risk of stigmatisation. Indeed, an UTT strategy may well increase long term costs [Dodd, 2010].

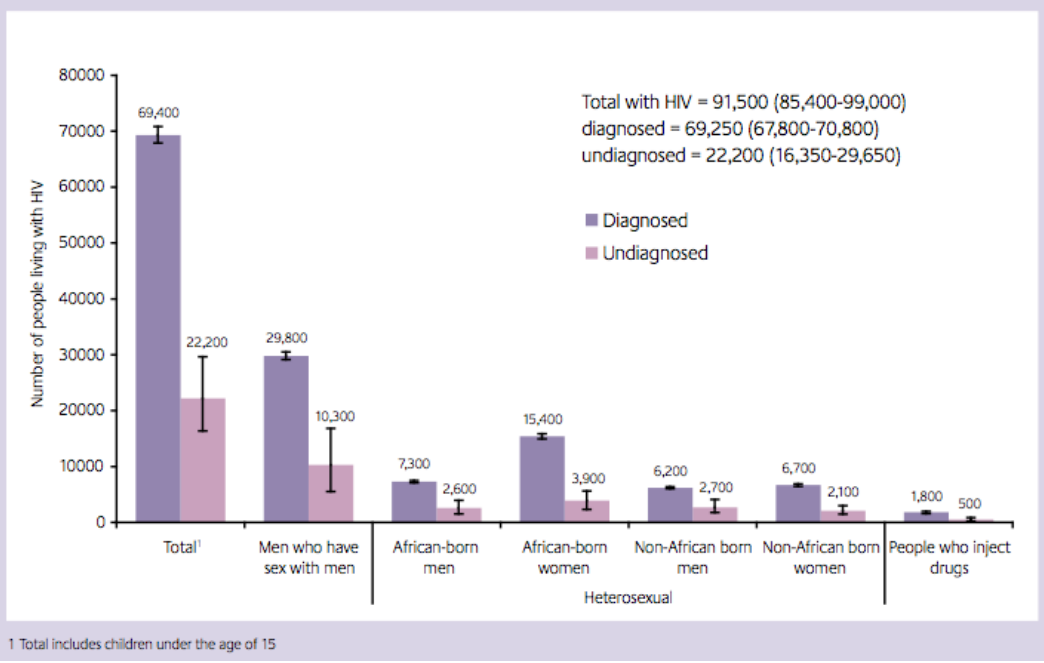
1.9 Potential barriers to treatment as prevention

1.9.1 HIV Testing coverage

Undiagnosed infection

Many individuals remain undiagnosed (see Figure 1.10). Anonymous HIV testing in UK GUM clinics suggests that 24% (range 19-30%) of the 91,500 HIV infections in the UK in 2010 remained undiagnosed - *i.e.* around 22,000 people were unaware of their infection and unable therefore to benefit from effective treatment [HPA, 2011]. An estimated 40,100 (35,300-46,700) MSM were living with HIV, of whom 26% (16-36%) were undiagnosed.

Figure 1.10 Estimated number of people living with HIV (both diagnosed and undiagnosed) in the United Kingdom: 2010 [Source: HPA, 2011]



HIV testing

HIV testing is recognised as a crucial part of all programmes for HIV prevention, especially in view of the potential prevention effect of HAART. Demand for HIV testing is a complex function of access to health care, perception of risk, fear and stigma [Medley, 2004]. In the USA, according to a 2008 CDC report, 16% of HIV-infected MSM had never previously tested for HIV [CDC, 2011a]. More recently, also in the USA, Margolis showed that 9% of MSM accessing an internet sexual networking site had never tested, including 24% in the age range 18-24 [Margolis, 2012]. UK surveys have shown that up to 30% of MSM and 38% of Black Africans living in the UK had never tested for HIV [HPA, 2011; HPA 2010].

Best practice guidance recommends that testing should be offered to all individuals at their first attendance at a genitourinary medicine (GUM) clinic and subsequently according to risk [BHIVA, 2008]. An estimated 2.1 million HIV tests were performed in 2010 in England. Approximately 931,700 (47%) and 657,500 (31%) of HIV tests were performed in GUM clinics and antenatal settings respectively. A further 260,000 were conducted in the primary care setting and 250,000 in secondary care; these settings were the source of one in four HIV tests in 2010.

Of all people who attended a GUM clinic in 2010, 69% received an HIV test. This coverage rate was higher among MSM: 82% compared to 69% in heterosexuals. Not all GUM attendees are offered or accept an HIV test at every visit. In 2010, of the 1,469,500 new visits among people not known to be HIV positive, 78% included the offer of an HIV test and one in five (22%) declined [HPA, 2011].

In 2011, the National Institute for Health and Clinical Excellence (NICE) published guidance for increasing the uptake of HIV testing among MSM and Black African communities [NICE, 2011a; NICE, 2011b]. The guidance takes further the recommendations made by BHIVA in advocating the development of local strategies for testing in the two higher-risk groups. Similar guidance has been issued by the European Centre for Disease Prevention and Control (ECDC) [ECDC, 2010a].

Evaluation of eight projects aimed at expanding HIV testing outside the GUM clinic and antenatal settings, shows that when appropriate training has been carried out, HIV testing is acceptable to both staff and patients [ECDC, 2011b]. More than 11,000 people were tested during the pilots and 51 people were newly diagnosed, giving an overall HIV positivity of 4 per 1,000 population. Most pilots had positivity rates of at least 1 per 1,000 population, the threshold for cost-effectiveness.

These findings provide an evidence base for the routine recommendation and offering of an HIV test to all general medical admissions and new registrants in primary care in high prevalence areas in accordance with the BHIVA national guidelines [Gazzard, 2008; BHIVA 2008]. In an attempt to reduce the pool of undiagnosed HIV infection, these guidelines recommend wider routine testing in any region where HIV prevalence is > 2 per 1,000 amongst 15-59 year olds. In 2010, 54 English local authorities had a diagnosed prevalence above the 2 per 1,000 threshold, of which 29 were in London.

Whilst the promotion of testing remains essential for the clinical outcome of individual patients, its potential to reduce current levels of HIV transmission appears uncertain. There are several reasons why increased testing might fail to achieve sufficient reduction in transmission. These include biased testing, incomplete testing coverage, a continuation or increase in sexual risk behaviour after testing positive *or* negative, delayed treatment and RHI. This is borne out by the fact that even though UK testing coverage and subsequent virological suppression in those infected and on treatment is amongst the highest in the world, HIV incidence in MSM continues to rise.

1.9.2 Behavioural disinhibition

There is a concern that for some individuals, some of the time, the use of HAART may promote increased risk-taking behaviour because of improvements in physical health, or diminished concerns regarding transmission.

However in the SMART study, where half the subjects were MSM, Burman reports that randomisation to start HAART was associated with a trend towards decreased high-risk behaviour [Burman, 2008]. Similarly, in an evaluation of 457 injection drug users within the Vancouver, Canada cohorts, initiation of HAART was not associated with increased unprotected intercourse or multiple sexual partnerships [Marshall, 2010]. Similar declines have been observed in heterosexual sub-Saharan African populations. Cohort data nested within the Partners in Prevention HSV/HIV Transmission trial also found diminished sexual risk behaviour associated with initiation of HAART [Donnell, 2010]. Analysis of 6,263 heterosexual men and women followed within primary care clinics in South Africa between 2003 and 2010 found that receipt of HAART was associated with a decrease in sexual activity, a decrease in unprotected sex and a decrease in multiple partnerships [Venkatesh, 2010].

Other cross-sectional studies have looked at the influence of taking HAART on sexual risk-taking behaviour. The results of individual studies differ, but a meta-analysis has shown no association between HAART use and risk behaviour [Crepaz, 2004]. Longitudinal cohort studies have also had disparate results, with some showing an increase in risk behaviour after starting HAART [Chen SC, 2006; Descqilbet, 2002] and others showing stable or decreased risk behaviour after starting [Bouhnik, 2007; Glass, 2004]. Other studies suggest a more complex effect of starting HAART on risk behaviour; women reported a decrease in number of sexual partners, but an increase in episodes of unprotected vaginal intercourse [Wilson TE, 2004].

Thus, whilst sexual behaviour on HAART has an influence in real-world transmission events it is difficult to model because of its complexity and unpredictability, as well as variability between individuals and in the same individuals over time.

1.9.3 Antiretroviral Resistance

One of the major concerns regarding implementation of treatment as prevention strategies is the development of resistance to antiretroviral

drugs. The emergence of drug-resistant strains poses clinical challenges, and may be important in the context of a community epidemic by being responsible for sustained transmission despite wide HAART coverage.

A mathematical model calibrated to represent the HIV epidemic in the MSM community in San Francisco determined that the majority of circulating resistant strains were capable of being transmitted and causing self-sustaining epidemics [Smith R, 2010]. Data from a Canadian Drug Treatment Program demonstrated significant decreases in drug resistance over the last decade [Gill, 2010]. This decrease is likely the result of more potent, well-tolerated regimens that achieve full virological suppression rapidly [Boyd, 2009].

As treatment coverage increases, so will the number of patients with drug-acquired resistance. The contribution of drug-acquired or TDR to subsequent TDR is not fully understood. If drug-acquired resistance is a major transmission pathway, then there will be a risk that TDR increases. This is further discussed in Chapter 3.

1.9.4 Sub-optimal adherence to HAART

Not surprisingly, success of potent HAART for HIV infection is compromised primarily by failure to maintain optimal levels of adherence over the long term [Moore, 2005; Paterson, 2000]. Sub-optimal adherence to HAART is a key barrier to optimal treatment response and a strong independent predictor of immunological progression, AIDS and death [Kalichman, 2011; Ford, 2010; Mocroft 2003]. Indeed, it is second only to the CD4 cell count as a predictor of progression to AIDS [Bangsberg, 2001] and it remains the most important potentially alterable factor that determines treatment outcome.

Adherence to a medication regimen is generally defined as the extent to which patients take medication as prescribed by their health care provider. Rates of adherence for individual patients are usually reported as the percentage of the prescribed doses of the medication actually taken by the patient over a specified period.

Research published in 2000 showed that an individual needed to be > 95% adherent to his or her HAART regimen in order to maintain full virological suppression [Paterson, 2000]. A review by Simoni reports on 77 articles published between 1996 and 2004 [Simoni, 2006]. A second meta-analysis by Nieuwkerk reports on 65 articles published between 1996 and 2003 [Nieuwkerk, 2005]. They examined the adherence predictors of plasma HIV RNA concentration outcomes among adult patients prescribed HAART using self-reported adherence measures. Across all studies, the pooled odds ratio (OR) of detectable plasma VL in non-adherent patients was 2.3 (95% CI 2.0–2.7) compared to adherent patients. Both articles suggest that the risk of HIV regimen failure is at least doubled among patients who are non-adherent to treatment.

However, there are little data on the newer agents that might be considered more "forgiving" of reduced adherence. It may be that >80% adherence to newer boosted PIs is sufficient, and some co-formulation NNRTI-based regimens could require lower adherence rates even than boosted PIs [Kobin, 2011] due to their mutually long half lives. However, recent RCTs suggest that >95% adherence remains critical. For example, the THRIVE study, which compared Truvada® and Efavirenz® against Truvada® and Rilpivirine®, showed that in those subjects who reported >95% adherence, treatment response was 89% and 90% respectively whereas in those who reported <95% adherence, response was only 64 and 62% respectively [Cohen CJ, 2011].

New formulations have dramatically reduced pill burden to as little as one pill taken once daily. Whilst there is no doubt that patients often prefer a once-daily regimen, these technological achievements only exerted a modest effect on adherence (+4.4%, p=0.001) and were non-significant to clinical outcome (+5.7%, p=0.27) in a recent meta-analysis [Parienti, 2009].

Despite many clinics in London reporting that between 95 and 98% of patients who are taking HAART are fully virologically suppressed [Gilson, 2011, *personal communication*; Fisher, 2011, *personal communication*], there is a significant minority of patients who are either unable to start or adhere to a HAART regimen. These individuals are often disengaged from

care, and so are not identified as non-adherent, and likely represent a serious risk for onward transmission.

Reasons for non-adherence

Whilst it is commonly accepted that "most of us are non-adherent some of the time" [Horne, 1999], predictors of non-adherence and the impact on viral breakthrough identify certain patient characteristics that are associated with medication non-adherence such as drug addiction, psychiatric comorbities (*e.g.* anxiety, depression), poor social support and even age [Sherr, 2008], while clinical variables of high baseline VL, low CD4 count and co-infections are associated with poorer treatment outcomes.

Adherence to HAART may be addressed by a dual "perceptions and practicalities" approach that considers the patient's beliefs about ART as well as their ability to follow the regimen. This approach suggests that, assuming appropriate resources are available, the health-seeking response of taking medication is essentially dependent on two factors: motivation and ability.

Most of the early research on medication-taking focused on ability, and non-adherence was often assumed to be *unintentional*. This includes a variety of practical barriers such as a misunderstanding of instructions, difficulties fitting the regimen into one's daily schedule, forgetting to take doses, or an inability to use the treatment because of impaired manual dexterity or cognitive impairment.

These factors are undoubtedly important, but it is now clear that this research tended to target ability and ignore motivation. Motivation would be concerned with the perceived benefit of the treatment versus the frequency and concerns about side effects. Therefore, sub-optimal adherence is often the result of an *intentional* decision on the part of the patient to avoid medication or to use it in a way that differs from the prescriber's instructions. An example of this is the patient who decides that it is better to reject treatment that will prolong life on the grounds that it might diminish their quality of life. Of course it is not so straightforward in reality - there can be some overlap. For example, adverse side effects of a

particular medication may increase the chance of forgetting to take a dose. However, such a distinction helps to maintain an accurate perspective on each patient's individual experience [Horne, 2007].

Adherence interventions

Levels of medication non-adherence and rates of treatment failure can be reduced by providing comprehensive and structured clinical management services, particularly those that address patients' problems with drug addiction and limited psychosocial resources. Such findings often result in the elimination of more problematic subpopulations such as drug users and homeless from trials and research summaries.

Beyond this, psychologists have developed several theoretical models to explain how patients initiate and maintain actions to preserve or improve health. These models share the common assumption that the motivation to engage in health-related behaviours arises from the beliefs that influence the interpretation of information and experiences. These are used in the design of behavioural interventions to improve adherence.

Leventhal's Self-Regulatory Model of illness behaviour is one such model [Leventhal, 1997]. This 3-stage model considers HIV infection as a threat to the individual's health, with uptake of and adherence to HAART being just one of any number of potential health-seeking responses available to counter that threat. Leventhal asserts that these health-seeking responses are governed by underlying beliefs, which vary between individuals and in the same individual over time. These beliefs are known as illness *representations* (1st stage) and are specific to each individual, playing a pivotal role in determining each health-seeking response (2nd stage), which can be as varied as medication-taking, dietary change or prayer. Each response is appraised in a continuous feedback loop (3rd stage).

The SRM model can be extended to specifically accommodate intentional non-adherence and is further explored in terms of a Necessity-Concerns framework (NCF) [Horne, 2007]. Validated research on the NCF has shown that beliefs about medicines can be summarised under 2 main themes: general and specific. General beliefs can be further subdivided in to

harm or *overuse* and, although often complex and diverse, patients' beliefs about specific medications can be grouped under two categories: perceptions of *necessity* for the treatment and *concerns* about adverse effects [Horne 1999a; Horne 1999b]. Tools are available to measure the patient beliefs that populate these models, such as the Illness Perception Questionnaire (IPQ) for the SRM [Weinman, 1996] and the Beliefs about Medicines Questionnaire (BMQ) [Horne, 1999].

Limitations of adherence research include the absence of standard approaches to define adherence; variability in the time periods over which adherence data are observed (days to years); variability of methods used to assess adherence; and the diversity of study samples. It is now commonly agreed that any intervention should be theory-based and well defined, according to a "taxonomy of behaviour change" [Abraham, 2008]. In addition to specific measurement and methodological problems, biologically-based interactions between medication adherence, viral replicative capacity, and medication pharmacokinetics in vivo preclude clear distinction between adequate and poor adherence behaviour.

Adherence and sexual risk taking

The association between HAART adherence and sexual risk behaviour is also complex, and are linked in different ways for different people. For some, non-adherence to HAART and therefore having a higher VL is related to risk-taking Kalichman, 2008b; Wilson TE, 2002] whereas for others, adhering to HAART and having an undetectable VL leads to increased sexual risk behaviour [Crepaz, 2004; Kalichman, 2001]. Thus, studies have reported both positive and negative associations between adherence and risk behaviours.

1.10 Research limitations of transmission studies

1.10.1 Imprecise identification of recent infection

Interpretation of and comparison between phylogenetic studies of HIV transmission using datasets derived from patients with RHI is problematic because of the limited methods available for identifying RHI. In all previous phylogenetic studies, identification of RHI has been sub-optimal [Bezemer, 2010; Brenner, 2007; Yerly, 2001]. Often, the diagnosis of RHI has depended on clinical suspicion of a vague 'flu-like illness coupled with sexual risk, with no explicit, consistent attempt made to determine incidence. This serves to skew the extent to which this population is seen to be generating transmission events.

Furthermore, in these studies, each RHI has been considered as fixed from diagnosis throughout the entire study period rather than the transient disease stage that it is. All patients will experience recent, followed by longstanding, infection, and the identification of possible transmission events (ascertained through phylogenetic reconstruction) between patients diagnosed *as* RHI does not necessarily mean transmission occurred *during* RHI. For example, a transmission event at time point B from an individual diagnosed with RHI at time point A (*e.g.* 3 years earlier) would be considered a transmission from RHI when in fact it was from longstanding infection. This would serve to overestimate the contribution of recent infection to new infections [Brown, 2009b].

Faithfully to explore the proportional contribution the different infection stages on transmission risk and the impact of HAART on prevention, it is essential to accurately diagnose RHI and to be able to account for its transient nature.

1.10.2 The limitations of phylogenetics

Phylogenetic reconstructions are not considered sufficiently accurate to confirm individual transmissions, for example in criminal cases [Bernard, 2007]. However, they can used to indicate viral linkage for public health analysis, if conservative cut-offs for possible transmission events are applied (*e.g.* bootstrap support of 99% or more and a genetic distance of less than 0.015 nucleotide substitutions per site) [Hué, 2005]. However, the robustness of phylogenetic methods for population level applications has not been rigorously assessed and there still remains significant debate about the suitability of phylogenetics for reconstructing HIV transmission events [Stürmer, 2004; Palmer, 2002].

The *pol* region is generally used for phylogenetic analyses out of convenience, since it is a by-product of routine HIV drug resistance testing. However there are concerns that the *pol* region is too genetically conserved, since it codes for regulatory genes involved in viral replication and consequently has insufficient genetic variability [Palmer, 2002]. Hué *et al.* [Hué, 2004] demonstrated that phylogenetic analysis of 140 HIV *pol* sequences produced the same results as analysis using HIV *env* and *gag* sequences from the same patients. There are relatively few full-length HIV sequences available from patients involved in a known transmission event, and large-scale phylogenetic analyses would be restricted by the sequencing costs of producing such a dataset and the computing power needed to analyse it.

Furthermore, even if two individuals did share a closely related virus, phylogenetic analysis could not verify the direction of transmission and it is difficult to rule out both individuals being infected via a third party or that a third partner was an intermediary contact between the two (because phylogenetic analyses rarely include sequences from every individual in the population). In Leitner *et al*'s phylogenetic analysis of transmission events, 1 out of 13 transmission events was incorrectly reconstructed (Leitner, 1996).

Further reasons why individuals may only be partially represented in population-based studies include HIV-diagnosed individuals being sexually

active in geographic areas where they do not attend clinics for care, not attending clinics or samples being unavailable (due to amplification problems etc.). There is also a need for concordance in calendar time. In the Brenner study [Brenner, 2007], while the chronically HIV-infected were sampled between 2001 and 2005, the dates of actual infection for these sequences were not known. The interval between infection dates within this population may span tens of years, increasing the likelihood that they had been obtained from different transmission networks. The consequences of not including every sequence within a local population for the purposes of phylogenetic reconstructions are not known. If the formation of robust clusters is affected by the diversity, representativeness and completeness of the entire sample, these factors could be crucial [Brown, 2009a].

1.10.3 Limitations and sensitivities of mathematical models

Mathematical models are often viewed as the only feasible method for predicting the effectiveness of future interventions, and there is increasing recognition of the importance in partnership between empirical data and modelling in HIV transmission. Whilst the central role of creating and running models is to develop our understanding of a system, the reliability of forecasts is affected by the limited quality of available data, uncertainty about parameter values, non-linearities in the system and chance events. Variables such as complex sexual networks, age-dependent increases or decreases in sexual activity, stratified risk behaviour, concurrent partnerships and polygamous relationships need to be considered. These are “input“ variables that are difficult to understand in real life, let alone in terms of model architecture, and they reinforce the need for more accurate empirical data to be embedded in these models. They may not be true independent variables, and this must be taken into consideration when interpreting mathematical models.

For example, it could be argued that most people become infected with HIV during a period of indiscretion in their lives, when they are having more sexual encounters in risky environments than during other times in their lives. There are a number of possible reasons for this: it might be a life

stage when young people are coupling with other young people; it might be an even more transient life stage than youth such as a period of relationship instability or recreational drug use; it might be a period of mobility with movement into unsupportive social environments that provide many sexual outlets. Such mobility might result in either adventurism or need for a relationship that lowers one's guard. Whilst it would be difficult to translate these behaviours into a parameter variable, such influences would be integral to any data that was collected empirically.

Furthermore, the different model populations, structures, assumptions and parameter ranges, as well as choice of model outcomes presented and the timescale over which the impact of HAART is measured, make comparison between HAART transmission models difficult.

On the intervention or "output side", a major difficulty with UTT transmission models is not so much that the challenges in the mathematical methods are insurmountable, but rather that the intervention parameter estimations are too often indefensible. First, there is the difficulty of testing large numbers of healthy people who are not attending health care services. Secondly, there is inadequate technology to detect people with RHI who are possibly the most infectious [Cohen MS, 2011b] (frequent HIV testing is not always an acceptable intervention). Thirdly, according to some models [Granich, 2009], not only does testing coverage need to be 100% and HAART initiation immediate for those diagnosed, but HAART must immediately reduce infectivity to zero (regardless of sexual risk behaviour) with nobody ever stopping or virologically failing HAART [Gardner, 2011].

Models of HIV prevention strategies would benefit from more plausible assumptions on both the input and output sides if they are reliably to inform real-world policies. Unrealistic assumptions risk skewing modelling outputs and run the risk of misleading policymakers.

Chapter 2

The Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) in a clinical cohort: 10 years' experience

This chapter describes the incorporation of STARHS into all new HIV diagnoses and transfers in to the clinic in Brighton over a 10-year period. It demonstrates the reliability of this method for ascertaining additional RHI diagnoses in subtype B-infected individuals and the need for clinical data to accompany assay results.

2.1 Abstract

Objectives: To investigate whether combining clinical data with the serological testing algorithm for recent HIV seroconversion (STARHS) reliably identifies otherwise unrecognised recent infections and observe their trends.

Design: Incorporation of STARHS into routine HIV diagnosis.

Methods: STARHS was applied to serum collected between 1996 and 2005 at HIV diagnosis and routine clinical/laboratory markers of recent infections were determined. The recent infections were identified by conventional means, by STARHS, and by both combined.

Results: Of 1,526 infections diagnosed, 812 were new. Of these, 604 were in MSM, 208 in heterosexuals; 88% had serum available for STARHS, which identified 88 recent infections that would otherwise have been unrecognised (12% of all new infections, 34% of all recent infections). Of these, 88% reported recent high-risk sex (UPAI or UPVI); 47% reported seroconversion symptoms. STARHS confirmed RHI in 71/74 (96%) known to be infected within 6 months by conventional methods. Combining both approaches, recent infections increased over time from 26% (1996) to 45% (2005) [$p<0.001$]. STARHS results from 3% of new diagnoses and 8% of previous diagnoses were deemed false incident (associated with HAART, advanced disease or undetectable VL). False incident results were inexplicable in only two individuals.

Conclusion: Adjunctive use of STARHS with clinical data identified a high and increasing proportion of new HIV diagnoses as RHI, confirming significant on-going transmission. Since 2002, 50% of new diagnoses among MSM were RHI. Identification of additional RHI by STARHS could enable effective interventions that may benefit the individual and reduce onward transmission.

** Recent infection in this study is defined as within 18 months of infection.*

2.2 Introduction

2.2.1 Real-time diagnosis of recent infection

Although 40-90% of acute infections are symptomatic [Kahn, 1998], the clinical manifestations are usually non-specific and either do not present to, or go unrecognised by, health care providers [Weintrob, 2003; Schacker, 1996].

Whilst the conventional techniques for identifying RHI at the time of diagnosis represent the gold standard, there are limitations to each: many individuals will not have previously tested for HIV [HPA, 2011]; the possibility of RHI may not have been considered at the time of clinical presentation and hence RNA/DNA testing not been performed [Burchell, 2003]; and presentation may be at a time when Ab responses and Western Blot profiles are already fully developed. Therefore, it has proved difficult reliably to find individuals with RHI either in longitudinal cohorts of subjects at high risk for acquiring the virus or through cross-sectional screening.

2.2.2 The Serological Testing Algorithm for HIV Seroconversion (STARHS) and Tests for Recent Infection (TRI)

The typically rapid immunological response to HIV infection means that within less than a month of anti-HIV seroconversion commencing, standard HIV test kits are unable to distinguish RHI from longstanding infections. However, a number of adapted techniques can be applied to individual specimens in which the presence of anti-HIV-1 Ab has already been confirmed.

The Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) is a generic term for several laboratory techniques that has been used to differentiate recent from longstanding infections. These are now more commonly termed *Tests for Recent Infection* (TRI). The STARHS or TRI approach offers a number of important advantages over other methods for determining HIV incidence. Unlike cohort studies that require repeated testing of individuals and where results may be biased by people leaving the study, STARHS testing can be carried out retrospectively on stored single

specimens from cross-sectional sero-surveys. In comparison with cohort studies, applying the STARHS approach is cheaper, quicker and simpler to perform. Furthermore, STARHS testing can be performed on a real-time basis thus allowing a measure of RHI at the time of a study as opposed to incidence derived from a cohort study which cannot be ascertained until after the follow-up sample has been collected and tested.

A gradual increase in anti-HIV titre occurs over several months and this is the basis of 'detuned' assays. This assay was the first assay to be described as being able to identify specimens from individuals recently infected with HIV for the purposes of incidence calculation. Employing the recommended assay cut-off, the technique recognises HIV seroconversions that have occurred on average four to six months prior to collection of the positive specimen [Kothe, 2003; Janssen 1998]. However, the period during which RHI can be identified can be altered by changing the cut-off applied to the assay. The method relies on the generalisation that anti-HIV titres in the plasma rise gradually, and at a similar rate in each infected individual, over a period of several months following seroconversion.

The STARHS approaches are applied to confirmed anti-HIV-1-positive specimens and, with the exception of the IgG3 and Inno-LIA approaches, rely on the marker employed (*e.g.* avidity) increasing over the first several months after seroconversion. If a test specimen gives a result below a pre-determined cut-off point, it is deemed to have been a recently-acquired infection. The cut-off point is set such that it provides an appropriate balance of sensitivity and specificity, and this is typically associated with an incidence window in the region of 3-6 months. However, there are uncertainties around this model, including subtype variability, the accuracy of the incidence window and the person-to-person variability shown by "outliers" with either a 'rapid' or a 'slow' response. The former may appear to have a long-standing infection some time sooner than the average incidence window, and the latter may appear to be a RHI for a considerably longer time [Murphy, 2008].

2.3 Methods

Subjects were those individuals who presented to the single combined HIV/GUM treatment centre at Brighton and Sussex University Hospitals NHS Trust over a 10-year period between January 1996 and December 2005. Screening for HIV infection was initially done using Abbott AxSYM HIV 1/2 g0, switching to Abbott AxSYM HIV COMBO 1/2 gO in July 2003 and finally Abbott ARCHITECT HIV Ag/Ab COMBO in May 2005. All reactive specimens were referred for confirmatory testing to the Virus Reference Department, Central Public Health Laboratory (CPHL), now the Health Protection Agency (HPA), London, UK. Demographic details (age, sex, risk factors for HIV acquisition, and ethnicity) were recorded at the time of HIV diagnosis. Where an individual had previously been diagnosed with HIV infection at another treatment centre, this was recorded. Conventional diagnosis of RHI was by a previous negative HIV test within 18 months, detectable HIV RNA with negative HIV Ab test, evolving HIV Ab pattern on serial samples, and/or a Western blot banding pattern that was limited to 4 bands, including at least p24 and gp160.

STARHS testing was performed at the National Reference Laboratory (HPA, London) with the Vironostika HIV-1 Microelisa System assay (bioMérieux UK Ltd, Basingstoke, UK) as previously described [Suligoi, 2002]. For this study an optical density of less than 1.0 was used to identify RHI, and this cut-off is associated with a seroconversion within 5-6 months. Testing was performed retrospectively for those diagnosed in 1996-2000, and prospectively thereafter.

For subjects for whom STARHS suggested RHI, further information was collected regarding clinical features of (symptomatic) AHI, prior HIV testing history, clinical status at diagnosis, sexual history, other laboratory features suggestive of RHI, CD4 cell count, VL and HAART status (if known previously to have been HIV positive).

Individuals were considered to have had a RHI if STARHS gave a result consistent with RHI, and this was confirmed by clinical and/or other laboratory features. The proportions of incident to prevalent diagnoses

were determined by year and by risk group, and trends over time were analysed using the Kruskal Wallis Test (SPSS software version 12.0).

2.4 Results

Study Population

Between January 1996 and December 2005, 1,526 individuals presented as anti-HIV-1 Ab positive to this treatment centre (Table 2.1). Of these, 714 had previously been diagnosed with HIV infection at another centre. Thus, for 812 this represented a new HIV diagnosis. Of these, 604 (74%) were MSM and 208 (26%) were heterosexual (83 male and 125 female), of whom 110 (53%) were known to have acquired their infection in countries of high HIV prevalence. The median ages were 36 years for the MSM group (range 19 to 82) and 34 years for the heterosexual group (range 17 to 73).

Conventional diagnosis of recent infection

Of those newly diagnosed with HIV infection, 175/812 (22%) were initially diagnosed as having RHI, based on a previous negative HIV Ab test within 18 months (n=150), evolving serological response (n=31), incomplete Western blot assay profile (n=8) or Ab negative/PCR positive discordance (n=1) [Table 2.1].

Application of STARHS

Of the 1,526 specimens collected at first presentation to this treatment centre, 1,112 (73%) were available for analysis by the STARHS method including 715/812 (88%) with newly diagnosed infection (Table 2.2). Among the 1,112 tested, 289 (26%) gave a result consistent with RHI using the STARHS assay. Considering only the 715 in whom this was their first HIV diagnosis, 228 (32%) individuals were preliminarily identified as incident infection by the STARHS assay.

Identification of false incident STARHS results:

Of the 289/1,112 individuals identified as incident by STARHS, 79 (27%) were considered to be incorrect based upon the clinical history (Figure 2.1). They comprised 38 individuals known to have longstanding HIV infection with an undetectable VL (<200 copies/mL) who had been receiving HAART for a median of 45 months (range 6-145). A further 5, of whom 4 had a

previous AIDS diagnosis, had been receiving HAART for a median of 15 months (range 3 to 100) and were sub-optimally suppressed with a median VL of 3.83 log copies/mL (range 2.72 to 5.47). A further 27 individuals were not on HAART but had advanced HIV infection with a median CD4 cell count of 37 cells/mm^3 (range 7 to 195) - 19 of these individuals presented with AIDS at diagnosis. A further five were known to have prevalent HIV infection (> 3 years since diagnosis) and were not receiving HAART but had very low levels of detectable virus (<50 copies/mL in four individuals and 388 copies/mL in another), and a further two had never tested previously but had undetectable VL (<50 copies/mL). For two, of unknown subtype, there was no apparent clinical explanation for an incident STARHS result.

Considering only the 228 individuals in whom this was their first HIV diagnosis, 23 (10%) were considered to be incorrectly identified as incident based upon the clinical history, and these have been excluded from the data (Table 2.2). Of these, 17/23 had a diagnosis of AIDS at presentation and a further 4 had a median CD4 count of 50 cells/mm^3 (range 5-130). The remaining two had undetectable VL (<50 copies/mL).

Combining STARHS and conventional methods to diagnose RHI

Of the 715 individuals in whom this was their first HIV diagnosis and STARHS was applied, excluding those 23 considered to be incorrectly identified as described above, 237 (33%) were determined as incident by incorporating the STARHS technique (Table 2.2). Without the application of STARHS, only 149 (21%) would conventionally have been identified as RHI. Therefore, a further 88 (60%) new diagnoses were determined by STARHS to have been infected recently that would otherwise have gone undetected.

Sensitivity and Specificity

Of those known to be infected in the previous 6 months by conventional methods (negative test in the previous 6 months, incomplete Western Blot or evolving serology) and where STARHS was performed, RHI was corroborated by STARHS in 71/74 (96%). In the 3 remaining cases a negative HIV screening test had been recorded between 4 and 5 months

previously (2 subtype B, one unknown) – towards the outer time "envelope" of the STARHS range. Behavioural and clinical data were available in 73/88 RHI diagnosed by STARHS alone: unprotected anal or vaginal intercourse with a partner from a high-risk group within the previous 6 months was reported by 60/73 (82%); and seroconversion symptoms by 34/73 (47%) individuals, as determined at the time of initial presentation.

Conversely, STARHS confirmed non-incident infection in 64/64 (100%) individuals known to have been diagnosed HIV-positive more than 18 months previously with a CD4 cell count of > 200 cells/mm^3, VL > 500 copies/mL and not on HAART.

Subtype and STARHS results

Of the 812 individuals in whom this was their first HIV diagnosis, 366 (45%) were infected with subtype B, 106 (13%) with non-B and it was unknown in 340 (42%). Of the 71 known to be infected in the previous 6 months by conventional methods and with an incident STARHS result, 58/71 (82%) were infected with subtype B and the remaining 13 had an unknown subtype but were UK-born Caucasian MSM. Eleven of 88 (13%) previously unknown RHI were non-B subtype - documentation was available in ten of these cases and UPAI/UPVI within the previous 6 months was reported in nine individuals and seroconversion symptoms in six.

Trends in recent infection

The proportion of individuals with diagnosed RHI (both by established methods and by STARHS) increased over time (Table 2.1). Considering only those individuals for whom specimens were available for STARHS testing (Table 2.2), this pattern was even more marked ($p<0.01$). There were clear differences between the proportions identified as RHI by risk category for HIV infection; in the heterosexual group the proportion identified as recent was stable ($p>0.1$, ns) and generally low whilst in the MSM group it was higher and increasing ($p<0.01$), accounting for the overall increase over time and reaching a level of over 50% of new diagnoses in 2002, 2004 and 2005.

Table 2.1: Additional diagnoses of RHI by STARHS over conventional methods (all subjects)

Year	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total
Total HIV diagnoses	125	107	107	109	155	139	176	200	199	209	1526
New HIV diagnoses	67	60	39	42	74	77	97	113	121	122	812
MSM	57	52	33	34	53	60	67	74	77	97	604
Hete (high prevalence)	2	3	1	2	7	9	23	20	28	15	110
Hete (other)	8	5	5	6	14	8	7	19	16	10	98
New HIV diagnoses, recent clinically	10	10	10	6	15	17	22	19	29	37	175
Previous –ve test	10	10	8	6	10	13	21	17	23	32	150
PCR +ve and Ab –ve	0	0	0	0	0	0	0	0	1	0	1
Evolving serology	0	1	2	0	4	4	1	4	7	8	31
Incomplete western blot	0	0	1	1	2	1	1	1	1	0	8
New HIV diagnoses, recent by STARHS	10	3	8	6	17	24	27	29	39	42	205
Confirming clinical diagnosis	0	1	6	3	13	14	15	15	21	29	117
Newly recognized as recent (false incident in brackets)	10 (4)	2 (5)	2 (0)	3 (5)	4 (1)	10 (2)	12 (1)	14 (1)	18 (2)	13 (2)	88 (23)
Total new HIV diagnoses, recent by both methods	20 (30%)	12 (20%)	12 (31%)	9 (21%)	19 (26%)	27 (35%)	34 (35%)	33 (29%)	47 (39%)	50 (41%)	263 (32%)

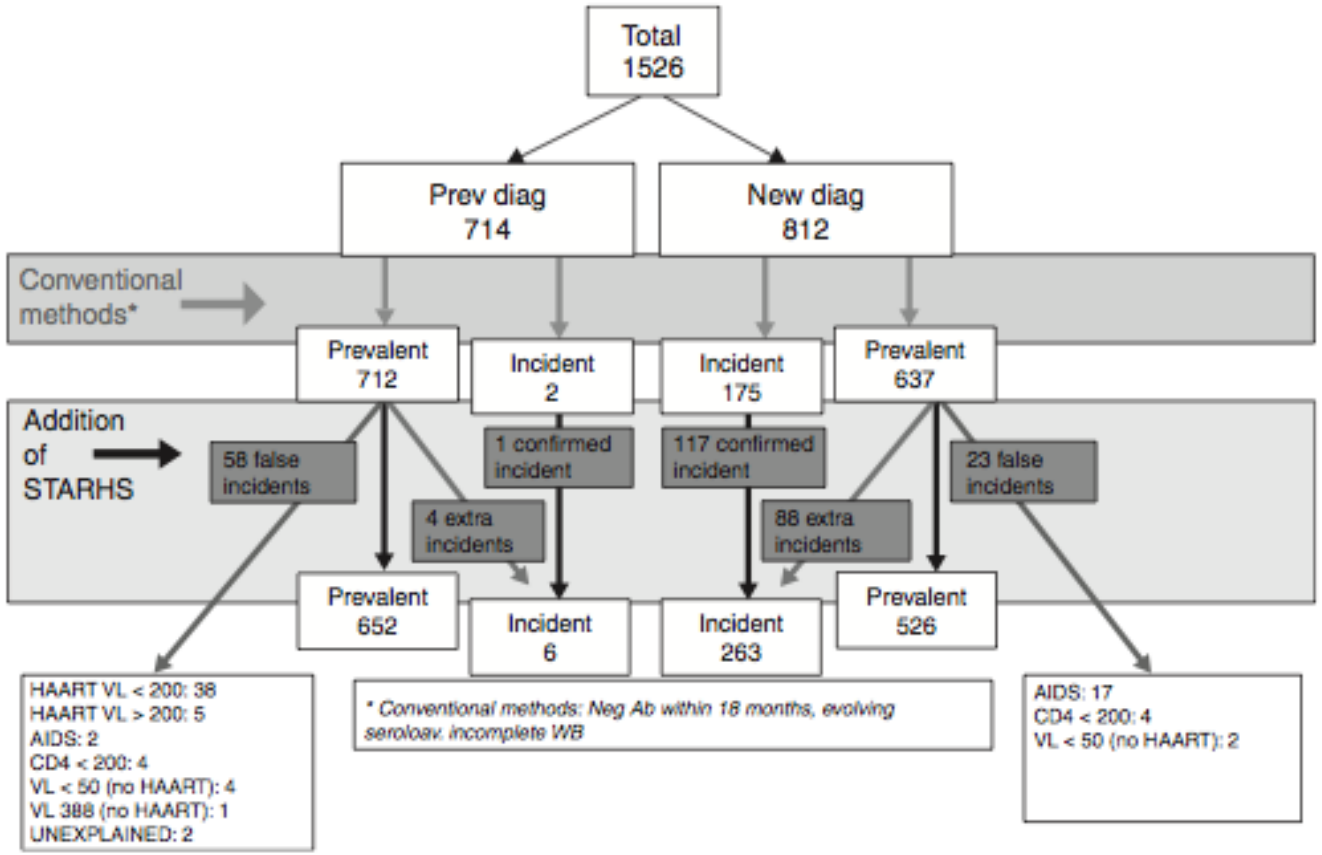
Ab, antibody; Hete, heterosexual; MSM, men who have sex with men.

Table 2.2: Additional diagnoses of RHI by STARHS over conventional methods (only subjects on whom STARHS performed)

Year	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total	
No. with specimen	47 (70%)	47 (78%)	32 (82%)	34 (81%)	70 (95%)	73 (97%)	92 (95%)	102 (90%)	108 (89%)	110 (90%)	715	
Recent clinically	2	4	8	3	15	16	21	17	27	36	149	
Recent by STARHS alone	10	2	2	3	4	10	12	14	18	13	88	
Total recent	12 (26%)	6 (13%)	10 (30%)	6 (18%)	19 (27%)	26 (36%)	33 (36%)	31 (30%)	45 (42%)	49 (45%)	237/715 (35%)	$P<001$
MSM	11/39 (28%)	6/40 (15%)	10/29 (34%)	5/27 (19%)	18/52 (35%)	23/58 (40%)	32/64 (50%)	28/67 (42%)	41/74 (55%)	47/92 (51%)	221/542 (41%)	$P<0.01$
Hete	1/8 (13%)	0/7	0/3	1/7 (14%)	1/18 (6%)	3/15 (20%)	1/28 (4%)	3/35 (9%)	4/34 (12%)	2/18 (6%)	16/173 (9%)	$P>0.1$

Hete, heterosexual; MSM, men who have sex with men.

Figure 2.1: Classification of all HIV diagnoses as recent by conventional methods and the addition of STARHS (1996–2005)



VL = viral load.
Conventional methods: Neg Ab within 18 months, evolving serology, incomplete western blot

2.5 Discussion

Over the study period we observed an increasing proportion of individuals newly diagnosed with HIV to have been recently infected, supporting the assertion that the rate of newly acquired HIV-infection in Europe had increased over recent years [Dukers, 2007; Giuliani 2005]. This trend is still particularly marked amongst MSM in the UK [HPA, 2011]. This demonstrated that on-going HIV transmission was occurring alongside changing sexual behaviour, despite the awareness of effective HIV prevention strategies and the potential for HAART to reduce the transmission of HIV. An alternative explanation is that, particularly among MSM, individuals were presenting more frequently for HIV testing [de Souza-Thomas, 2005], thereby making recognition of RHI more likely.

These data demonstrate that the use of the STARHS technique enables identification of RHI over and above that which would have otherwise been possible. Utilising conventional clinical and laboratory techniques for diagnosing RHI, 21% of new HIV diagnoses were determined to be recent, but with the addition of the STARHS technique a further 12% of the study population gave a result consistent with recent HIV infection. Thus 60% more RHI were diagnosed, representing an increase in overall RHI from 21% to 33%. Such improved detection of recent versus longstanding infection offers the opportunity for discussion of early intervention studies for the individual, and it is likely that sources of infection and contacts at risk of onward transmission may be more easily identified. Furthermore, the incorporation of STARHS into a surveillance scheme that monitors antiretroviral resistance [Delpech, 2011; Cane, 2005] should permit a comparison of primary resistance between recent and longstanding infections, enabling more accurate prediction of future resistance patterns. The utility of methods such as STARHS to determine RHI in non-B subtypes requires validation before it can be routinely introduced [Sakarovitch 2007, Young 2003].

It has been argued that since a STARHS (or similar methodology) result consistent with RHI can occur in individuals with known prevalent infection this test should not be utilised in a clinical setting [Schwarcz,

2007], and our findings confirm the impact of HAART and advanced disease stage on the accuracy of STARHS [Kellogg, 2005]. Although STARHS was adjudged to have given a false result of RHI in 79/1526 (5%) HIV-infected individuals, it was only in 9 of these 79 (11%) that an obvious cause for the false incident result could not be easily recognised with adjunctive use of clinical or other laboratory data. Therefore, we found that, when used together, laboratory and clinical data led to a false recent infection rate of only 9/1526 (0.6%). Thus our data demonstrate that false incident results can be reliably identified and, conversely, suggest that significant limitations may be associated with surveillance applications [UNAIDS, 2005] if relevant clinical data are not available for individual specimens.

Alternative serological techniques have been described that identify RHI such as an Ab avidity assay [Suligoi, 2002], the BED ELISA [Parekh, 2005], IDE-V3 [Barin, 2005], IgG3 Anti-HIV and Inno-LIA HIV Adaptation [Schüpbach, 2011]. These assays employ different biological principles which may be less affected by HAART, advanced disease and subtype and may, either in isolation or in combination with each other, offer more accurate differentiation of recent from longstanding HIV infection. However, these assays require further validation before their usefulness can be fully assessed (see Chapter 7).

Finally, Ambrose *et al.* at University College London (UCL) has developed a "recent infection classifier" based on percentage nucleotide ambiguity, validated against clinical data and avidity assay results, which with a sensitivity of 85% and specificity of 80% is comparable to the accepted laboratory-based assays used to identify RHI [Sakarovitch, 2007]. This is based on the theory that it may be possible to use population sequence ambiguity as a marker of age of infection [Batorsky, 2011; Kouyos, 2011].

Chapter 3

What is the drug resistance mutational burden in an HIV-1 prevalent cohort and its relationship to the incidence of transmitted resistance?

Having reliably identified additional RHI diagnoses in Chapter 2, studies on individuals primarily infected with transmitted drug resistant (TDR) virus were made possible. The long-term persistence of mutations was observed and an exploratory attempt made to compare the community resistance mutational burden with incident TDR.

3.1 Introduction

The enhanced detection of RHI by STARHS described in Chapter 2 facilitated the study of transmitted drug resistance (TDR) - *i.e.* primary infection with HIV that carries resistance - in the Brighton cohort.

At an individual level, infection with TDR virus has been shown to significantly prolong the period required for virologic suppression [Grant, 2002; Little, 1999], although this is less of a problem as more potent antiretroviral agents become available [Boyd, 2009]. TDR is increasingly recognised as distinct from drug-acquired resistance in two key ways that can affect the onward transmission of resistance. First, because it is a primary infection with resistance virus, TDR is always associated with the highly viraemic period of RHI. Secondly, there is evidence that certain TDR mutations remain detectable for a longer time compared to equivalent drug-acquired mutations. Both suggest that TDR can be expected to be highly transmissible at a population level. Other host and/or viral factors not accounted for by VL or specific mutation are also likely to influence transmission of resistance.

TDR has been well documented in many countries for many years [Brenner, 2000; Pillay, 2000; Boden, 1999; Little, 1999]. Initially, only extremely low levels of TDR were observed in the 1990s, but these increased to up to 25% of new cases of RHI in some populations by 2001 [Grant, 2002; UK Collaborative Group, 2001]. It has been well documented that the key ZDV resistance mutations, T215Y/F, seldom persist long-term after transmission in an untreated patient. Instead, 215D, -S, -N, or -C replaces 215Y/F within a few months, but these mutations may then persist for up to 3 years [de Ronde, 2001; Yerly, 1998]. These changes restore fitness to the virus as well as susceptibility to ZDV but are more able, more quickly, to revert to 215Y/F under drug pressure because of the shorter mutational distance required [Garcia-Lerma, 2001]. K70R, which also confers reduced susceptibility to ZDV, has been observed in transmitted virus and shown to be stable for >1 year [Conlon, 1994]. Brenner *et al.* [Brenner, 2002] reported variable stability of multidrug-resistant virus (MDR) ranging up to 5 years.

With regard to surveillance, most patients are diagnosed some time after RHI, and several studies have examined new longstanding diagnoses to determine the prevalence of resistance in treatment-naive patients. Variable levels of resistance were also found [Perno, 2002; Wegner, 2000].

However, estimates of both recent and longstanding infection with TDR virus are difficult to compare with each other because of different criteria being used to define resistance. For example, estimates of TDR using genotypic tests are difficult to compare because of differences in significance given to particular mutations and the different algorithms used to interpret genotypic data [De Luca, 2003].

Shafer *et al.* defined a list of mutations suitable for epidemiological studies [Shafer, 2007]. Mutations are included provided they meet the following criteria: commonly recognised as causing or contributing to resistance; non-polymorphic in untreated persons; applicable to all HIV subtypes. However, the list does not include all clinically relevant mutations. This list has recently been updated [Bennett, 2009].

If the probability of transmission of TDR is proportional to the VL in the infection source individual, it is therefore likely that this association can be extrapolated to the community VL of a cohort. This would suggest that mutational patterns of incident TDR would reflect the community resistance mutation "burden" rather than simple mutation frequency. This might mean that the incidence of TDR would decrease as HIV treatment outcomes improve and the community resistance mutation burden decreases, but only if TDR came predominantly from infection source individuals with drug-acquired resistance. If TDR came from infection source individuals who themselves also had TDR, better treatment outcomes would have little effect on transmission of resistant virus.

3.2 Aims and objectives

Aims

To follow up patients infected with TDR virus to determine the stability of resistance-associated mutations, and to estimate the community resistance mutational burden in TDR relative to drug-acquired resistant virus.

Objectives

Objective 1:

To determine the stability of TDR-associated mutations over time

Objective 2:

To estimate the community resistance mutation burden in TDR compared to drug-acquired resistance

3.3 Objective 1: to determine the stability of TDR-associated mutations over time

The purpose of this study was to observe the persistence of resistance-associated mutations after transmission in ART-naïve patients. The study included patients with RHI (within 18 months in this study) with virus showing NRTI, NNRTI, PI resistance or MDR.

3.3.1 Methods

Study Samples

Patients were identified as having RHI through having had a negative Ab test 18 months previously, laboratory evidence of acute seroconversion illness such as an evolving Ab response, or STARHS suggestive of RHI, along with clinical markers indicating RHI (as described in Chapter 2). Follow-up samples were obtained from 1 to 36 months after diagnosis of RHI. Details of periods between initial and subsequent samples are shown in Table 3.1.

Table 3.1: Persistence of Drug Resistance–Associated Mutations Over Time After Primary Infection

Patient	Months After First Sample	NRTI Mutations	NNRTI Mutations	PI Mutations
A	0		Y181C	L63P, V77I, I93L
	25			L63P, A71T, V77I, I93L
B	0	T69N		M36L, L63P
	15	T69N		M36L, P63P/L
C	0, 7	M41L, T215L		L63P, V77I/V, I93L
D	0, 1, 9, 17	M41L, E44D, V118I, 210W, T215Y, K219R	K103N, V179L	L10I, L24F, L33F, I54V, L63P, A71V, G73S, V77I, V82A, L90M
E	0	M41L, T215Y		D60E, L63P
	21	M41L, T215C		D60E, L63P/S
	33	M41L, T215C		D60E, L63P
F	0		K103N, V108I/V	L33V
	23		K103N	L33V
G	0	T69N		L63P
	32	T69N		L63P, V77I
H	0, 28	M41L		L10I, L33M, L63N, I93L
I	0	A62V		L63P
	2			L63P
J	0	K219Q		L10V, M36I
	9, 22, 36			L10V, M36I
K	0, 11	T215D		M36I
L	0, 18	T69N, K219Q	A98G, V106I, Y188L	L10I, I54L, L63P, A71V, G73T, I84V, L90M, I93L
M	0, 16	T69N, V118I	A98S	L63P, V77I, I93L
N	0, 13	T215D		M36I

Analysis

Sequence was obtained by in-house methods from the entire protease gene and first 230 codons of RT as previously described [Pillay, 2000]. Briefly, RNA was extracted from pelleted virus and a nested RT–polymerase chain reaction (PCR) assay performed. Sequencing was carried out using a CEQ 2000 sequencer (Beckman Coulter), and sequences were edited using Sequencher software (Gene Codes Corporation). Differences from the subtype B consensus sequence were derived using the Stanford database [Shafer, 2000].

3.3.2 Results

Drug resistance-associated mutations were observed in 14 patients with evidence of RHI for whom follow-up samples were available, with the patients remaining untreated. The mutations observed in the initial and follow-up samples are summarised in Table 3.1, together with the time intervals between samples. All patients were infected with subtype B virus.

Nucleoside Reverse Transcriptase mutations

M41L was detected in three patients and was still present in the last samples tested from all these patients, which were obtained between 7 and 33 months later. A62V was observed alone in one patient. This mutation is usually associated with the multi-NRTI resistance complex based on Q151M. It is unlikely that this mutation alone confers resistance, and its presence may not represent TDR. Nevertheless, the mutation rapidly disappeared, becoming undetectable after 2 months. T69N was observed in the first sample from four patients, including one patient with MDR virus. This mutation was still present in all subsequent samples between 15 and 32 months later. One of the patients showed V118I in addition (patient M), which was also unchanged after 16 months.

Codon 215 variants were observed in five patients at diagnosis. Patient C had T215L along with M41L, and no change was observed after 7 months. Patient D had T215Y in conjunction with MDR virus, and no change was observed after 17 months. Patient E had T215Y together with M41L,

and this was replaced with T215C in a sample taken 21 months later. Patients K and N showed T215D alone at diagnosis, and this was still present 11 and 13 months later, respectively.

K219Q was observed in two patients, and K219R in one. One patient with 219Q and another with 219R, both with many other resistance mutations, showed retention of these mutations after 18 and 17 months, respectively. One patient with 219Q alone showed loss of the mutation within 9 months; further testing of samples at 24 and 36 months showed continued absence of this mutation

Non-nucleoside Reverse Transcriptase Inhibitor mutations

Two of the patients studied had NNRTI resistance alone: patient A showed only Y181C, which had disappeared 25 months later. The second patient (patient F) showed K103N, which was still present after 23 months, although a mixture at codon 108 (I/V) became fully wild-type during this period. Two patients were infected with MDR virus, including K103N and V179L (patient D) and A98G, V106I, and Y188L (patient L), and these mutations were unchanged after 17 and 18 months, respectively.

Protease Inhibitor mutations

No patients in the study had PI resistance alone. The two MDR patients showed L10I, L24F, L33F, I54V, L63P, A71V, G73S, V77I, V82A, and L90M (patient D) and L10I, I54L, L63P, A71V, G73T, I84V, L90M, and I93L (patient L) in protease, and as with the NNRTI mutations in these patients, these PI mutations were un- changed 17 and 18 months after diagnosis, respectively. In general, little or no change occurred in secondary PI mutations during the course of these observations.

Multidrug resistance and viral load

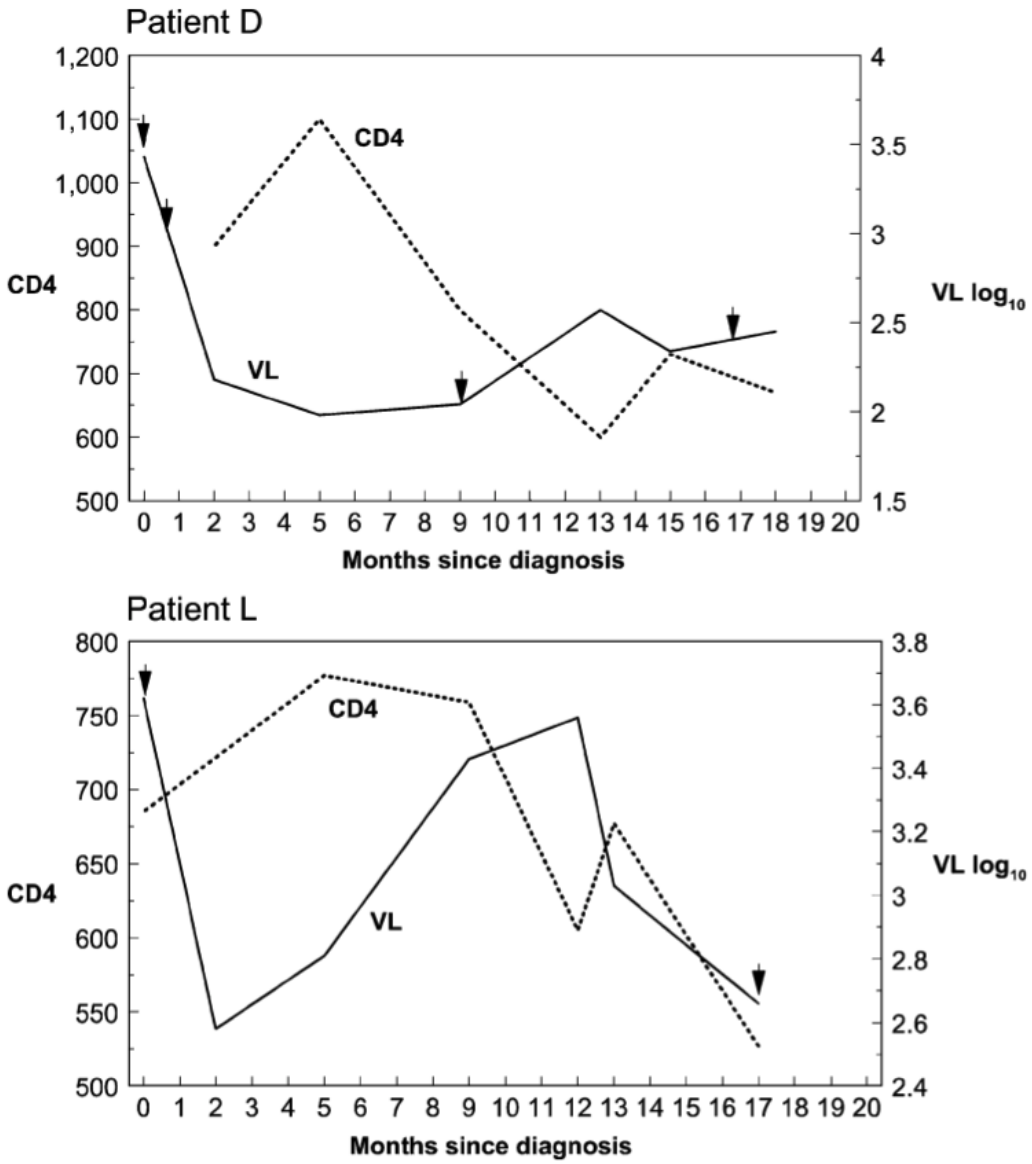
As described previously, two patients (D and L) were infected with MDR virus. The viral loads and CD4 count for these patients are shown in Figure 3.1. VL remained low off-treatment for some time for both patients.

Patient D's VL on diagnosis was 2500 copies/mL, fell to 150 copies/mL after 2 months (no treatment), and remained below 1000 copies/mL for the subsequent 18 months. His CD4 count has declined from a peak of 1100 to 670 cells/mL during this period. No change in resistance-associated mutations was observed in this period.

Patient L's VL was 4140 copies/mL at diagnosis, and 456 copies/mL 18 months later, although his CD4 count showed little change during this period (from 685 to 527 cells/mL). As with patient D, no change was observed in the resistance-associated mutations during this period.

Figure 3.1: Graphs showing viral load and CD4 counts with time of patients infected with multidrug-resistant virus

Arrows indicate where genotyping performed



3.4 Objective 2: to estimate the community resistance mutation burden for TDR compared to drug-acquired resistance

The mutational patterns observed in TDR cases do not directly correlate to the most frequent resistance mutations observed in treated individuals. For instance, M184V is a very common drug-acquired mutation but is rarely observed in the TDR group. We explored the basis of this discordance by estimating the community infectious burden of common resistance mutations in both groups, and attempted to compare this with patterns of TDR in incident infections.

3.4.1 Methods

Subjects

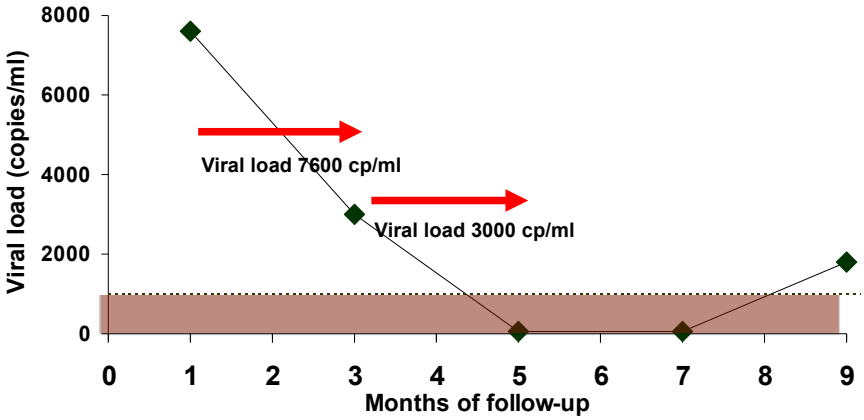
We studied the same predominantly MSM cohort as described in Chapter 2, served by a single health service provider and with approximately 80% of HIV-1 infections diagnosed (unlinked anonymous survey data). Subjects were HIV-1 positive attending between 2000 and 2004, who had at least one VL measurement. From study start, data were contributed from the date of first VL to the last attendance at clinic, or death, up to 31st December 2003.

Resistance mutational burden

Each patient's follow-up was divided into 1 month periods. We calculated the person-years of follow-up (PYFU) for each patient in the cohort in each year from 2000 to the end of 2004. The PYFU was then stratified according to whether the patient's VL at the time was detectable (> 1000 copies/mL) or not, and whether they were known to have had RHI (Figure 3.2).

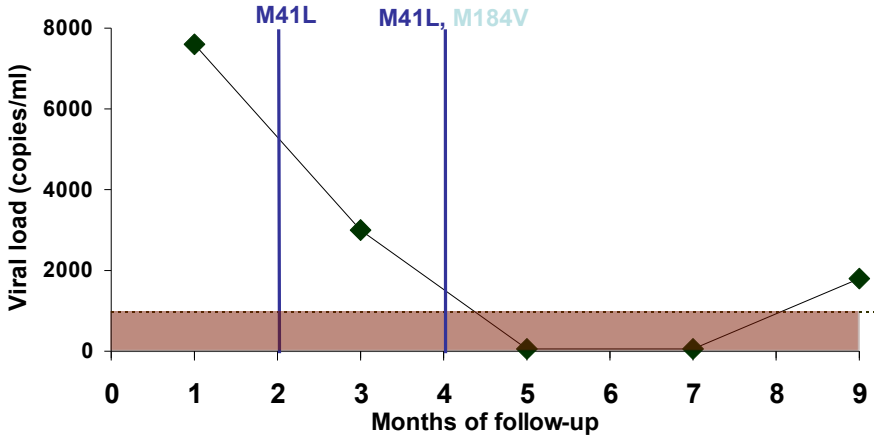
We then considered the follow-up among individuals with a detectable VL and stratified this according to the presence or absence of each mutation (a mutation was considered present if the patient had had a resistance test on or before the date of the detectable VL, with different sensitivity analyses). The VL nearest to the start of each month was identified. Therefore, each patient-month was classified by presence of mutation (Figure 3.3).

Figure 3.2: Each patient's follow-up divided into 1 month periods



Month	1	2	3	4
Viral load	7600	7600	3000	3000
Days in month	31	31	31	31
Total viral burden	235600	235600	93000	93000

Figure 3.3: Each patient-month classified by presence of mutation



Month	1	2	3	4	5	6	7	8
Total viral burden	235600	235600	93000	93000	0	0	0	0
With M41L	0	235600	93000	93000	0	0	0	0
Without M41L	235600	0	0	0	0	0	0	0
With M184V	0	0	0	93000	0	0	0	0
Without M184V	235600	235600	93000	0	0	0	0	0

Note:

Where VL>1000 copies/mL, monthly mutational burden= viral load x days in month

Where VL<1000 copies/mL, monthly mutational burden = 0 (transmission risk assumed zero)

Analyses

Main analysis A: presence/absence of each mutation determined on basis of prior resistance test results, assuming varying persistence of mutations with different sensitivity analyses by an "area under the curve" analysis (VL years of specific mutant). VL in the absence of prior resistance testing (typically untreated individuals) was assumed to represent wild-type virus. Active ascertainment and resistance testing for incident infections was undertaken from year 2000 onwards.

Sensitivity analyses:

Analysis B: once detected, resistance mutations persist, even if not detected in subsequent genotype;

Analysis C: resistance mutations disappear after 6 months, virus remains wild type until next genotype;

Analysis D: A combination of B and C where TDR mutations persist indefinitely but drug-acquired mutations disappear after 6 months.

3.4.2 Results

We included 1,286 patients who were under active follow up after 2000 (Table 3.2). Of these, 84% were Caucasian, 87% were men, and the predominant route of transmission was sex between men (75%).

Table 3.2 Demographics

Number of patients		1286	(100.0)
Sex	female	161	(12.5)
Ethnicity	White	1083	(84.2)
	Black African	122	(9.5)
	Other/not known	81	(6.3)
Risk group	Homosexual	964	(75.0)
	Heterosexual	251	(19.5)
	Other/not known	71	(5.6)
Age	Median (range)	35	(16 – 71)
Years of follow-up*	Median (range)	3.1	(0 – 4.9)
Number of viral load measurements	Median (range)	12	(0 – 49)

* Calculated from first clinic visit, date of 16th birthday or 1/1/2000 (whichever was latest) to date of last clinic visit

Viral load and genotype testing

In the 1,286 individuals, a median of 12 (range 1-49) VL results were undertaken. From January 2000 to end of 2003, 857 (67%) individuals had a VL>1000 copies/mL. Of these, 448 (52%) had a genotypic resistance test performed (median 1, range 1-4 tests). In total, 654 resistance tests were performed:

- 152 (23%) among patients exposed to ART
- 502 (77%) among ART-naïve patients

Those untested were predominantly longstanding ART-naïve individuals diagnosed pre-2000 or those on ART throughout study period with undetectable VL.

Over time, the proportion of follow-up spent with a detectable VL gradually decreased, in line with a gradual increase in the proportion of time spent on HAART. The proportion of follow-up with unknown VL status decreased dramatically as VL testing became more routine (Table 3.3).

Table 3.3 Person-years of follow-up amongst patients in the cohort

	Total PYFU	*Detectable VL (>1000 copies/mL) (% of PYFU)*			*Known primary infection (% of PYFU)*		
Year		**No**	**Yes**	**Not known**	**No**	**Yes**	**Not known**
2000	627.89	339.99 (54.1)	246.96 (39.3)	40.94 (6.5)	22.75 (3.6)	12.65 (2.0)	592.49 (94.2)
2001	709.46	407.28 (57.4)	263.89 (37.2)	38.29 (5.4)	61.75 (8.7)	34.45 (4.9)	613.27 (86.4)
2002	810.44	483.32 (59.6)	291.37 (36.0)	35.75 (4.4)	104.94 (12.9)	63.82 (7.9)	641.68 (79.2)
2003	969.58	609.38 (62.8)	333.43 (34.4)	26.77 (2.8)	164.26 (16.9)	97.73 (10.1)	707.58 (73.0)
2004	700.85	463.99 (66.2)	226.23 (32.3)	10.63 (1.5)	131.66 (18.8)	79.12 (11.3)	490.07 (69.9)

Resistance mutational burden

For each mutation, we multiplied the total days of follow-up when the VL was detectable by the VL at each time point, to obtain the area under the VL curve. Although the total proportion of PYFU spent with each cohort was small, the proportion did generally increase over time (Table 3.4).

Table 3.4 Person-years of follow-up in individuals with detectable (>1000 copies/mL) viral load and with the presence of each mutation

By main analysis A

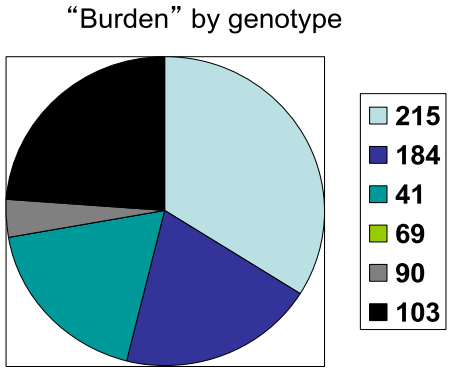
	Total PYFU spent with each mutation present whilst with a detectable viral load (% of total PYFU in year)					
Year	**M41L**	**T69D/L**	**T215Y/F**	**T215S/D/E**	**L90M**	**K103N**
2000	0.90 (0.14)	0.49 (0.08)	0 (-)	0.25 (0.04)	0.33 (0.05)	0 (-)
2001	2.79 (0.39)	1.15 (0.16)	0.25 (0.04)	1.56 (0.22)	0 (-)	0.69 (0.10)
2002	3.42 (0.42)	1.97 (0.24)	0.57 (0.07)	3.18 (0.39)	0 (-)	2.14 (0.26)
2003	3.94 (0.41)	3.37 (0.35)	0.74 (0.08)	6.98 (0.72)	0.25 (0.03)	3.45 (0.36)
2004	3.53 (0.50)	2.37 (0.34)	0 (-)	4.75 (0.68)	0.64 (0.09)	2.90 (0.41)

Patterns of mutations in longstanding vs. recent infection

The relative frequency of specific resistance mutation in the longstanding (clinical) cohort was T215any > M41L > K103N > M184V > L90M. During the same period, 24/150 RHI with resistance were identified, with frequency of mutants K103N > 215any > M41L > L90M. Of note, there was no M184V seen (See Figure 3.4).

Figure 3.4 Patterns of mutations in longstanding vs. recent infection

- Frequency in clinical genotypes:
  - 184>215>103>41>69=90
- Frequency in RHI:
  - 103=215>41>90
  - No 184 or 69

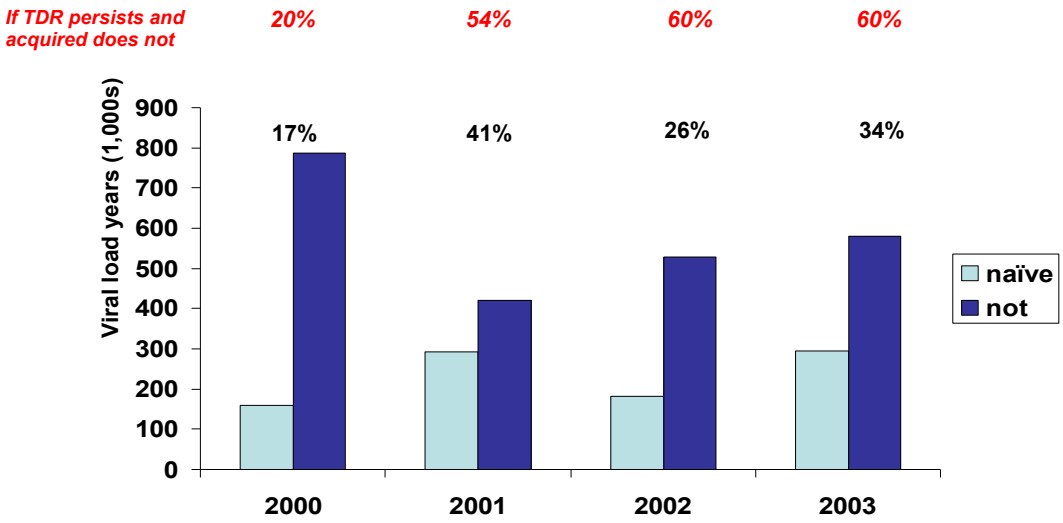


Contribution of TDR vs. drug-acquired mutations to the community mutational burden

In main analysis A, the contribution of TDR to the resistance mutational burden remained below 50% for each study year. Under analysis D, however, where TDR mutations persist but drug-acquired mutations disappear after 6 months, TDR was estimated to contribute to more than half the mutational burden between 2001-2003 (Figure 3.6).

Figure 3.6: % mutational burden that is contributed by TDR (ARV-naïve) individuals

Figures in red: Analysis D (TDR mutations persist but drug-acquired mutations disappear after 6 months)



3.5 Discussion

This chapter describes the duration of persistence of TDR-associated mutations after sexual transmission (objective 1), and estimates their relative community mutational burden (objective 2).

Objective 1

Resistance mutations that arise as a result of ART rapidly become undetectable when treatment is stopped. This may mainly be a result of overgrowth of wild-type virus originating in the viral reservoirs rather than true reversion of the mutant virus *per se* [Brenner, 2002; Delaugerre, 2001].

As previously described, virus from patients primarily infected with ZDV-resistant virus showing mutations at codon 215 of RT frequently demonstrated onward mutation at this codon resulting in T215S/D/C. These variants were shown in this study to be quite stable. Most other mutations were also found to persist over the period of study. The exceptions were A62V, Y181C, and K219Q when these mutations occurred singly.

We have therefore observed that TDR mutations often persist for a considerable time after primary infection. This observation correlates with the findings that the prevalence of resistance in new diagnoses is similar to that observed in seroconverters. This persistence of resistance-associated mutations for prolonged periods after infection underscores the importance of performing a resistance test at baseline [Pozniak, 2003], even when evidence of RHI is not present.

Care must be taken with interpretation algorithms, however, particularly with respect to mutations at codon 215, where the classic resistance mutations T215Y and T215F are usually rapidly replaced with variants restoring fitness to the virus. Such variants do not confer resistance *per se* but are indicators of primary infection with resistant virus, and some interpretation algorithms may not highlight such mutations; thus, there may be an overestimation of potential response to a drug regimen. It may be that algorithms need some modification for analysis of samples from untreated patients.

Objective 2

Results for objective 2 were mainly inconclusive because the numbers of mutations were insufficient and the differentiation between patterns of resistance in TDR vs. non-TDR groups impossible without phylogenetic analysis. Nevertheless, the sensitivity analyses did suggest that the persistence of TDR would increase its contribution to onward transmission of resistance as HAART coverage widened. It was also interesting to observe that the mutational burden of M184V was around half of that for T215 variants in prevalent infections and further, that it was absent altogether in TDR samples.

Limitations

We were unable to achieve our original objective to compare trends in transmitted versus acquired resistance over time, with a view to ascertaining the origin of TDR. There are several significant limitations associated with objective 2, such that the results can only be viewed as an preliminary exploration. These limitations can be categorised as follows:

Subject numbers: the number of individuals with resistance mutations was too low, and no transmissions could be phylogenetically confirmed.

Linkage: without phylogenetic analysis, the transmission source of TDR cannot be ascertained

Virology: there were assumptions made about persistence of mutations, even though they are likely to vary not only between TDR and acquired, but also independently between specific mutations.

Statistical analysis: not all patients were genotyped. There were missing data, predominantly in untreated patients, which is likely to underestimate the community mutational burden of TDR.

Sexual risk activity or STI: this may differ between those individuals who are virologically suppressed or not, or those on ART or not.

Future work

These preliminary findings warrant several modifications and improvements:

- updated genotypic data to include more retrospective as well as more recent samples
- inclusion of data on HAART regimen changes
- "on/off" rather than "ever" analysis of mutations
- variable assumptions of persistence by specific mutation
- broaden to include UK-CHIC and UK Resistance databases
- phylogenetic mapping of RHI to other RHI versus longstanding infections

In conclusion, consideration of community resistance mutational burden may explain *some* of variation in TDR rates. If burden does relate to TDR, rates are likely to decrease as HAART outcomes improve. However, even if most treated patients are virologically suppressed, TDR incidence may increase disproportionally if it is introduced into a community with high rates of onward transmission because of role of TDR in begetting further TDR. Therefore the main implication of these findings is to highlight the increasing potential for onward transmission of resistant virus from untreated patients.

Acknowledgements

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Chapter 4

The utility of STARHS in determining the prevalence of undiagnosed recent HIV infection presenting to primary healthcare

Enhanced identification of RHI in the Brighton Cohort allowed a better understanding of the degree to which symptomatic AHI remained undiagnosed in the community, despite presentation to primary health care services.

4.1 Abstract

Objectives: To investigate the extent to which symptomatic AHI presents to healthcare providers and the degree to which it is unrecognised.

Methods: All individuals diagnosed with RHI between 2003 and 2005 were identified (based on the following criteria: an evolving Ab response, negative HIV test within 18 months or STARHS. Symptoms of AHI and previous presentation to other health care providers were ascertained from HIV clinic notes and laboratory records (a single laboratory performing all of the HIV tests in the area).

Results: Of the 108 subjects, 103 (95%) were male and 93 (86%) were MSM. Seventy-six of the 108 (70%) individuals reported symptoms of seroconversion. Of these, 40 (53%) presented to a health care provider during the symptomatic period. Of these, 21 (52%) were diagnosed with AHI at first presentation. In the 19 patients (48%) in whom a diagnosis of AHI was not made at first presentation, 15 were seen in primary care, 3 in accident and emergency (A&E), and 1 in GUM.

Conclusions: The diagnosis of AHI is often missed. Individuals in high-risk groups need to be informed to access health care when they experience symptoms of seroconversion. Non-HIV/GUM healthcare providers (especially primary care) may benefit from training in case recognition to improve rates of diagnosis.

** Recent infection in this study is defined as within 18 months of infection.*

4.2 Introduction

An estimated 50-90% of individuals with RHI are thought to develop a symptomatic seroconversion illness, or AHI [Weintrob, 2003; Schacker, 1996]. However, as previously discussed, these symptoms are non-specific in nature and mimic many common febrile illnesses. Correct diagnosis at this stage of HIV infection is important: it may be the only chance to diagnose HIV in an individual prior to developing advanced disease; it allows consideration of early therapeutic intervention which may represent a unique window [Rosenberg, 2000]; and it offers a significant opportunity to prevent onward transmission at a time of increased infectiousness [Wawer, 2005; Pilcher, 2004, Tovanabutra, 2002]. The extent to which those recently infected present to primary health care providers in the UK, and the degree to which it is recognised, is unknown. This study investigates missed opportunities for diagnosing symptomatic AHI.

4.3 Methods

Patients with RHI between 2003 and 2005 were identified from an HIV clinical database. RHI was defined in this study by one or more of the following: a previous negative HIV Ab test within 18 months (note the longer duration of RHI by consensus at the time of study), an evolving Western blot or Ab response, or by application of STARHS.

At initial presentation to the HIV clinic, data were collected on the following:

- recall of any seroconversion symptoms (*e.g.* fever, rash, sore throat [Hecht, 2002; Daar, 2001; Kahn, 1998]
- timing of the seroconversion illness and any self-reported prior presentation to health care providers

For those individuals who did not self-report presentation to any health care provider, we analysed laboratory records to determine whether any blood tests (not just HIV tests, but any blood sample) had been requested by those healthcare providers (*e.g.* general practitioner (GP) or A&E) at the time of seroconversion symptoms and before presentation to the HIV clinic.

4.4 Results

Of 356 individuals diagnosed anti-HIV Ab positive during the study period, 117 (33%) were classified as RHI. Clinic notes were accessible for 108 (92%). Of these, 103 (95%) were male and 93 (86%) were MSM. RHI was identified by application of the STARHS assay in 60 (56%), a previous negative Ab test within 18 months in 11 (10%), an evolving serological response in 3 (3%) or by combination of the above in 34 (31%).

Seventy-six of the 108 patients (70%) reported symptoms of seroconversion, of whom 91% were MSM. The most common symptoms reported were fever (55%), rash (37%), sore throat (33%), diarrhoea (28%), swollen glands (21%), arthralgias (12%) and headache (11%) and "'flu-like symptoms" (46%). Of the 76 symptomatic individuals, 40 (53%) accessed healthcare at the time. Of these, 21 (52%) patients were correctly diagnosed with AHI at first presentation: 12 (57%) by GUM; 5 (24%) by other hospital teams (4 by general medical teams); and 4 (19%) by their GP.

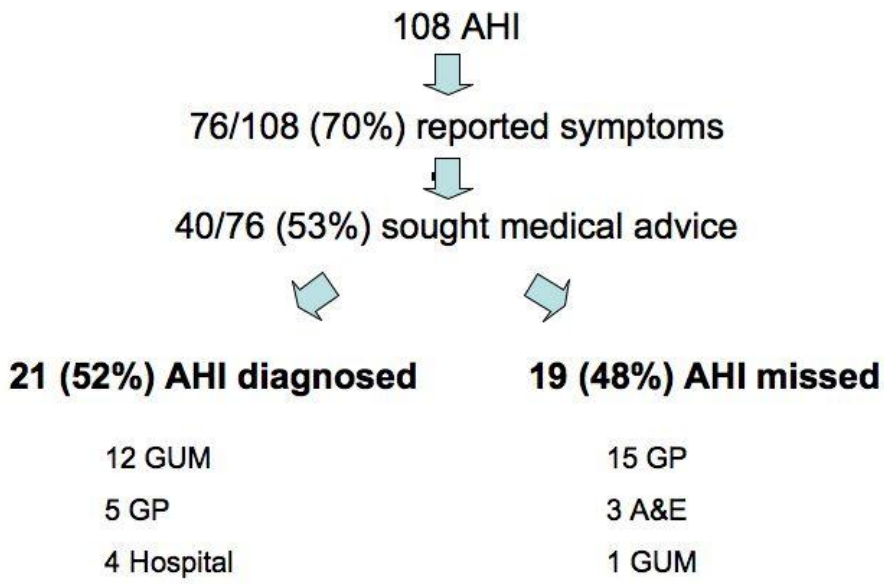
Nineteen of 40 (48%) patients had a prior presentation to health care at the time of seroconversion, yet a diagnosis of AHI was missed (details in Table 4.1); 17 (89%) of these were MSM. Fifteen (79%) had presented to their GP, 3 (16%) to A&E and 1 (5%) to GUM (see Figure 4.1). Of the 19 "missed" cases, 13 were identified as "missed" because the patient reported prior presentation to healthcare with symptoms suggestive of seroconversion. Diagnoses given to the patient at these consultations included bacterial infection (n=4), tonsillitis (n=2), glandular fever (n=1), viral infection (n=1), thrombocytopenia of unknown cause (n=1) and in 4 cases, no diagnosis was given. Six of the 19 "missed" cases were identified as such because laboratory records showed blood tests requested by health care providers at the time of the reported seroconversion symptoms. In one of these six cases an HIV test had been performed. This patient had presented to GUM where a HIV test (4th generation combined Ab / p24 Ag test) was negative at the time of presentation but the patient was not retested until a subsequent presentation 3 months later. (See Table 4.1)

Table 4.1: Details of the 19 missed opportunities for the diagnosis of symptomatic AHI

Cases 1–13: self reported prior presentation to health care with seroconversion symptoms				
Case	**Ethnicity, sexuality, gender**	**Symptoms**	**Diagnosis patient recalls being given**	**Healthcare provider**
1	White MSM	Fever, sore throat, swollen glands, arthralgia	Tonsillitis	GP
2	White heterosexual female	Fever, sore throat, diarrhoea, headache	Tonsillitis	A&E
3	White heterosexual female	Rash, sore throat, swollen glands, weight loss	Glandular fever	GP
4	White MSM	Fever, rash, flu-like symptoms	Viral infection	GP
5	White MSM	Fever, abdominal pain, flu-like symptoms	Low platelets	GP
6	White MSM	Fever, sore throat, malaise, weight loss, headaches	Bacterial infection	GP
7	White MSM	Fever, sore throat	Bacterial infection	GP
8	White MSM	Fever, sore throat, flu-like symptoms	Bacterial infection	GP
9	White MSM	Rash, sore throat, flu-like symptoms	Bacterial infection	GP
10	White MSM	Fever, rash, flu-like symptoms	Not specified	GP
11	White MSM	Fever, rash, sore throat, lymphadenopathy, mouth sores	Not specified	GP
12	White MSM	Flu-like symptoms	Not specified	GP
13	White MSM	Fever, sore throat, diarrhoea,	Not specified	A&E
Cases 14–19: laboratory evidence of prior presentation to health care with seroconversion symptoms				
Case	**Ethnicity, sexuality, gender**	**Clinical information on blood form**	**Laboratory tests requested**	**Healthcare provider**
14	White MSM	Rash, streptococcal	ASOT titre	GP
15	White MSM	History malaise, cough, swollen glands	FBC, U&E, LFTs, CMV+ EBV serology, monospot	GP
16	White MSM	Fever returning from Kenya	FBC, U&E, LFTs, blood film	GP
17	White MSM	Diarrhoea, malaise	FBC, U&E, LFTs, ESR, stool culture	GP
18	White bisexual male	Sore throat 3 weeks	FBC, U&E, LFTs, monospot test	A&E
		Infectious mononucleosis	Throat culture	
19	White MSM	HIV	Fourth generation HIV test	GUM clinic

A&E, accident and emergency; ASOT, antistreptolysin-O-test; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ESR, erythrocyte sedimentation rate; FBC, full blood count; GP, general practitioner; GUM, genitourinary medicine; LFT, liver function tests; MSM, men who have sex with men; U&E, urea and electrocytes

Figure 4.1: Flowchart of study



4.5 Discussion

This study shows that the diagnosis of AHI is often missed and highlights two points at which this occurs. First, almost half (47%) of the symptomatic individuals did not seek medical attention when they were experiencing seroconversion symptoms. Secondly, in almost half (48%) of the symptomatic individuals that presented to healthcare the diagnosis of AHI was not made; the majority of these missed diagnoses were in primary care (79%). Despite the fact that MSM represents the major risk category for acquiring HIV infection in the UK, our results show that AHI remains largely undiagnosed in this group.

Comparison with previous studies

This was the first UK study of its kind. Melzer *et al.* had previously showed that AHI may not always be diagnosed by health care providers: they anonymously tested the sera of 238 attendees with "symptoms consistent with seroconversion" and found 2 cases of undiagnosed AHI [Melzer, 2001]. Our findings are also consistent with two North American studies that showed that AHI is infrequently considered as the diagnosis when symptomatic patients first present to healthcare [Weintrob, 2003; Shacker, 1996].

The other principal finding in our study was that only 53% of the symptomatic patients accessed healthcare at the time of their seroconversion illness. This is in contrast to Schacker *et al.* [Schacker, 1996], who showed in a cohort of 46 patients with RHI, (of whom the majority were MSM), 94% sought healthcare when they had symptoms. Of note, that study was completed before the advent of HAART and in an entirely different health care system. Furthermore, our findings are supported by a questionnaire study in North America, which showed that although most HIV negative men could identify symptoms of AHI, relatively few would access healthcare for such symptoms [Stekler, 2006].

Raising the awareness of AHI amongst MSM is currently an active component of the National AIDS Trust (NAT) prevention strategy.

Limitations

Our study used the patients' recall of prior presentation to health care, therefore reporting may not be completely accurate. The effect of this reporting bias is highlighted by identification of further missed opportunities using laboratory records (Table 4.1, cases 15-19). In this study, a significant proportion of individuals did not access healthcare throughout the duration of their AHI. This includes 32 individuals who did not report seroconversion symptoms, as well as 36 symptomatic individuals who did not access healthcare at the time. We are unable to comment on the specific reasons why these individuals did not access healthcare initially, or the factors leading them eventually to present.

In addition, further research is needed to explore strategies to detect those individuals who remain undiagnosed in the community. From a provider perspective, it was not possible to access primary health care records due to reasons of patient confidentiality. Hence we could not clarify if healthcare professionals had considered HIV as a diagnosis, completed a risk assessment (including knowledge of sexual orientation), or if HIV testing was offered and refused at time of presentation. Of relevance, a survey of over 13,000 MSM showed that only 28% believed that their GPs had knowledge of their sexuality [Sigma Research, 2004]. Our study was conducted in an urban centre with a large white MSM community, and may not be representative of the populations served by most primary care physicians. However, our findings show that in two instances, the diagnosis of AHI was missed in heterosexual females, suggesting the need for a high index of suspicion for the diagnosis of AHI in all populations.

Implications

Strategies to improve these outcomes need to consider both patient and provider factors. From a patient perspective, the likelihood of future diagnosis could be improved by encouraging at-risk groups (*e.g.* MSM and UK Black Africans) to access health care when they experience symptoms of seroconversion or following high-risk exposure. From a provider perspective, training for non-GUM specialists should include recognition of

both the acute seroconversion illness and recent high-risk behaviour. In the current era, reducing stigma and enabling discussion of potential risk factors for HIV acquisition is likely to facilitate the diagnosis of AHI. In this study, a combination of awareness of sexuality with recognition of classical symptoms of AHI may have enabled the diagnosis in 16/19 (84%) of "missed" cases that had presented to healthcare.

In one "missed" case (case 19, Table 4.1), the diagnosis of AHI was not made even though a HIV test had been performed. Of note, this was in a GUM clinic. Although the latest fourth generation combined Ab / p24 Ag test provides improved sensitivity for detecting RHI, those presenting very early after onset of symptoms may still be missed. In such instances where AHI is suspected and there has been sexual risk, follow-up HIV testing 1 week later is necessary to rule out AHI as the cause of those symptoms. This will not necessarily rule out asymptomatic HIV seroconversion, and the 3 month window period for HIV Ab testing still needs to be observed.

Chapter 5

Transmission of HIV during RHI: relationship to sexual risk and sexually transmitted infections. A pilot study of phylogenetic relatedness in recent infection.

This chapter describes the pilot study in which the database was designed and phylogenetic analysis limited to those with RHI only. In order further to understand the role played by RHI in sexual transmission we carried out phylogenetic characterisation of RHI and collected relevant epidemiological data regarding sexual behaviour, clinical features and STI.

5.1 Abstract

Objective: To study recent HIV infections (within 18 months), using molecular and epidemiological approaches in order to assess correlates of transmission in this population.

Methods: Individuals with RHI were recruited prospectively from a discrete cohort of 1,235 individuals under follow-up in a well-defined geographical area between 1999 and 2003. RHI was diagnosed by one of the following: negative HIV Ab test within 18 months, evolving Ab response, or application of STARHS. The *pol* gene was sequenced to identify genotypic resistance and facilitate molecular epidemiological analysis. Clinical data were collected and linked in an irretrievable fashion when informed consent was obtained.

Results: A total of 103 individuals with RHI diagnosed between 1999 and 2003 were included in the study; 99 (96%) were male and 90 (91%) were MSM. Viruses from 35 out of 103 (34%) appeared within 15 phylogenetically-related clusters. Significant associations with clustering were: young age, high CD4 cell count, number of sexual contacts, and UPAI in the 3 months before diagnosis ($p<0.05$ for all). High rates of acute STI were observed in both groups with a trend towards higher rates in those individuals with viruses within a cluster (42.9 versus 27.9%; $p<0.13$).

Conclusion: High rates of partner change, UPAI and STI are factors that facilitate onward transmission during RHI. More active identification of individuals during RHI, the management of STI and HAART may all be useful methods to break transmission networks.

** Recent infection in this study is defined as within 18 months of infection.*

5.2 Methods

Study recruitment

Individuals were recruited from a cohort of 1,235 HIV-positive individuals attending the single GUM clinic for follow-up from 1999 to 2003. The department is the sole local provider of HIV and STI care, and national surveillance data confirm that over 90% of individuals with HIV infection resident in the area attend this institution [SOPHID, 2010; Brown, 2007, *personal communication*].

Individuals with RHI were identified by one or more of the following: previous negative HIV Ab test within 18 months, evolving Western blot or HIV Ab response, or application of the STARHS assay, as described in Chapter 2.

Clinical data collection

In those from whom written informed consent was obtained, information regarding clinical status was collected from clinic case notes: the date of diagnosis, CD4 cell count, CD4 cell percentage, HIV VL, the presence and nature of STI in the 3 months before the diagnosis of RHI (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, non-specific urethritis, early syphilis, herpes simplex and *Trichomonas vaginalis*) and the absence or presence of seroconversion symptoms. Information relating to the individual's HIV acquisition risk group, sexual behaviour (including estimated number and nature of sexual contacts in the 3 months before diagnosis of RHI) was also recorded. These data are routinely collected for all new HIV diagnoses within this clinic.

Serological Testing Algorithm for Recent HIV Infection

STARHS testing was performed using the bioMérieux Vironostika HIV-1 assay (bioMérieux UK Ltd., Basingstoke, UK) as previously described in Chapter 2. A standardized optical density for each specimen was determined. For this study a standardized optical density of less than 1.0 was used to identify RHI, and this cut-off equates to an estimated seroconversion within the previous 4–6 months.

Phylogenetic analysis

The HIV *pol* gene was sequenced from plasma obtained at the time of HIV diagnosis. These sequences were used for phylogenetic analysis, a method previously shown in Chapter 2 to have utility in reconstructing transmission events. Full-length sequences from the protease gene (295 nt) and the first 230 codons of RT were aligned using the program Clustal X (available from http://www.ebi.ac.uk/Tools/msa/clustalw2/ - accessed January 21, 2012) and then adjusted manually with the software BioEdit (available from http://www.mbio.ncsu.edu/ - accessed January 21, 2012). Sequences that could not be unambiguously aligned or were of insufficient length were excluded from the study.

Phylogenetic relationships between the pol sequences were reconstructed using the neighbour-joining followed by maximum likelihood methods. An initial neighbour-joining tree was built under the Hasegawa-Kishino-Yang (HKY85) model of evolution with a ratio of transversion to transitions of 2:1 using the tree-building software Paup® (available from http://paup.csit.fsu.edu/about.html - accessed January 21, 2012).

The best fitting model of nucleotide substitution was estimated on the basis of the neighbour-joining tree topology using a maximum likelihood ratio test with Modeltest version 3.0. The derived parameters of the selected model were then used to perform a heuristic search for a maximum likelihood tree with Paup. The construction of the tree was done according to the general time reversible (GTR) model of evolution, with a proportion of invariable sites and gamma distribution. An HIV-1 subtype K sequence (Genbank accession number AJ249239) retrieved from the Los Alamos HIV database (http://hiv-web.lanl.gov - accessed January 21, 2012) was used as an outgroup and six pairs of follow-up sequences from the same individuals were used as controls. The robustness of the neighbour-joining trees was evaluated by bootstrap analysis, with 1000 rounds of replication.

Statistical analysis

Statistical comparisons of those in a cluster with those not in a cluster were performed using Chi-squared tests, Fisher's exact tests or Mann-Whitney U tests, as appropriate. Multivariable logistic regression was used to identify factors independently associated with belonging to a cluster. All statistical analyses were performed using SAS version 8 (available from http://v8doc.sas.com/sashtml - accessed January 21, 2012).

The study was approved by the Brighton and Hove Local Research Ethics Committee and the Health Protection Agency Ethics Committee. Confidentiality and anonymity were protected by irreversibly unlinking clinic and laboratory from the study ID number using a firewall system managed by the local public health laboratory. Written, informed consent was obtained from all participants.

5.3 Results

Study population description

The prospective cohort included over 2,100 patients with HIV infection, with 1,235 being seen during the study period. Of these, 86% were Caucasian, 89% were men, and the predominant route of transmission was sex between men (79%).

A total of 103 individuals with RHI diagnosed between 1999 and 2003 were included in the epidemiological and phylogenetic analysis. Of these, 73 (71%) had a STARHS assay suggestive of infection within the previous 4-6 months. Almost all (99/103, 96%) were men and 90/99 (91%) men were MSM. All the men and two out of four women were Caucasian with a median age of 36 years (range 21-67). The median age was 36 years (range 21-67). Six individuals (6.1%) reported a history of injecting drug use (2 MSM, 2 heterosexual men and 2 heterosexual women). The median CD4 cell count (available in 101/103) was 526 cells/mm^3 (range 195-1477) and the median CD4 cell percentage (available in 81/103) was 28% (7-42). The median plasma HIV VL was log 4.95 copies/mL (2.03-6.00). Thirteen MSM (12.6% of total patients) were infected with viruses that contained primary antiretroviral resistance-associated mutations. STI were diagnosed concurrently with RHI in 34 of the 89 individuals (38.2%) for whom information was available. Among the 90 MSM, 61 (68%) reported UPAI in the 3 months before RHI diagnosis; no information was available regarding sexual practices in the period preceding this.

Cluster comparison

Viruses from 35/103 individuals (34%) appeared within 15 transmission clusters, comprising one cluster of five individuals, two of three and 12 of two (full results shown in Table 5.1 and Fig. 5.1). All were men and 32 (97%) were MSM. For individuals within 11 out of 15 clusters, the diagnosis of RHI was made within 12 months of each other, giving supporting evidence that transmission occurred during the RHI period. Those in the cluster group had a higher CD4 cell count (p=0.005), higher CD4 cell percentage (p=0.003), were younger (p=0.05), reported a higher number of

different sexual contacts in the previous 3 months (p=0.006), and were more likely to have engaged in UPAI in the 3 months before the RHI diagnosis (p=0.05) in comparison to those individuals not within a cluster. High rates of STI at the time of RHI were observed in both groups, with a trend towards higher rates in those individuals with viruses in a cluster (42.9 versus 27.9%, p=0.13). Multivariable logistic regression analyses identified the CD4 cell percentage [odds ratio (OR) 1.14, 95% CI 1.04–1.23, p=0.003] and having more than five sexual partners (OR 3.38, 95% CI 1.13–10.10, p=0.03) as the only independent predictors of belonging to a cluster. Six individuals (17%) had antiretroviral-associated resistance mutations, of whom two (both T215D in RT) belonged to a linkage pair.

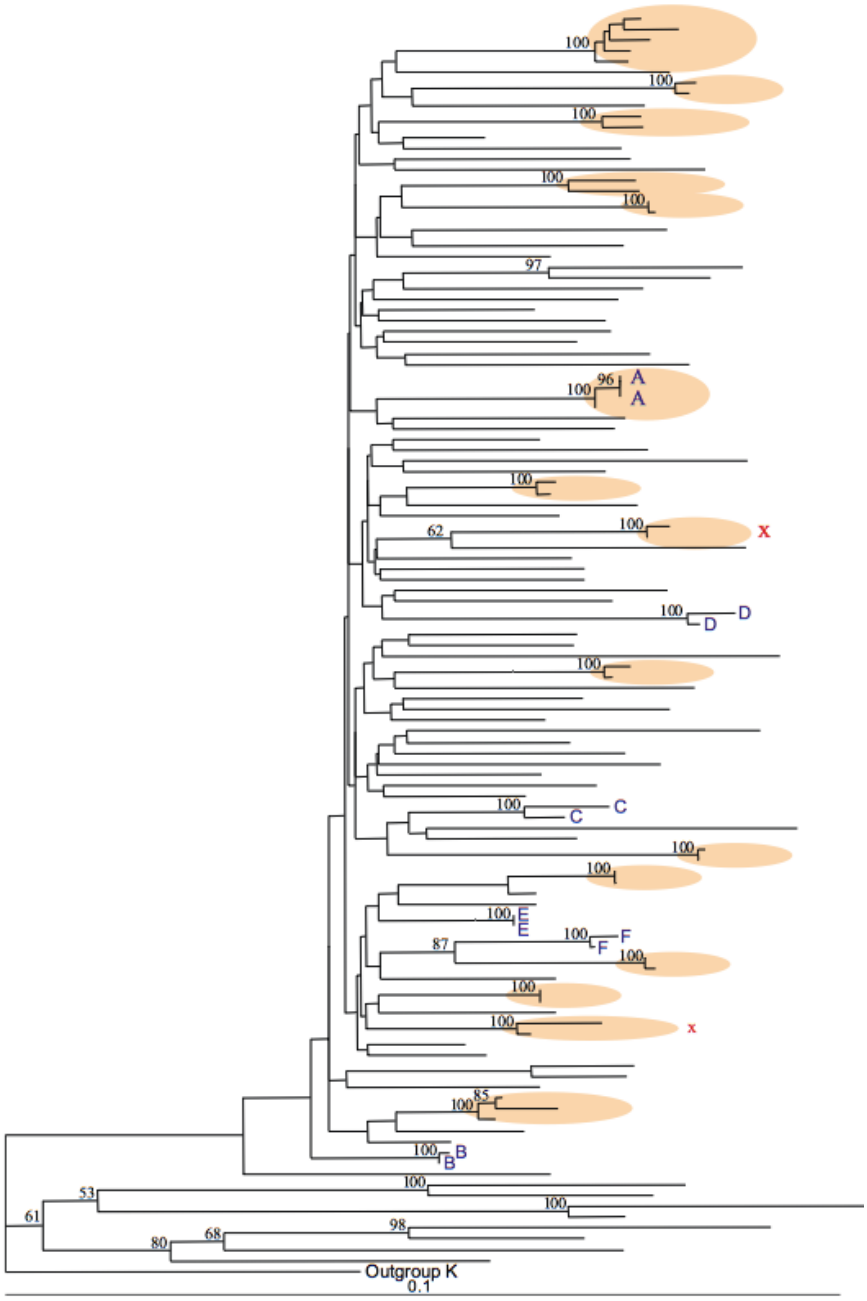
Table 5.1: Comparison of features associated with patients in the cluster and non-cluster groups

	In cluster	Not in cluster	*P* value[a]
Number of patients	35	68	
Male sex	35 (100%)	64 (94.1%)	0.30
Age (years): median (range)	34 (23–54)	37 (21–67)	0.05
Number of contacts in 3 months before diagnosis: median (range)	3 (1–100)	2 (1–36)	0.006
Homosexual risk group	32 (97.0%)	58 (85.3%)	0.10
Highest reported risk in the 3 months before diagnosis of PHI			
Unprotected oral intercourse	25 (78.1%)	36 (73.5%)	0.83
Protected anal intercourse	2 (6.3%)	5 (10.2%)	0.70
Unprotected anal intercourse	28 (87.5%)	32 (65.3%)	0.05
Unprotected vaginal intercourse	0 (–)	8 (16.3%)	0.02
STI in 3 months before diagnosis			0.31
Yes	15 (42.9%)	19 (27.9%)	
No	18 (51.4%)	37 (54.4%)	
Not known	2 (5.7%)	12 (17.7%)	0.13
CD4 cell count (cells/mm^3): median (range)	612 (195–1477)	474 (196–1259)	0.005
CD4 cell percentage: median (range)	31 (12–40)	26.5 (7–42)	0.003
Viral load ($\log_{10}$ copies/ml): median (range)	4.97 (2.03–6.00)	4.94 (2.30–6.00)	0.90

[a]Entries in table are *n* (%) unless otherwise specified.

Figure 5.1: Maximum likelihood phylogenetic tree based on pol sequences from 103 individuals with primary HIV infection

Possible transmission clusters are circled. Linkages confirmed by clinical data are indicated by a red cross. Transmission clusters were identified if the bootstrap value was equal or greater than 99% and the average genetic distance (i.e. branch length) was lower than 0.015 nucleotide substitutions per site. Six pairs of multiple sequences from single patients were used as controls for relatedness and are indicated by letters (e.g. A indicates multiples sequences from patient A). Bootstrap values higher than 50% are indicated on the branches.



5.4 Discussion

The high rates of clustering observed within our study support the assertion that RHI may be associated with an increased risk of onward transmission. The associations we found with younger age, high rates of UPAI, and sexual partner change identify this as a high-risk group for HIV transmission. There was a trend towards higher rates of STI in the cluster group on a background of extremely high STI rates in the study population, supporting the argument for increased STI surveillance, particularly of high-risk groups.

The highly significant correlation with CD4 cell count may represent the early disease stage, or rapid contact tracing and testing of sexual partners of individuals diagnosed with RHI. The plasma VL at diagnosis was not predictive of clustering, and it is possible that the seminal VL in men is a more consistent correlate of infectiousness, particularly in the context of genital tract inflammation, with plasma/genital tract discordance playing an important role [Pilcher 2004; Pilcher 2001; Coombs, 1998; Leynaert 1998].

The presence of the same antiretroviral resistance mutation in one cluster pair, neither of whom had received HAART, illustrates the potential for the secondary spread of such resistant strains, as we have previously documented [Taylor 2004; Yerly 2001,].

Our results do not exclude the possibility of a common source for each cluster, rather than transmission within clusters. However, a phylogenetic tree comprising viruses from these 103 recent infections, together with more than 2000 *pol* sequences from prevalent infections throughout the UK only identified one further potential linkage, and that involved a RHI case not within an existing cluster (data not shown).

Only 31 of the non-cluster group (64.6%) reported UPAI, but it should be noted that this is only in the time window 3 months before diagnosis with RHI. Interestingly, routinely collected data on recent sexual contacts only confirmed three of the linkage pairs that were revealed in the phylogenetic analysis, emphasising the high rates of anonymous sexual partners and the difficulty in obtaining a reliable sexual history.

These results provide evidence that the active management of RHI will reduce HIV transmission. HIV prevention programmes have been heavily focused on protecting susceptible individuals, but accumulating biological and modelling data suggest that reducing the infectiousness of HIV-positive individuals may also be an effective strategy.

A large proportion of RHI remains undiagnosed in the community [HPA, 2011; Pilcher 2004; Melzer 2001], and these findings support the view that as a disease stage RHI represents a public health threat. Efforts should be re-focused on improving rates of diagnosis of individuals during RHI, timely contact tracing, risk reduction, the management of STI, and possibly early treatment with HAART in an effort to break transmission networks during this unique and possibly crucial stage of HIV infection [Cates, 1997]. Furthermore, consideration should be given in information and awareness campaigns to highlight the possible symptoms of AHI in groups with high rates of onward transmission, to encourage such individuals to present to appropriate health care providers to enable the timely diagnosis and management of this important disease stage.

Chapter 6

The determinants of HIV-1 transmission in men who have sex with men: a combined clinical, epidemiological and phylogenetic approach

This chapter describes the extension of the database and the phylogenetic exploration of the entire Brighton MSM cohort. It sets out to characterise the clinical characteristics of potential source individuals to those with recent infection and ascertain the proportional contribution of transmission determinants, including undiagnosed infection.

6.1 Abstract

Aim: To identify the biological characteristics of HIV infection source individuals at the likely time of transmission, and estimate their proportional contribution to onward transmission in MSM

Design: A longitudinal phylogenetic analysis of HIV from an MSM cohort, incorporating clinical and epidemiological data.

Methods: Potential individuals were HIV-infected MSM attending a sexual health clinic between 2000 and 2006. Individuals were classified such that they could move from recent to longstanding infection categories. HIV-1 *pol* gene sequences were obtained from plasma virus or proviral DNA and clusters estimated by maximum likelihood and conservative genetic distance differences. The single most-likely infection source individual infecting each individual with RHI (RHI now defined as within 6 months) was ascertained, and risk factors around time of likely transmission explored using Poisson regression modelling.

Results: From 1,144 HIV-infected MSM, pol sequence data were obtained for 859 (75%); 159 out of 859 (19%) were recently HIV infected at diagnosis. A single most-likely infection source individual was identified for 41/159 (26%), of which 11 were recently infected (27%) and 30 chronically infected. Factors associated with transmission in multivariable analysis were: younger age {rate ratio per 5 years older 0.68 [95% confidence interval (CI) 0.54–0.86], p<0.0009}, higher VL [rate ratio per log higher 1.61 (95% CI 1.15–2.25), p<0.005], RHI [rate ratio 3.88 (95% CI 1.76–8.55), p<0.0008] and recent STI [rate ratio 5.32 (95% CI 2.51–11.29), p<0.0001]. HAART was highly protective in a univariate model, RR 0.14 (95% CI 0.07–0.27, p<0.0001).

Conclusion: Onward transmission of HIV among MSM is significantly associated with RHI, STI and higher plasma VL, and reduced by effectively-taken HAART. The majority of new infections appear to occur from individuals whose infection was undiagnosed at the time of transmission.

** Recent infection in this study is defined as within 6 months of infection*

6.2 Introduction and aims

Introduction

Previous phylogenetic studies [Bezemer, 2010; Brenner, 2007; Yerly, 2001] have shown high levels of clustering among individuals diagnosed during RHI, estimating that between 24 and 49% of these infections are linked to other RHI. However, risk factors present at diagnosis do not necessarily remain over time. For example in the case of RHI, in all studies so far, each RHI has been considered as fixed from diagnosis throughout the entire study period rather than for a transient time interval. Furthermore, previous studies have seldom been able to include other relevant risk factors for transmission (VL, HAART and STI status) and, if they have, not throughout the entire study period nor linked to specific transmission events.

If these limitations of phylogenetic analysis can be overcome and it is accepted that transmission by a third party (either infecting both individuals in a cluster or being an intermediary between the two) cannot entirely be excluded, a more accurate estimation of transmission dynamics is possible.

Aims

We aimed to improve upon these limitations by allowing for RHI to progress into longstanding infection over time and by collecting accurate risk factor data. By doing so we aimed to identify, in an MSM cohort covering a relatively small geographic area, the single most-likely infection source individual (or "transmitter") to each individual with RHI by time-specific phylogenetic analysis. By describing the biological characteristics of these infection source individuals at the time of transmission, we set out to determine the proportional contribution of these biological characteristics to onward HIV transmission.

6.3 Methods

Study population

The study population was a cohort of HIV-infected MSM attending the single GUM/HIV treatment clinic at the Brighton and Sussex University Hospitals NHS Trust between 2000 and 2006; all were eligible for baseline genotypic resistance testing. The study period was stratified into a series of 3-month intervals and the characteristics of patients under follow-up were summarised at the start of each interval. Clinical data considered for each individual under follow-up for each 3-month period were date of diagnosis, disease stage (recent or longstanding infection, changing over time), age group, ethnicity, latest CD4 cell count, latest VL, prior receipt of HAART and diagnosis of an STI. The STI included in the analysis were *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, nonspecific urethritis, primary syphilis, genital herpes simplex and *Trichomonas vaginalis*.

Study period

The study period 2000 – 2006 was stratified into a series of three month calendar quarters and the characteristics of patients under follow-up were summarised at the start of each quarter. For each patient, data were collected for the first calendar quarter of attendance during the study period and updated for each consecutive calendar quarter throughout the study period. Where patients did not attend during a calendar quarter, the data from the previous quarter were carried forward. Patients who did not attend for 12 consecutive months were considered lost to follow-up and excluded from the study period from their last quarter of attendance.

Characterisation of RHI

RHI was defined on the basis of:

- negative HIV Ab result within 6 months
- p24 Ag-positive result in conjunction with a negative anti-HIV Ab test
- limited western blot (4 bands, including p24 and gp160)
- STARHS if the viral subtype was B and patient not presenting with advanced disease or on HAART.

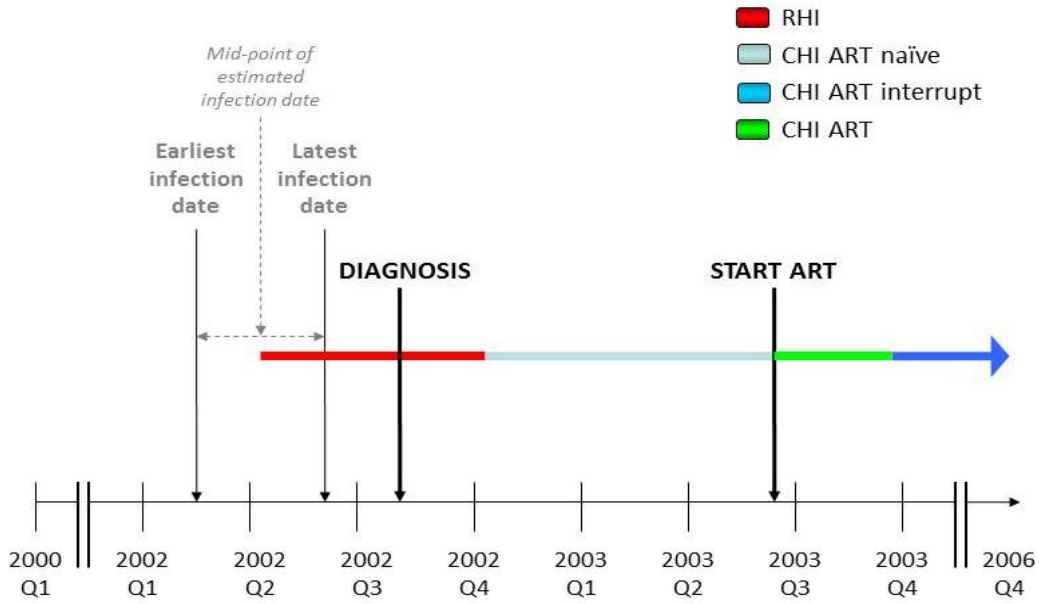
STARHS, combined with clinical data including subtype and CD4 cell count, was available for over 95% of individuals, so that categorisation of newly diagnosed infections as recent or longstanding was high. STARHS testing was with the bioMérieux Vironostika HIV-1 assay (bioMérieux UK Ltd., Basingstoke, UK), as previously described in Chapter 2. For this study, an optical density of less than 1.0 was used to identify RHI, and this cut-off is associated with a seroconversion within 4-6 months. Testing was performed retrospectively for those diagnosed in 1996-2000, and prospectively thereafter.

The study design allowed for individuals to progress from RHI to longstanding infection during the study period. For all recently infected individuals, an earliest and latest infection date was calculated according to the assay that had been used to identify RHI:

- 60-183 days prior to diagnosis for STARHS
- 1-30 days prior to diagnosis for Ab-negative/p24 Ag-positive
- 30-60 days prior to diagnosis for evolving Western Blot

For the main analysis, an estimated infection date was taken as the midpoint between the earliest and latest infection date for each patient. Depending on these window periods, as well as intervals between first-positive and last-negative HIV tests in some cases, the calendar period dates that each individual was potentially experiencing seroconversion (6 months) were defined, after which they were reclassified as longstanding infection (see Figure 6.1). Sensitivity analyses were also performed using earliest and latest infection dates, rather than midpoint estimates, as described later.

Figure 6.1: Flow diagram showing how infection category was ascertained and updated over time for each subject



Comparison Group

The clinical characteristics of each most-likely infection source individual were compared with the study group and included every patient for every calendar quarter following diagnosis. Patients were not included if they were lost to follow-up (no attendance for 12 months). Where data were unavailable, data from the previous quarter were carried forward.

Phylogenetic analysis

Pol sequences were generated from the plasma RNA of new diagnosis specimens. Only the infection source individuals clustering with individuals diagnosed during RHI were sought, in order to clarify as far as possible direction of transmission. When baseline genotype specimens were initially unavailable (*i.e.* if an individual had low or no detectable viraemia throughout the study period), sequences were obtained from an EDTA cell pellet. *Pol* sequences were generated through an in-house assay, spanning from position 1 in protease to at least position 300 in RT. Sequences were

aligned using a Sequence Analyser. Because of the large sample size, a neighbour-joining tree was constructed with gamma rate heterogeneity set at 0.5 and 500 bootstrap replications.

Clusters were defined as sequences that shared a common node that had bootstrap support of over 99% and genetic distance under 0.015 nucleotide substitutions per site. Those sequences that formed a cluster were grouped with 50 random sequences that did not cluster and run through ModelTest. A heuristic search was conducted for a maximum likelihood tree using the selected model (K81uf+I+G) and its derived parameters (proportion of invariable sites set at 0.4477 and the heterogeneity set at 0.6946). The same definition of cluster was used; those clusters that were identical between the neighbour-joining and the maximum likelihood trees were retained.

Reconstruction of transmission events

Only the *single most-likely* infection source individual for each RHI individual was sought. All individuals, regardless of disease stage, were considered potential infection sources, and the period of potential transmission from these sources was determined to be from the earliest possible date of infection (in the case of RHI) or from the date of diagnosis (for longstanding infections) through to study end.

Potential infection source individuals for each RHI individual were defined as having any other sequence that was in the same robust phylogenetic cluster and who was diagnosed before or in the same calendar quarter as the recently-infected individual. When there was more than one candidate infection source individual, the candidate sequence with the shortest genetic distance was chosen as the most likely subject for the infection source. *No other linkages were explored.*

Where a transmission event was identified between two individuals both diagnosed as recently HIV-infected during the same calendar quarter, the individual identified as recently HIV-infected using the marker with the longest window period was selected as the infection source.

Excluded transmission events

Any individual in the cluster whose earliest date of infection was probably after the latest time of transmission to the RHI individual was not considered a potential infection source individual in that particular cluster. Potential infection source individuals were also excluded if they had been diagnosed during chronic infection and their quarter of diagnosis occurred after diagnosis quarter of the recently HIV-infection patient with which it was linked. This is because the direction of transmission could not be ascertained (*e.g.* it could not be established whether the recent infection generated the chronic infection or vice versa). Similarly, where the same marker of recent infection had been used for 2 RHI individuals in a cluster, the pair was excluded from analysis since it was impossible to determine the direction of transmission between the two. Potential infection sources that appeared to transmit after they were lost to follow-up were also excluded.

Figure 6.2: Schematic examples of valid transmission events

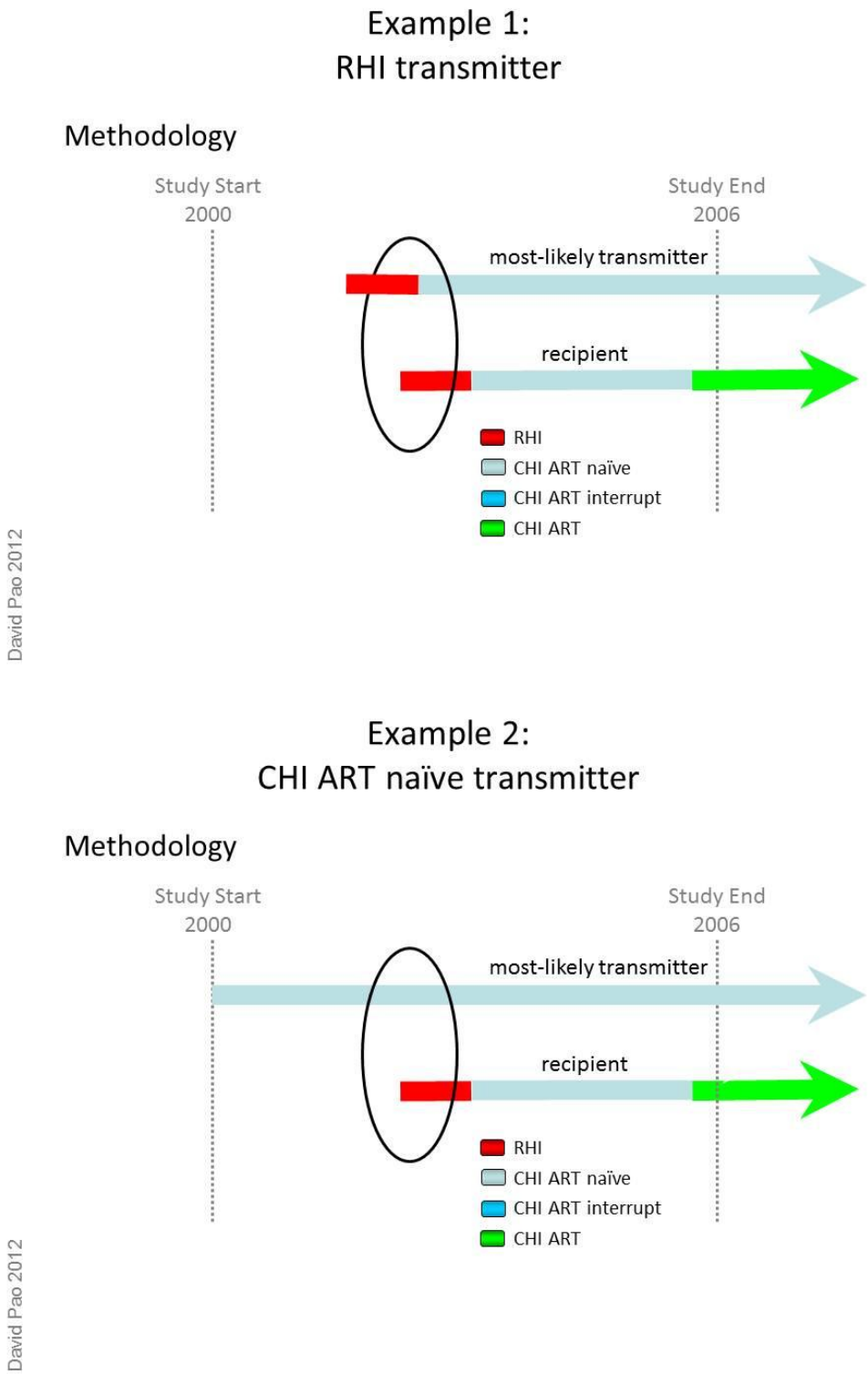
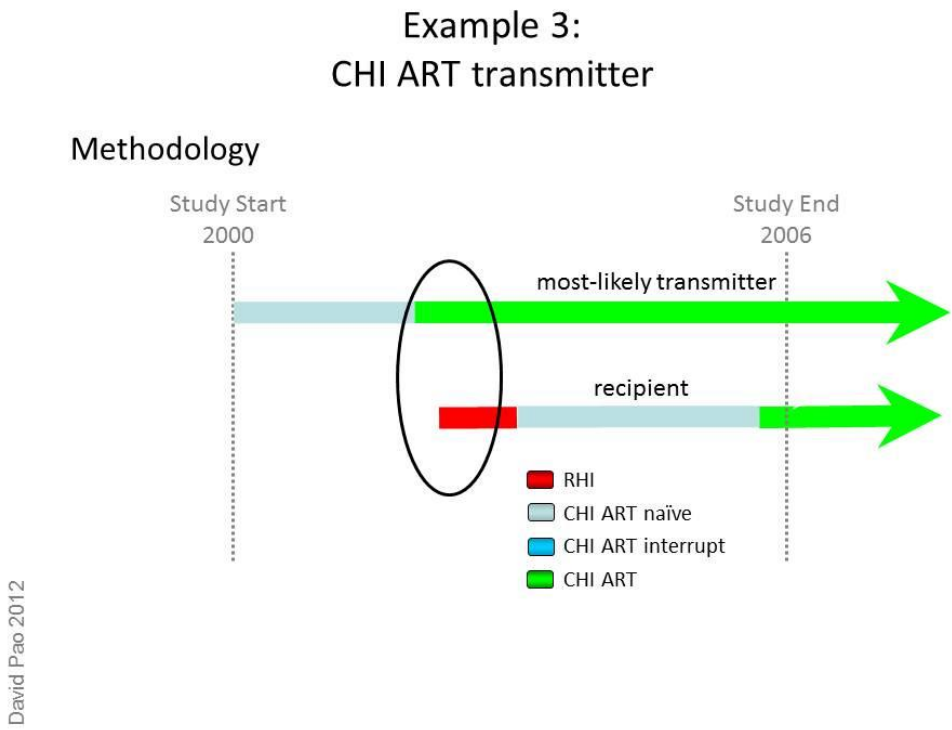
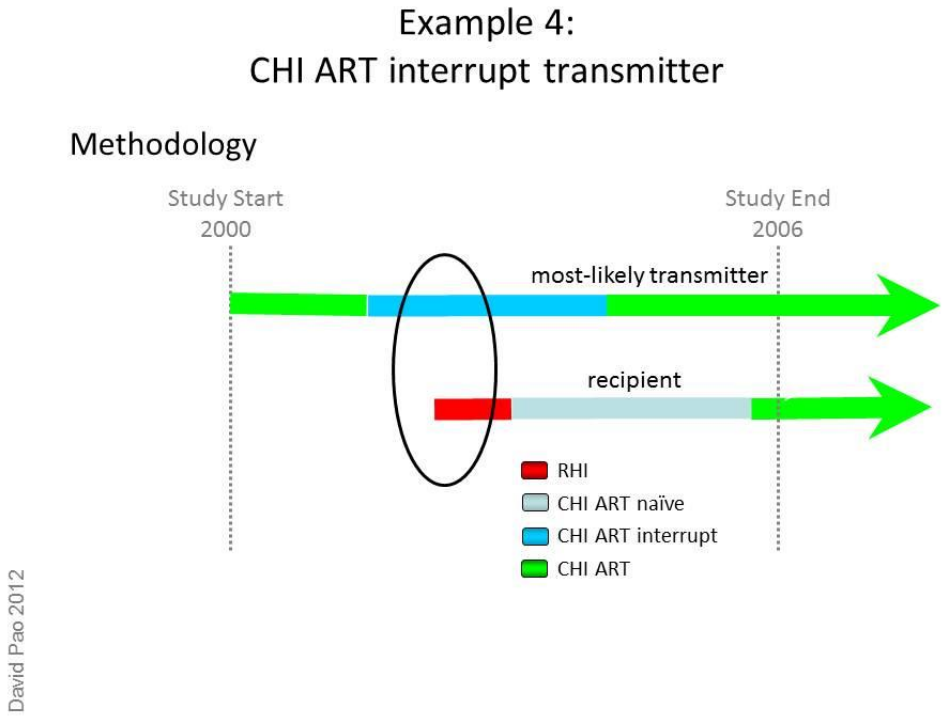


Figure 6.2 (cont.): Schematic examples of transmission events





Statistical analysis

Over 90% of VL and CD4 cell count data were complete for each patient per calendar quarter.

Using the information from most-likely infection source individuals during each 3-month period, and those who were thought not to have transmitted HIV during that period, we identified factors independently associated with transmission using multivariable Poisson regression models (SAS version 9.1; SAS Institute Inc., Cary, North Carolina, USA).

The rate of transmission was calculated for each risk factor, per 100 person years of follow-up (PYFU). The PYFU were calculated through summing every calendar quarter of the study period for each patient from their first attendance to study end or the patient's last attendance if lost to follow-up. Transmission rates were represented as the number of transmissions that occurred divided by the total person-years of follow-up (PYFU) within each strata of interest (and expressed per 100 PYFU).

To do this, each 3-month period of follow-up for an individual was included as a separate observation in the analysis with the outcome of interest being a binary covariate that indicated whether the individual was thought to have transmitted HIV during that period or not. Thus, each individual contributed as many observations to the analyses as 3-month periods when he remained under follow-up as a potential infection source individual.

Patients who were diagnosed with HIV infection nearer the end of the study period would be represented in fewer calendar quarters compared to those diagnosed before the study period. This will affect those recently HIV-infected at diagnosis disproportionately and for this reason transmission rates per PYFU were calculated.

Factors included in the model were age at the start of each period (categorised as < 35, 35-44 and > 45 years and also treated as a continuous covariate), latest VL (unknown, < 50, 50-1,000, 1,001-10,000, 10,001-100,000 and >100,000 copies/mL and also treated as a continuous covariate), latest CD4 cell count (unknown, < 200, 201-350, 351-500 and >500 cells/mL and also treated as a continuous covariate), disease stage

and receipt of HAART combined (RHI, longstanding untreated, longstanding treated and longstanding treatment interruption), whether the individual had a diagnosis of AIDS at the start of the period, whether the individual had an STI in the 3-month period, and calendar year (2000-2001, 2002-2003 and 2004 onwards). In this way, each individual's covariates (including disease stage) were allowed to change over time as his infection progressed.

Sensitivity analyses were conducted to assess the impact of the methods used to allocate infection category. Rather than use a midpoint for date of infection, different analyses were carried out for earliest possible and latest possible dates of infection for the RHI subjects. Multivariate analyses were repeated while adjusting relevant variables.

Ethical approval and procedures

The study was approved by Brighton and Hove Research Ethics Committee (06/Q1907/93). When additional specimens were required over and above routine clinical care (*i.e.* for generation of sequences from proviral DNA), individual informed consent was required. All clinical information and patient identifiers were anonymised prior to phylogenetic analysis, so that individuals would not be identifiable to the researchers once phylogenetic analysis had been performed. By aggregating data into 3-month intervals, we also limited identification opportunities prior to data linkage (*e.g.* dates of CD4 and VL tests, AIDS events and STI).

6.4 Results

Study participants

Between 2000 and 2006, 1,144 MSM were seen at least once in the clinic. *Pol* sequence data were obtained for 859 (75%) and included in the phylogenetic analysis. There were no significant differences in the characteristics of those for whom a sequence could not be obtained. Of the remaining 285 individuals, 118 had incomplete *pol* sequences (and excluded from the analysis) and 167 did not have any sequence available. Of these, 134 (80%) were diagnosed before the study period.

Over 90% of VL and CD4 cell count data were complete for each patient per calendar quarter. Between 2000 and 2006, the average number of patients represented was 606.7 (range 451-724) for each calendar quarter. On average, patients had data available for 21.2 consecutive calendar quarters (range: 3-28, SD 8.8 calendar quarters) and 8 patients were lost to follow-up every calendar quarter.

Clustering of participants

Of the 859 participants' sequences, 209 (25%) fell into discrete clusters in the neighbour-joining tree (Figure 6.3). These sequences were selected with a random selection of 50 sequences that did not form a cluster in the initial tree and underwent a maximum likelihood reconstruction. Of the 209 original cluster sequences, 129 were retained in the maximum likelihood tree, resulting in 15% (129/859) of sequences clustering overall.

Ascertainment of transmission events within clusters: 159 (19%) of the 859 participants with sequence data available were classified as being RHI at diagnosis, and were, therefore, infections for whom an infection source individual was sought. Out of these, 47 (30%) fell into clusters and a single most-likely infection source individual was identified for 41 (26%) [transmission rate 0.66/100 PYFU, 95% confidence interval (CI) 0.46-0.87]. During the 3-month period, when transmission was thought to have occurred, 11 out of 41 (27%) of these infection source subjects were categorised as RHI and 30 (73%) as longstanding infections (Figure 6.4).

Figure 6.3: Phylogenetic reconstruction of HIV transmission events among diagnosed HIV-infected MSM attending Brighton clinic, with most-likely infection sources highlighted, 2000-2006

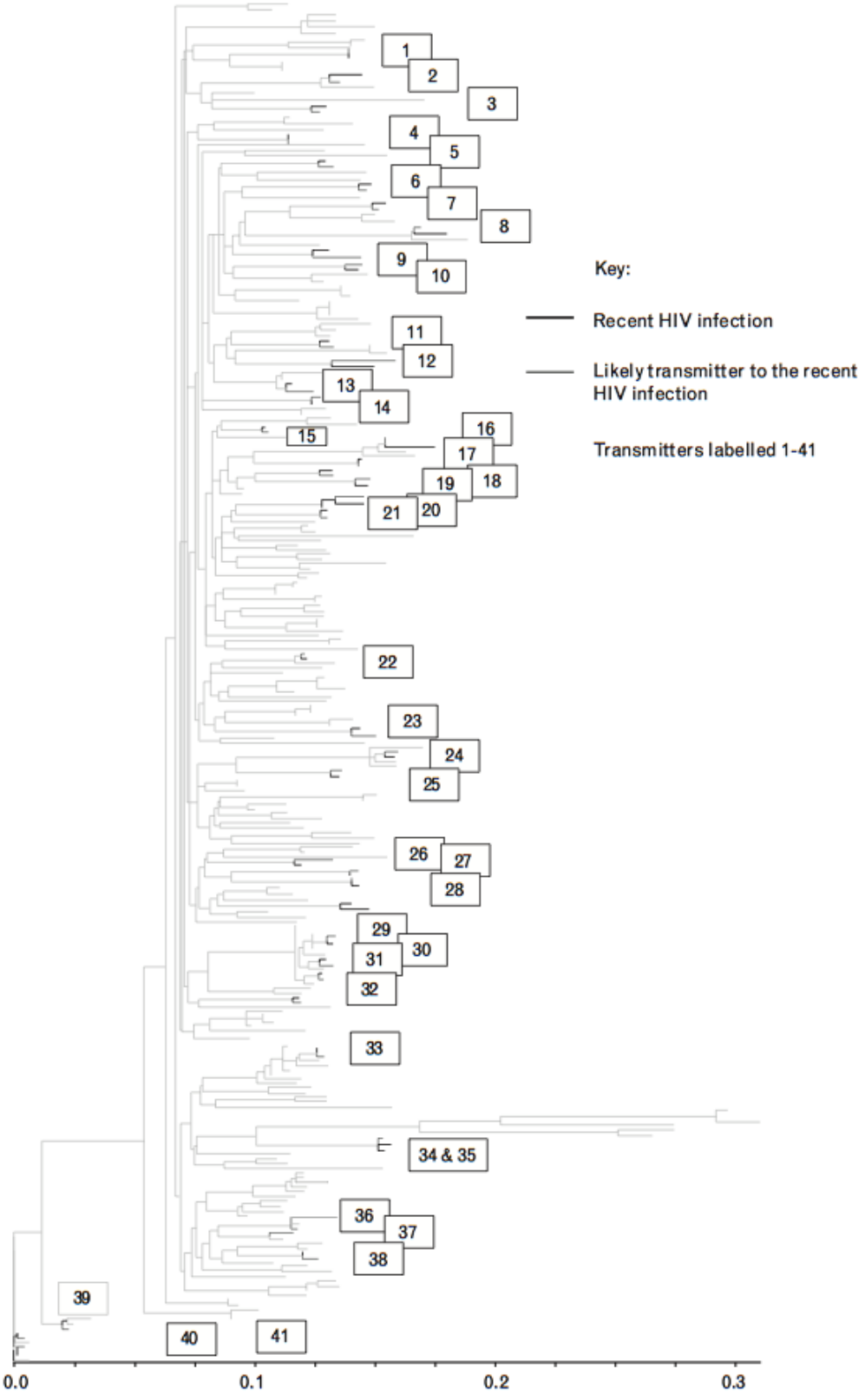
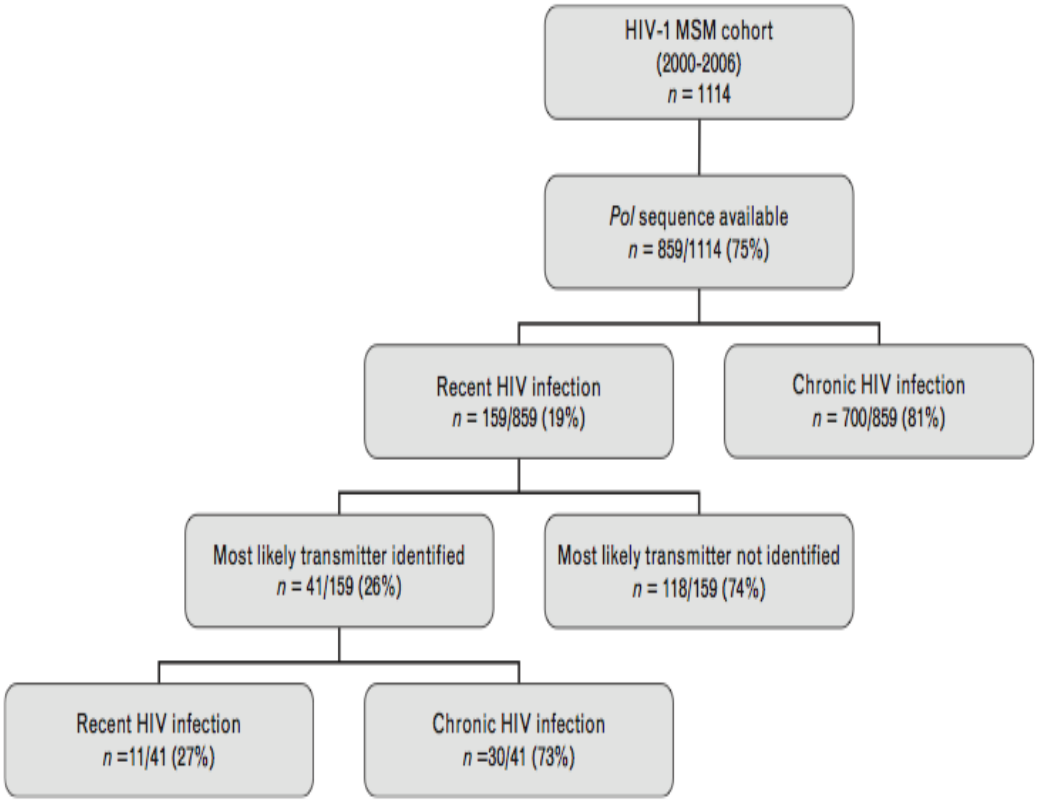


Figure 6.4: Phylogenetic clustering and determination of most-likely transmitter by infection stage



Transmission rates

Transmission rates were higher in those aged less than 35 years, those with VL > 10,000 copies/mL, those with recent or chronic untreated infection, those with CD4 cell counts > 350 cells/mm^3 and those experiencing an STI during the 3-month interval (Table 6.1).

Univariate analyses confirmed that younger age (rate ratio per 5 years older 0.52, 95% CI 0.41-0.65, p=0.0001), higher VL (rate ratio per log higher 2.32, 95% CI 1.79-3.01, p=0.0001), RHI (rate ratio 4.44, 95% CI 2.11-9.33, p=0.0001) and a recent STI (RR 12.13, 95% CI 5.95-24.74, p=0.0001) were all associated with transmission risk (Table 6.2).

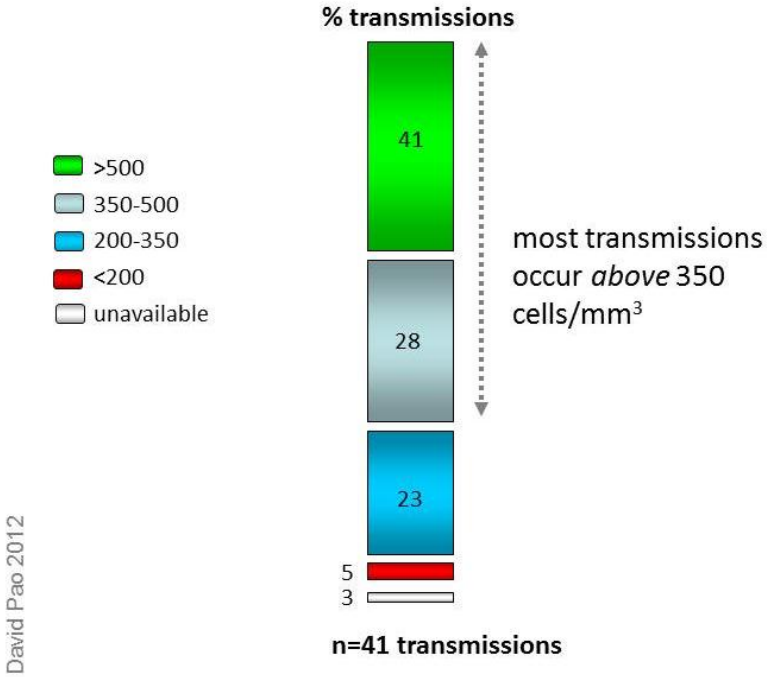
In multivariate analyses, younger age (rate ratio per 5 years older 0.68, 95% CI 0.54-0.86, p=0.0009), higher VL (rate ratio per log higher 1.61, 95% CI 1.15-2.25, p=0.005), RHI (rate ratio 3.88, 95% CI 1.76-8.55, p=0.0008) and a recent STI (rate ratio 5.32, 95% CI 2.51-11.29, p=0.0001) were confirmed as independent risk factors for transmission (Table 6.3).

Although use of HAART was significantly associated with transmission risk in univariate analyses (rate ratio 0.14, 95% CI 0.07-0.27, p=0.0001) (Table 6.2), this effect was attenuated towards unity after adjustment for the other covariates (including latest VL) in a multivariate model (rate ratio 0.77, 95% CI 0.35-1.69, p=0.51) (Table 6.3). Each patient had an estimated infection date calculated as the midpoint of their earliest and latest infection date, based on the markers used to ascertain RHI.

CD4 cell counts

70% 0f transmissions occurred from infection source individuals who had a CD4 cell count > 350 cells/mm^3 (Figure 6.5).

Figure 6.5: Proportional contribution to transmission by CD4 count



Sensitivity analyses

Two sensitivity analyses were undertaken. For the first sensitivity analysis, the earliest date of the estimated transmission period was chosen as the estimated infection date. The association with RHI stage again was reduced (rate ratio 1.65) and did not retain its significance (p=0.46), but the relationship with VL did (rate ratio 1.60, p=0.004). For the second analysis, the latest possible infection date from the estimated transmission period was chosen as the infection date. The associations between increased transmission rate and infection category and VL were both retained (rate ratio 3.25, p=0.005 and rate ratio 1.63, p=0.004, respectively).

Table 6.1: Transmission rate (per 100 person-years of follow-up) and factors associated with the 41 infection source individuals of recent HIV infections identified through phylogenetic analysis

	Transmission rate			Univariable analysis			Multivariable analysis		
Factor	Transmissions	PYFU	Rate/100 PYFU	Rate ratio	95% CI	*P*	Rate ratio	95% CI	*P*
Overall	41	6167	0.66	–	–	–	–	–	–
Age group									
<35	28	1502	1.86	4.83	2.35–9.94	0.0001	–	–	–
35–44	10	2592	0.39	1	–	–	–	–	–
>45	3	2082	0.14	0.37	0.10–1.36	0.13	–	–	–
Age (per 5 years older)	–	–	–	0.52	0.41–0.65	0.0001	0.68	0.54–0.86	0.0009
CD4 cell count (cells/μl)									
<200	2	582	0.34	0.49	0.10–2.25	0.36	–	–	–
201–350	9	1269	0.71	1	–	–	–	–	–
351–500	12	1563	0.77	1.08	0.46–2.57	0.86	–	–	–
>500	16	2242	0.71	1.01	0.44–2.28	0.99	–	–	–
Not known	2	521	0.38	0.54	0.12–2.51	0.43	–	–	–
CD4 cell count (per 50 cells higher)	–	–	–	1.00	0.94–1.07	0.96	–	–	–
Viral load (copies/ml)									
<50	2	3176	0.06	0.05	0.01–0.28	0.0005	–	–	–
50–1000	2	482	0.42	0.35	0.07–1.83	0.22	–	–	–
1001–10 000	5	427	1.17	1	–	–	–	–	–
10 001–100 000	15	941	1.59	1.36	0.49–3.75	0.55	–	–	–
>100 000	14	611	2.29	1.95	0.70–5.43	0.20	–	–	–
Not known	3	540	0.56	0.47	0.11–1.98	0.31	–	–	–
Viral load (per log higher)	–	–	–	2.32	1.79–3.01	0.0001	1.61	1.15–2.25	0.005
Infection category									
Recently HIV–infected	11	194	5.67	4.44	2.11–9.33	0.0001	3.88	1.76–8.55	0.0008
Chronic infection, untreated	19	1485	1.28	1	–	–	1	–	–
Chronic infection, treated	2	3556	0.06	0.14	0.01–0.19	0.0001	0.25	0.05–1.30	0.10
Chronic infection, treatment interruption	9	941	0.96	0.75	0.34–1.65	0.47	1.65	0.71–3.82	0.25
AIDS									
No	40	4972	0.81	1	–	–	–	–	–
Yes	1	1204	0.08	0.10	0.01–0.75	0.02	–	–	–
STD diagnosis									
No	31	6016	0.52	1	–	–	1	–	–
Yes	10	160	6.25	12.13	5.95–24.74	0.0001	5.32	2.51–11.29	0.0001
Year									
2000–2001	6	1463	0.41	0.48	0.20–1.17	0.11	0.35	0.13–0.93	0.03
2002–2003	9	1663	0.54	0.64	0.30–1.36	0.24	0.49	0.22–1.09	0.08
>2004	26	3050	0.85	1	–	–	1	–	–

CI, confidence interval; PYFU, person-years of follow-up; STD, sexually transmitted disease.

Table 6.2: Univariate analysis of transmission rate ratio using poisson regression model, generated by infection sources with estimated transmission dates, 2000-2006

Factor		Rate ratio	95% CI	p-value
Age-group	<35	5.37	2.53-11.37	0.0001
	35-44	1		
	>45	0.41	0.11-1.53	0.19
	Per five years older	0.51	0.41-0.65	0.0001
CD4	<200	0.49	0.10-2.25	0.36
	201-350	1		
	351-500	0.99	0.41-2.39	0.99
	>500	1.01	0.44-2.28	0.99
	Not known	0.81	0.22-3.00	0.75
	Per 50 cells per mm^3 higher	1	0.94-1.07	0.93
Viral load	<50	0.07	0.01-0.37	
	50-1000	0.44	0.08-2.42	0.002
	1001-10,000	1		0.35
	10,001-100,000	1.7	0.24-2.04	
	>100,000	2.44	0.56-5.13	0.34
	Not known	0.79	0.8-7.42	0.12
	Per log 10 higher	2.38	1.82-3.11	0.74
Infection category	Recently HIV-infected	4.03	1.88-8.68	0.0001
	Chronic infection, untreated	1		0.0004
	Chronic infection, treated	0.04	0.01-0.19	0.0001
	Chronic infection, treatment interruption	0.75	0.34-1.65	0.47
AIDS	No	1		
	Yes	0.11	0.01-0.77	0.03
STI diagnosis	No	1		
	Yes	12.53	6.13-25.64	0.0001

Table 6.3: Multivariate analysis of transmission rate ratio from infection sources with estimated transmission dates, using poisson regression model, using the earliest possible infection date, 2000-2006

Factor		Rate ratio	95% CI	p-value
Age (per five years older)		0.68	0.54-0.86	0.001
Viral load (per log higher)		1.64	1.16-2.31	0.005
Infection category	Recent	3.06	1.32-7.08	0.009
	Recent, untreated	1		
	Chronic treated	0.26	0.05-1.38	0.11
	Chronic, treatment interruption	1.66	0.71-3.86	0.24
STI diagnosis	No	1		
	Yes	6.07	2.83-12.99	0.0001

6.5 Discussion

Our findings show that HIV infections are disproportionately generated by those who are recently infected, untreated and with a concomitant STI. Furthermore, it seems likely that the majority of infections come from those individuals who are unaware of their HIV infection.

The undiagnosed

We could not ascertain a likely transmitter for 74% of recently infected individuals. With such a high ascertainment of viral sequence data from the study cohort, these data suggest that the largest source of new infections is the undiagnosed population. We cannot, of course, exclude infection derived from other geographical areas of the UK and beyond. On-going work in terms of broader phylogenetic analyses comparing these sequences with those from other MSM in the UK (through the UK Resistance Database [Dunn, 2007] will quantify the degree of cross-transmission between Brighton and other cities in the UK.

Recent infection

We have demonstrated an association between RHI and onward transmission, consistent with modelling, serodiscordant couple studies and supportive of assertions from previous phylogenetic studies [Bezemer, 2010; Brenner, 2007; Yerly, 2001]. This enhanced role of RHI in generating new infections is demonstrated by 11/41 (27%) transmitters being classified as RHI despite RHI only representing 194 out of 6,176 PYFU (2%) of time under follow-up within the study period (a 13-fold increase).

It should be noted that RHI remains independently associated with transmission, suggesting that factors other than higher viraemia also contribute to disproportionate transmission during this disease stage. In particular, these would include behavioural factors such as alcohol, drug use and high risk sex or the viral characteristics of the founder virus that facilitate sexual transmission [Fischer, 2010).

The untreated

We have shown an association between VL and onward transmission, consistent with that expected by biological plausibility and that seen in heterosexual serodiscordant couple studies [Wawer, 2005, Tovanabutra, 2002]. We found use of HAART to be significant in the univariate but not in multivariate analysis, consistent with the hypothesis that the impact of HAART on reducing transmission is mediated through its reduction in viraemia.

The untreated population (containing an uncertain, but elevated proportion of the recently HIV-infected individuals) was responsible for generating approximately half of the transmissions identified. Furthermore, the use of treatment was significantly associated with a reduced risk of transmission. Around 20% of transmissions originated from those currently interrupting treatment. Those with the low CD4 counts (<200 copies/mm^3) were least likely to generate transmission. This could be because such individuals are more likely to be treated (and/or more likely to have symptomatic illness), which may impact on their sexual activity.

Treatment interruption

Nine out of 41 transmitters were undergoing a treatment interruption at the presumed time of transmission. This is consistent with modelling within the Strategies for Management of Anti-Retroviral Therapy (SMART) study [Burman, 2008], suggesting that, unless accompanied by changes in risk behaviour, treatment interruption may be associated with increased onward transmission.

Two of the transmitters identified in this study were classified as having a VL below detection limits at the estimated time of transmission. For one individual, the next available VL was above detection, suggesting that transmission may actually have occurred with detectable viraemia. For the other, there is no evident explanation for apparent transmission while undetectable on HAART. Virological rebound during the interval between undetectable VL or seminal/plasma discordance [Sheth, 2009; Marcelin, 2008] cannot be excluded. Alternative explanations include that the

reconstruction was incorrect or that the most-likely infection source individual was incorrectly identified. Nevertheless, given the large size of the cohort – of whom approximately 70% (*circa* 800) were receiving HAART - the absolute risk of transmission associated with an undetectable VL was low.

Sexually Transmitted Infections

A quarter of transmission sources had an STI diagnosis during the calendar quarter or the previous calendar quarter. Transmitters with estimated transmission dates were more likely to ever have an STI diagnosis during the study period, compared to the rest of the HIV-infected population. This suggests that the transmitters may display riskier sexual behaviours compared with the rest of the population. Others have identified the importance of STI in increasing infectivity [Aral, 2005], as we do. It is not possible in this analysis to determine whether this is a marker of higher risk sexual activity or the result of increased transmission risk due to increased genital HIV shedding.

Sensitivity analyses

The robustness of the association between RHI and onward transmission was examined by two sensitivity analyses, which varied the estimated infection dates of each potential infection source individual (which in the original analysis was the midpoint of the window period of whichever test determined RHI). Varying the length of RHI from this midpoint to the earliest and latest possible date of infection substantially impacted upon the association between infection category and transmission risk.

When a later date was given to RHI (sensitivity analysis 2), there was a higher likelihood that the transmission occurred from an infection source individual with RHI. When an earlier date of RHI is given (sensitivity analysis 1), there was less chance that the transmission occurred from an infection source individual experiencing RHI. This demonstrates that the timing and duration allocated to infection stages need to be meticulously considered in such analyses, as they have the potential to substantially affect results.

Methodological improvements in this study

A better understanding of the relative impact of the biological drivers of the on-going MSM epidemic is urgently required. Previous work has focused on phylogenetic approaches to identify linkages between individuals and groups, but the absence of integrated demographic and clinical data represents a major limitation to interpretation. This study addresses many of these limitations as far as possible and contains a number of methodological improvements.

First, results from the STARHS assay was available for over 90% of individuals to ensure that RHI was maximally ascertained. This meant that we were able to assign variable duration of RHI by specific assay type (date last negative, incomplete western blot, evolving Ab response, STARHS etc.). This enabled precise disease stage evolution – *i.e.* as the study period progressed, a subject would cease to be categorised as RHI and be recorded as a longstanding infection.

Secondly, the search for potential infection source individuals of only the recently HIV-infected enabled the direction of transmission to be ascertained.

Thirdly, the exclusion of every potential infection source individual bar the "most-likely" maximised as far as possible the fidelity of each transmission event, so that the observed proportional contribution of each determinant remained as accurate as possible.

Fourthly the database integrated all clinical, HAART and STI data chronologically over the study period, allowing clinical data and risk attributes of the infection source individual to be matched to the precise calendar quarter of each likely transmission event.

Finally, participants were from a geographically confined cohort where 88% of MSM attend a single HIV clinic for both HIV and STI treatment (SOPHID, 2010; Brown, 2007, *personal communication*). Selection bias was minimised because baseline genotypic testing (>75% coverage) had been routine since the beginning of the study. When baseline genotype specimens were unavailable (*i.e*. if an individual had low or no detectable viraemia throughout the study period), sequences were obtained from proviral DNA.

Limitations of this study

There are four main limitations to this study, in addition to the limitations inherent to phylogenetics described in Section 1.10.2.

First, the focus was on obtaining an accurate estimation of proportional contribution to onward infection rather than absolute numbers. We did not set out to identify all possible transmissions, but only those in which we can be confident about direction of transmission. Therefore, no attempt was made to interpret multiple phylogenetic clusters and we only looked at pair-wise relationships where direction of transmission could be confirmed by excluding all but the single most-likely infection source individual to each RHI individual. While this served to improve the likelihood that the events actually occurred, and therefore relative accuracy, it also served to greatly underestimate transmissions.

Secondly, it was assumed that the Brighton population is closed, with random sexual mixing patterns. The dataset only included those known to be HIV-positive and attending the single clinic. The sequences therefore do not realistically include those undiagnosed in the population or sexual partners not known to the clinic. The dataset might also over-represent certain HIV subpopulations such as the recently infected (due to increased testing frequency or symptomatic presentation for testing). However, this does not affect the observed relative increased contribution of RHI to onward transmission.

Thirdly, the assays used to define RHI varied in their accuracy and range of estimated infection dates. This affected the reconstruction of transmission events, particularly where STARHS was used. Furthermore, the use of calendar quarters led to a loss of accuracy in linking all data to potential transmission events. This was essential to anonymise data and reduce the risk of deductive disclosure.

Finally, we do not have enough behavioural data to allow accurate independent interpretation of transmission rates. As discussed in Sections 1.7.1 and 1.7.4, sexual risk behaviour plays a key role in governing sexual transmission of HIV and is known to vary significantly between different disease stages [Dodds, 2004].

Acknowledgements

Dr Alison Brown (HPA UK), who coordinated the phylogenetic and methodological analysis; Professor Caroline Sabin (UCL), for the statistical analysis; Professor O. Noel Gill and Dr Valerie Delpech (HPA UK), for critical guidance; Dr Gillian Dean (Brighton), who set up the original Brighton database; Dr Kate Nambiar and Stuart Tilbury (Brighton), for maintaining the Brighton database; Professor John Parry & Gary Murphy (HPA UK) and Denis McElborough & Gary Homer (Brighton), for laboratory work.

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Chapter 7

Conclusions

7.1 Thesis summary

- **Identifying and characterising RHI**
- **Determinants of transmission**
    - **Undiagnosed infection**
    - **RHI**
    - **HAART**

7.2 Relevance to treatment as prevention strategies

- **Undiagnosed infection**
- **RHI**
- **Earlier treatment considerations**
- **Adherence**
- **The accuracy of mathematical models**
- **Combination prevention strategies**

7.3 Future Research

7.4 Closing statement

The final chapter describes how this thesis contributes to each research area and how it relates to current prevention strategies. Limitations of each study have been discussed previously in each individual chapter. Potential directions for future research are outlined.

7.1 Thesis summary

The overarching aim of this research was to ascertain the proportional contribution of undiagnosed HIV infection, RHI and longstanding untreated infection to onward transmission, and to determine the degree to which effective HAART reduces transmission. The thesis has two distinct sections. The first (Chapters 2, 3 and 4) sets out to identify and understand RHI and the second (Chapters 5 and 6) to explore the relationship of RHI to other biological factors, within the context of transmission.

In the first section, it was necessary to demonstrate the reliability of STARHS in identifying RHI in order more accurately to quantify the transmission dynamics of HIV infection. We attempted to characterise the relationship between the community burden of resistance and incident TDR, and health-seeking behaviour in RHI.

The second section describes the construction of an irreversibly-unlinked patient database that allowed for the increased accuracy unique to this group's approach. From this, we were able to identify the clinical characteristics of infection source individuals at the time of specific, phylogenetically-confirmed transmission events, and hence ascertain the proportional contribution of the major biological factors to onward transmission.

7.1.1 Identifying and characterising RHI

Identifying RHI

RHI is difficult to diagnose, both in the acute setting and retrospectively for epidemiological purposes. Wider use of tests for recent infection (TRI) as part of routine testing of all new diagnoses will increase our understanding of the dynamics of the epidemic and offer a more accurate and timely measurement of HIV incidence.

Desirable characteristics of a TRI are that it is easy to use, accurate, rapid, with an appropriate window period, with a low false incident rate, useable with different subtypes and not expensive. Our evaluation of STARHS over 10 years has demonstrated that it is a valid and reliable technique when application is limited to subtype B infections and when samples from

individuals with advanced disease or taking HAART have been excluded. Linkage to clinical data is of critical importance.

Newer TRI assays employ different biological principles which are less affected by subtype, advanced disease and HAART. For example in the UK, a guanidine-based AxSYM Ab avidity assay [Suligoi, 2002] is currently being explored by the HPA which, at and avidity index of ≤ 0.75, reproducibly identifies seroconversion within the previous 125 days (95% CI 85-164 days). False incident rate is 3.2% [Chawla, 2007].

The UK is the only country where TRI results are given to the patient.

Characterising RHI

We have shown that, when applied to a specific cohort, STARHS can improve our understanding of RHI as a clinical disease stage.

The persistence over time of genotypic mutations beyond those of acquired resistance, for example (Chapter 3), has provided solid evidence to support the clinical utility of baseline resistance testing of all new diagnoses, which has been included in the BHIVA Guidelines for the care of HIV-infected individuals since 2003 [Pozniak, 2003].

Dunn is conducting further analyses to examine the *relative* persistence of mutations, to explore predictors of persistence (*e.g.* patient VL) and whether persistence of an individual mutation is affected by the presence of other mutations [Dunn, 2011]. Our study group is continuing to develop methods and apply them using next generation sequencing [Shaw, 2012].

However, our attempt to explore the origins of TDR by comparing the community resistance mutational burden of TDR and drug-acquired resistance was rudimentary and lacked methodological sophistication. Not only were there insufficient patient numbers to generate enough specific mutations to be studied but there was difficulty in ascribing accurate values to the mutational burden over time. This concept has been taken further, with additional phylogenetic analysis, by Brown [Brown, 2009a].

STARHS has also enabled the study of RHI as a clinical phenomenon. We demonstrated that half of individuals with symptomatic RHI do not

present to primary health care and of the half that do, a further 50% are not tested for HIV (Chapter 4). Such evidence supports initiatives to increase both public and medical awareness of RHI, and to recommend more emphatically testing for HIV where the population prevalence exceeds 2 per 1000 [BHIVA, 2008].

7.1.2 Determinants of transmission

Chapter 5 describes the pilot study and was limited to the phylogenetic analysis of RHI subjects. This pilot study represented the first use of the phylogenetic methodology on this cohort, although as all the subjects were recently infected we were unable to estimate direction of transmission. The high rates of clustering observed within this pilot study supported the assertion that RHI may be associated with an increased risk of onward transmission and the significant association we found with younger age, high rates of UPAI and sexual partner change identified this as an important group for potential intervention.

In the full phylogenetic study, we have demonstrated how improved and novel methodology, described in Section 6.5, has in many ways overcome the main limitations of previous studies and provided a robust estimation of the proportional relationship between onward transmission and disease stage and HAART status.

Transmission rates were higher in those MSM aged < 35 years, those with VL above 10,000 copies/mL, those with RHI or longstanding untreated infection, and those with CD4 cell counts above 350 cells/mm^3. Rates were dramatically lower in those individuals taking suppressive HAART.

Contribution of undiagnosed HIV to transmission

A most-likely infection source individual could not be identified from within the cohort for 74% of recent infections, suggesting that individuals with undiagnosed HIV represent a major source of onward transmission. Our findings make a clear case for improved HIV testing being of paramount importance in preventing further transmissions and support research into

the treatment as prevention strategies that are currently being modelled and investigated (see below).

Contribution of RHI to transmission

We have emphatically shown RHI to be a highly infectious disease stage - representing only 2% of total follow-up time yet 27% of all transmissions (rate ratio 3.04 compared with 1 from the untreated longstanding HIV-infected). This concords very closely with the most robust empirical data available [Wawer, 2005] and affirms the reliability of our methodology. We have also shown that individuals with RHI are difficult to diagnose and will therefore be over-represented in the pool of undiagnosed infection. When one further considers data that individuals with RHI exhibit behavioural traits that increase transmission, RHI becomes a profound example of biological and behavioural synergy amplifying the risk of transmission.

The high proportional contribution of undiagnosed infection and RHI and the likely synergy between the two states stands as a major barrier to the success of treatment as prevention. This suggests that the reduction in transmission afforded by HAART may be offset significantly by inadequate testing coverage. Universal testing coupled with increased awareness of HIV indicator diseases [BHIVA, 2008] are welcome advances in population HIV testing strategies but are difficult and costly to implement, and might not reduce the undiagnosed pool sufficiently to make a significant difference in incidence (see *community viral load*, Section 1.8.4). Current initiatives should be strengthened and coverage of testing widened, using an opt-out testing strategy wherever possible.

Effect of HAART on transmission

In Chapter 6, the majority of transmissions *from known sources* were generated by the currently untreated population, regardless of infection category - with the risk of transmission from the treated population being extremely low.

Being on suppressive HAART significantly reduced onward transmission, with a rate of 0.06 versus 1.4/100 PYFU for those not on

HAART (either not yet needing HAART, or not adhering to HAART). This finding echoes the findings of many studies and in particular the HPTN 052 which demonstrated that early HAART reduced transmission in discordant heterosexual couples by 96%.

However, 70% of transmissions were generated from patients with a CD4 cell count > 350 cells/mm^3. This is particularly relevant as current UK and European guidelines recommend starting HAART at a CD4 cell count of 350 cells/mm^3, meaning that treatment would have to start at a higher CD4 cell count to be effective at preventing transmissions [EACS, 2011; WHO, 2010; Thompson, 2010; Gazzard, 2008]. The argument for starting treatment at a higher CD4 cell count is strengthened by these findings.

7.2 Relevance to a UK treatment as prevention strategy

Evidence has been accumulating that people with an undetectable VL, most commonly achieved with HAART, are a much less likely to transmit HIV than those who remain untreated. Two pivotal discordant studies - albeit in heterosexual couples - have demonstrated that HIV-positive people who are on HAART are > 20 times less likely to transmit the virus to their partners than people who are not taking treatment [Cohen MS, 2011a; Donnell, 2010]. However until recently, and despite considerable international discussion, there has been little engagement towards achieving a consensus regarding the use of HAART as prevention in UK policy.

7.2.1 Undiagnosed infections

Perhaps the key controversy surrounding treatment as prevention models is that to be successful it requires widespread HIV testing, although the threshold for effective coverage is undefined and variable. The UK is a good example of a country with relatively high rates of testing and very high rates of successful viral suppression in those diagnosed (and engaged with care) but where HIV prevalence, and in key groups incidence, has continued to climb [Delpech, 2012, *personal communication*; HPA, 2011].

The detection of a high proportion of the HIV-positive population in order to assure a sufficient population level reduction in VL remains challenging. A detection rate of more than 90% of HIV-positive individuals outside of clinical trial settings has not yet been reported. Such a level of detection may not be possible without both a strong commitment to testing availability and also a concerted effort to tackle the stigma and perceived or real marginalisation associated with HIV infection [MacPherson, 2012].

Even if less than 90% identification of HIV-positive individuals is achieved, if associated with a high uptake of HAART and high adherence, this may still lead to a larger impact on HIV incidence than any previously tested HIV prevention strategy [Granich, 2010; Garnett, 2009]. However, although encouraging, whilst there is compelling evidence for the effectiveness of treatment as prevention on an individual level, its impact on a population level is as yet unproven. Evidence that widespread HAART coverage is

starting to bring down HIV incidence is hard to find, because the proportion of the HIV population that needs to be diagnosed and treated in order to bring down the community VL sufficiently far to make a difference is so high that it has rarely been achieved anywhere in the world [Das, 2010; Montaner, 2010a].

7.2.2 Recent infections

The real-time and reliable identification of the transient but highly infectious disease stage of RHI is even more difficult, but as demonstrated in this research, achievable.

Since 2009 the HPA has had in place a Recent Infection Testing Algorithm (RITA) incorporating >90 clinical centres and >50 laboratories with good geographical coverage. Its aim is to incorporate TRI as part of the routine public health monitoring of all newly diagnosed HIV infections in the UK, and will also collect data on CD4 cell counts <200 mm^3, AIDS diagnoses and HAART use. In 2009-2010, of 1,426 new MSM diagnoses, 343 (24%) were deemed recent within 6 months [Delpech, 2011].

Potential clinical benefits of a RITA include the possibility of modifying clinical care, better prioritisation of contact tracing (also a public health benefit) and the ability to identify individuals for recruitment into clinical trials of interventions to prevent infection or treat RHI. Public health benefits include a better understanding of how infections are acquired, the ability to calculate incidence estimates (at relatively low cost using a single sample), better targeting of behavioural interventions to groups most at risk of acquiring HIV at that moment in time and more sophisticated evaluation of the impact of HIV prevention measures.

7.2.3 Earlier treatment for population prevention

A number of possible approaches around treatment and population prevention are relevant and have been considered nationally and internationally:

1. Increase the proportion of those diagnosed with HIV who start treatment when clinically recommended (and then adhere)

2. Improve HIV testing to reduce the proportion of people undiagnosed and increase the number of those with HIV who can access treatment when clinically recommended
3. Introduce earlier treatment than currently recommended in clinical guidelines so as to increase the proportion of people with HIV with an undetectable VL
4. Universal voluntary testing and treatment at any CD4 count

Options (3) and (4) above highlight the second key controversy surrounding the earlier treatment of HIV-infected individuals for the explicit purpose of reducing onward transmission – and therefore at higher CD4 cell counts than is current standard of care in almost all countries except the USA (*i.e.* >350 cells/mm^3). At present, UK guidelines on treatment commencement are based on the best available data about individual benefit of HAART initiation at different CD4 cell counts. The 2008 BHIVA HIV Treatment Guidelines already noted that observational studies suggested that there *may* be a benefit to commencing at a CD4 cell count of 500 cells/mm^3 [BHIVA, 2008].

Earlier HIV treatment for population prevention reasons raises important ethical questions. Earlier treatment must be seen to be rational, feasible and not harmful. Whilst concerns over pill burden, side effects and medium-term toxicities have declined as treatment has advanced [Boyd, 2009], there remain uncertainties over the longer-term impact of HAART. If earlier initiation of HAART carries the possibility of a greater risk of longer-term ill-health, through toxicity or drug resistance for example, it does not seem right to recommend earlier treatment simply for public health benefit.

The draft version of the new edition of the 2012 BHIVA HIV Treatment Guidelines recommends that evidence for the effectiveness of HIV treatment as prevention should be discussed with everyone living with HIV" and further, that “the early initiation of treatment to reduce the risk of onward transmission may be appropriate as part of a risk reduction approach for some individuals” [BHIVA, 2012].

Even if it is agreed that an individual has a right to discuss possible risks and weigh them against prevention benefits (whether to a known HIV-

negative regular partner or as yet unmet casual partners), considerations in relation to treatment guidelines for all HIV-infected individuals are very different.

7.2.4 Adherence

Even where there is not considered to be any harm to the patient from starting treatment early, there is the risk of some of those individuals displaying sub-optimal adherence, potentially and paradoxically leading to increased transmission.

Those infected individuals who decline to start HAART, those who are sub-optimally adherent and those who discontinue treatment are likely to contribute significantly to transmission. Moreover, these individuals have a higher chance of transmitting drug-resistant virus. These groups represent a transmission risk that will grow disproportionately if treatment is started earlier at a higher CD4 cell count. Better characterisation and identification of this group, coupled to more effective behavioural interventions to increase adherence and engagement with care (even if small and expensive), could have important consequences on transmission.

Improved monitoring of adherence to HAART and research into the factors affecting adherence are needed to help individuals achieve and maintain an undetectable VL. Across the UK, outcomes should be monitored and published on the proportion of people with HIV who commence treatment when clinically recommended, numbers lost to follow-up, the proportion of people who have an undetectable VL 6 months after commencement of treatment and the overall proportion of people on treatment who have an undetectable VL. The Supporting Uptake and Adherence to Antiretroviral Therapy for HIV (SUPA) trial is currently underway in the UK, an NIHR-funded study that measures these outcomes and tests behavioural interventions to improve adherence. The NHS Outcomes Framework also needs to reflect the importance of achieving and maintaining an undetectable VL for people living with HIV.

7.2.5 Accuracy of mathematical models

Whilst accepting that reducing the proportion of people undiagnosed and increasing treatment uptake and adherence are important for clinical HIV management, the effectiveness of any treatment as prevention strategy on HIV incidence would depend on many other issues, including:

- Uptake and frequency of testing
- Effect of early HAART on individual health
- Proportion diagnosed during RHI
- Adherence to HAART
- Durability of VL suppression on HAART
- Development of drug resistant strains
- Changes in sexual risk behaviour post-diagnosis
- Changes in sexual risk behaviour when viral suppression achieved
- Reduction in infectiousness with HAART in MSM and in relation to
- anal sex, condom and PrEP use

Where assumptions have been made and models used to predict the effect of these variables, conclusions have varied wildly, often requiring much higher and probably unrealistic rates of testing and treatment for populations with high rates of sexual partner change. Model outputs are extremely sensitive to all the variables listed above but arguably sexual risk behaviour in particular. One issue that highlights this sensitivity is the lack of data available to explain whether the often-observed reduction in transmission after diagnosis is due to post-diagnosis behaviour change or the reduction in viraemia from starting HAART. Model output will *always* depend on the quality of data input.

To date, no RCT powered to address the effectiveness and cost-effectiveness of a treatment as prevention approach has commenced. The Preventing Sexual Transmission of HIV with Anti-HIV Drugs (START) trial, which will compare the effects of starting treatment with a CD4 cells count above 500 cell/mm^3 versus waiting until the CD4 cell count is below 350 cells/mm^3 is not due to report for another 2 years [START, 2012].

7.2.6 Combination prevention

As awareness of the preventive contribution of HAART to safer sex increases, there will be a need for clear, accessible and consistent information around complex questions of risk, and there will need to be clear advice to individuals on the benefits and limitations of treatment as part of a wider prevention strategy. This advice will need to include the importance of adherence and risks of treatment discontinuation – in both individual health and transmission contexts - as well as advice on legal issues around criminal liability for reckless HIV transmission.

This need to address the role of treatment in relation to sexual behavioural risk is being addressed by the new BASHH/BHIVA UK National guideline on safer sex advice [Clutterbuck, 2011]. These draft guidelines recommend that discussion with people living with HIV, their sexual partners and those from groups with higher incidence of HIV infection should include:

- Taking effective HAART and having an undetectable plasma VL significantly reduces the risk of HIV transmission
- Even with a routine undetectable plasma VL, a residual risk of transmission is likely to exist and this is likely to be higher for UPAI than UPVI or UPOI
- Transmission risk is increased with reduced HAART adherence or the presence of STIs in either partner. The risks can be reduced by using condoms and having regular sexual health screening

7.3 Future research

This research highlights at least two areas in the current prevention arena for future research and suggests novel research ideas.

7.3.1 Current prevention arena

Better testing coverage

Future efforts to increase uptake of HIV testing will include protocol and strategy interventions, but would also benefit from research into the behavioural aspects of testing. First, testing would ideally be matched to behaviour, rather than an arbitrary testing frequency, and be able rapidly and accurately to detect RHI. Secondly, even if HIV testing were available on every street corner, the rate-limiting step is an individual's motivation to test and understanding this remains a complex goal. There is a significant body of research on the cognitive and emotional response to being diagnosed with HIV (*e.g.* Leventhal's SRM, see Section 1.9.4) which explains variations in health-seeking behaviour such as adherence to HAART and possibly engagement with care [Horne, 2007; Leventhal, 1997]. In the individual who perceives himself or herself to be HIV-negative and is about to test, that response, or illness representation, are probably skewed by even more inaccurate beliefs and fears about being HIV positive than are seen in the diagnosed individual. Understanding how these beliefs influence testing behaviour are critical to develop effective strategies that actually help people achieve independence in their decision to test.

Impact of earlier treatment

Significant effort remains focused on secondary prevention, and in particular the question of which CD4 cell count threshold to start HAART, for individual and public benefit. Recommendations for initiating HAART for individual benefit may change in the near future. We propose a re-analysis of the data from our phylogenetic study, re-stratifying the transmitters into CD4<350, CD4 350-500 and CD4>500 cells/mm^3 to represent the potential change in individual treatment guidelines and to give an idea of the impact of changing the treatment threshold to 500 cells/mm^3.

7.3.2 Novel areas for further research

Social and sexual network dynamics

Almost by definition, the undiagnosed subgroup has been difficult to study. Exploring the dynamics of the social and sexual networks of those who are sub-optimally adherent and disengaged from care might reveal transmission behaviours that overlap with the undiagnosed subgroup. In other words, there is probably a significant fraction within the sub-optimally adherent subgroups that demonstrate the same behavioural characteristics as the undiagnosed population. Network analysis [Drumright, 2010] would shed light on what is arguably the most important HIV transmission group. Questions to ask include: *Who are they? With whom do they associate? What are their personal and behavioural characteristics? Can one predict sexual from social networks?*

Adherence research

The behavioural aspects of HIV transmission remain as critical and as difficult as ever to research. The value of researching adherence to HAART goes beyond reducing morbidity, mortality and infectiousness, however, as adherence to medication parallels self-care and engagement with care. Furthermore, many of the factors that drive non-adherence probably drive other high-risk behaviours such as high-risk sex [Finlayson, 2011; Wilson TE, 2009; Diamond, 2005; Kalichman, 2003; Wagner, 2002], drug and alcohol use [Klein, 2010; Parsons, 2008], and faith or religious fanaticism [Kagee, 2010; Kremer, 2009; Habib, 2009], sharing common roots in cognitive-emotional conditions such as low self esteem, anxiety and fear of social disconnection or shame [Moscowitz, 2011; Pence, 2008; Whetten, 2008; Brown B, 2006; Stein, 2005].

These behaviours are culturally specific and vary between individuals, and often in the same individuals over time. Understanding the beliefs that underlie these behaviours will come from qualitative interviews and robust, evidence-driven psychological research - without which even the most potent biological interventions may fail to be effective.

7.4 Closing statement

There are no clearly demonstrated strategies that halt or effectively slow HIV epidemic growth. Although HAART has demonstrated massive potential to prevent sexual transmission, for it to be effective on a population level infection source individuals need to be maximally identified and targeted. We set out to quantify the proportional contribution to onward transmission of individuals with undiagnosed and recent HIV infection, which appears greater than previously estimated. We have also observed that HAART considerably reduces transmissions in an uncontrolled cohort, which gives optimism in line with some current research findings. However HAART will be only one element, albeit a critical one, of a combination prevention strategy that must follow an evidence-based, biopsychosocial approach [Engel, 1977]. This approach will ideally address behavioural change (HIV testing, adherence and sexual risk behaviour) as well as incorporating a variety of biomedical strategies, supported by a coordinated political and social consensus.

37, 085 words

David Pao

July 2012

APPENDICES

Appendix A: **STUDY PROTOCOL Version 1.2 (January 3, 2003)**
A study to determine the evolution of primary drug resistance mutations in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)

Appendix B: **STUDY PROTOCOL *Version 1.2 (January 2, 2003)***
A study to determine the similarity of viral sequences between individuals recently infected with human immunodeficiency virus type 1 (HIV-1)

Appendix C: **STUDY PROTOCOL *Version 1.0 (November 8, 2006)***
The use of virological, clinical and epidemiological parameters in understanding the dynamics of HIV-1 transmission in men who have sex with men

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STUDY PROTOCOL
Version 1.2 (3 January 2003)

A study to determine the evolution of primary drug resistance mutations in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)

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Hypothesis

HIV-1 genotypic mutations in patients with *primary* antiretroviral drug resistance may persist into the chronic stage of the disease, suggesting the need for resistance testing before treatment initiation at all disease stages.

Background

The use of highly active antiretroviral therapy (HAART) in patients infected with human immunodeficiency virus type 1 (HIV-1) reduces morbidity and mortality[1-3] and often results in substantial recovery of impaired immunologic function[4,5]. With time on treatment, the evolution of (*secondary*) drug-resistant virus poses a significant challenge to effective pharmacological intervention by adversely affecting both the magnitude and duration of treatment response[6]. Transmission of drug-resistant virus to another individual has been recognised for more than a decade and leads to what is known as *primary* drug resistance in the recipient host. Whilst primary drug resistance is not thought to affect the natural course of the disease, it does have an adverse effect on both the magnitude and duration of treatment response.

A number of cohort studies have shown steady increases in the prevalence of primary drug resistance in the UK[7] and elsewhere (for example, in one study from the USA the prevalence of primary drug resistance increased from 16.7% to 27.6% between 1996 and 2001[8]). Local unpublished data from Brighton demonstrates a prevalence of 33%. Increases have not been observed in France, Switzerland or Spain, however, and are believed to be a result of new HIV diagnoses occurring among immigrants from countries with less access to HAART.

It is well established that *secondary* drug resistance mutations acquired in individuals as a result of HAART revert to original or "wild-type" after treatment (and hence selection pressure) is withdrawn. It is not clear, however, whether this same reversion will be seen and whether, if it does, at what rate this will happen, in individuals with primary drug resistance. Investigating studies have been small, cross-sectional and have used different definitions for genotypic resistance. One study has reported a lower prevalence of primary drug resistance in established versus recent infection[9], but another did not confirm this observation[10]. The only longitudinal data, from just six patients[11], shows baseline primary drug resistance in 33% of cases, with persistence of NNRTI drug resistance mutations to just under a year and persistence of NRTI and PI drug resistance to just over a year.

The development of automated assay technology for rapid genotypic characterisation of HIV now makes it feasible to use these assays in the management of HAART. Information from this study will help to clarify the relevance of resistance testing in individuals at *any disease stage* prior to commencing HAART, for which current treatment and resistance guidelines are poorly informed Additionally, it may help to determine the potential significance of individuals with primary drug resistance in determining the *subsequent* frequency of further cases of primary drug resistance.

Method

Individuals (registered at the Lawson Unit from 1995 to date) identified as having primary drug resistance (as part of routine clinical practice) by the following criteria:

(a) *recent* HIV-1 infection by *one* of the following criteria:

- Previous negative HIV antibody test within 18 months
- Positive HIV-1 antibody test *in association with* a negative "detuned" HIV antibody assay (which is suggestive of infection within the previous 5-6 months)
- An evolving Western Blot or HIV antibody response

and

(b) evidence of primary drug resistance mutations from first visit blood specimen (test already performed)

A retrospective and longitudinal analysis of HIV-1 viral isolates for genotypic drug resistance mutations from samples obtained during routine clinical practice. An estimated 25 subjects will be eligible for inclusion. All specimens will have been collected / will be collected at routine venesection (as per clinical practice) at the Lawson Unit or Elton John Centre in Brighton. For individuals from whom informed consent has been obtained, an HIV genotypic test will be performed on the most recent available specimen (this blood specimen will have already been taken) and compared to the initial specimen as described in (b) above. If there is seen to be partial or complete loss of resistance mutations, an HIV genotypic test will be performed on intermediate specimens to determine the nature and chronology of this evolution. If, however, the most recent specimen indicates persistence of resistance mutations, a further specimen at a later date will be analysed. This process will be repeated until all resistance mutations have disappeared or until the subject withdraws informed consent. If the subject subsequently commences HAART their response to therapy will be monitored prospectively and further genotypic testing performed in accordance with treatment guidelines and compared to those obtained as part of this study.

Individuals will be identified by their confidential clinic number only, and will already be aware of their resistance status. The individual will be given their results, if requested. The subject's general practitioner will be informed of their participation in the study, only if permitted to do so by the subject. The overall results of the study will be made available to participants at its conclusion.

Data Analysis:

Specimens will have been spun / will be spun and stored at the Microbiology Department in Brighton, as per current routine clinical practice. The relevant specimens will have been batched / will be batched and sent to the Antiviral Susceptibility Unit in Birmingham where genotypic testing will be performed at the accredited laboratory (laboratory protocol available).

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Patient Information Sheet

Study title: **A study to determine the evolution of primary drug resistance mutations in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

1. What is the purpose of the study?

The use of antiretroviral treatment has meant people with HIV live healthier and longer lives than ever before. Unfortunately, treatment combinations become much less effective with time because the virus *changes* (evolves) to become drug resistant (known as "*secondary* drug resistance"), the drug stops working and the patient's condition can deteriorate. It is now known that a patient does not even need to have taken treatment in order to develop drug resistance, as in up to a third of cases they may be infected *in the first place* with a resistant virus (this is known as "*primary* drug resistance").

When we analyse blood specimens from patients who have this drug resistance, we can see tiny changes in the genetic structure of the virus, which we call "drug resistance mutations". The results of this "resistance test" can tell us which drugs will work, and which will not.

If the drug resistance comes about because of treatment (secondary drug resistance), the drug resistance mutations disappear quickly from the blood when the treatment is stopped, although that same treatment will not work again. Recent evidence has shown that in the case of primary drug resistance (where the virus is drug resistant in the first place), the drug resistance mutations can stay in the blood for a much longer time (maybe for more than one year). Clearly, if this were to be the case, patients who have been infected for some time would benefit from resistance testing so the appropriate drugs could then be started, to which the virus will respond, and so the drugs will be effective for longer. Long term therapy can then be individualised to ensure maximum efficiency and durability of therapy for the patient.

The aim of this study is to find out how long primary drug resistance mutations persist in blood.

2. Why have I been chosen?
You are being invited to take part in this study because you were known to have recently acquired HIV when you first attended the Lawson Unit and have primary drug resistance already present.

3. Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect in any way the standard of care you receive.

4. What will happen to me if I take part?
No extra clinic visits, questionnaires or time will be required from you.

We will analyse your latest blood sample (which has already been taken) to see if the mutation(s) which we found from your first ever visit has disappeared or changed. If it has, we will look at a sample taken from a time in-between these two samples to see more clearly how they have changed over time. Some sections of your medical notes will be looked at for basic information such as your CD4 count, viral load and treatment history.

If we find that the same resistance mutations are still present in both the earliest and latest samples, we would like to continue to look for them in subsequent samples we take (if this is necessary, we would like to take an extra two teaspoons of blood from you during routine blood taking, which will be stored in the laboratory.) Taking part in this study will not affect your care in any way. For example, if you do need to start antiretroviral treatment at any stage, you can continue on the study (although the information we have obtained in the study may be useful and may help us with your treatment choice).

5. What are the possible disadvantages and risks of taking part?
None.

6. What are the possible benefits of taking part?
Your participation will increase our knowledge of how best to choose HIV treatment combinations.

7. Will my taking part in this study be kept confidential?
Yes, your confidentiality will be respected at all times during and after the study
.

8. What will happen to the results of the research study?
Your individual results will be given to you, if you wish. Your general practitioner will be informed of your participation in this study, with your permission. The overall results of the study will be made available to you at its conclusion and may be published in a medical journal (your confidentiality will be respected at all times).

Brighton and Sussex University Hospitals NHS Trust

CONSENT FORM

Title of Project: A study to determine the evolution of primary drug resistance mutations in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)

Patient Identification Number for this trial:

Name of Researcher: Dr Martin Fisher

1. I confirm that I have read and understand the information sheet dated 27 November 2002 (version 1.1)for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

1. I understand that sections of any of my medical notes may be looked at by researchers ☐

   from the Lawson Unit as is relevant to my taking part in research. Information will remain strictly confidential. I give permission for these individuals to have access to my records.

4. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

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STUDY PROTOCOL
Version 1.2 (2 January 2003)

A study to determine the similarity of viral sequences between individuals recently infected with human immunodeficiency virus type 1 (HIV-1)

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Other local investigators: Dr Gillian Dean
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Antiviral Susceptibility Unit
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Birmingham
(Responsible for the viral sequencing analaysis)

Dr John Parry
Central PHLS
Colindale

Hypothesis

There is viral genetic similarity or association between individuals recently infected with HIV-1, as a result of high transmission rates in this group.

Background

Despite a decline in the mortality and morbidity in individuals infected with human immunodeficiency virus (HIV) since the introduction of highly active antiretroviral therapy (HAART), the incidence of *new* infections has increased[1]. Furthermore, rates of transmission of resistant HIV strains (which compromise treatment success[2]) are up to 28% of cases in many countries[3,4]. Some of the highest rates occur in the UK[5], in particular from this treatment centre.

It is unclear what is driving this increase in new cases. These new cases are often assumed to have occurred in other parts of the world, particularly in the African and Asian continents, but this assumption can hide the smaller growing epidemic of new infections occurring within the UK. From epidemiological data, we can see that most new infections in gay men have occurred in this country[6], and there is growing evidence that these cases of new infection initiate a chain of sequential *exponential* infections:

1. Mathematical modelling indicates that eliminating contagiousness in early infection has more effect than eliminating contagiousness during any other disease stage. For example, a 5% reduction in contagiousness in high-risk groups (i.e. early infection, see (2) below) could decrease the total infection in the population by 28%[7]. These models suggest that growth of an epidemic from transmission in the later stages of disease is less likely, and that early infection is the core factor in the propagation of an epidemic.
2. Individuals with early infection have a much higher plasma HIV viral load, which generally correlates with genital tract viral load, and are up to 1000 times more infectious than during any other stage of disease[8,9]. In addition, these individuals may show no symptoms of disease and so be unaware of the risk they pose to partners, often engage in high-risk sexual practices[10,11], with a higher number of sexual contacts[12], and have a higher rate of sexually transmitted infections (STIs) which increase HIV transmission probability still further[13].
3. Reports from Switzerland and the USA have suggested that a significant proportion of new HIV infections have been acquired from individuals who themselves have early infection. In one study of 197 cases from Switzerland[14], genomic "clustering" was seen in 29% of cases indicating a positive relationship between viruses.

The above evidence does not, however, provide us with complete knowledge of HIV transmission systems within a population. The mathematical modelling in (1) requires specific data regarding behavioural and social factors to confirm theory applies in practice. The work done on HIV relationships (3) does not tell us important information, for example whether the clustering is the result of rapid, serial transmissions or multiple, parallel infections by core transmitters. Nevertheless, similar cluster genotyping studies have been used successfully in

tuberculosis outbreaks in the USA[15,16], leading to significant changes in public health control practices. Only when this information is available will cost-effective ways to implement transmission interruption and control strategies be possible.

The aim of the study is to ascertain the extent to which virus from individuals with proven recent HIV infection is genetically similar. In addition, limited and non-traceable epidemiological data will be collected. The **unique** position of Brighton HIV Services in investigating these factors cannot be emphasised strongly enough. There is a relatively high incidence and prevalence of HIV in what is essentially a **single treatment centre**, enabling a better interpretation of genetic similarity without the confounding influence of large numbers of individuals approaching different treatment centres for their care. Furthermore, a reliable system of epidemiological data collection already in place can provide much of the information needed.

It is anticipated that the findings of this study will help guide health promotion strategies, both locally and nationally, and determine the extent to which HIV testing should strive to reduce the amount of undiagnosed infection in the community.

Method

Individuals (registered at the Lawson Unit from 1999 to date) will be selected who have been identified (as part of routine clinical practice) as having *recent* HIV-1 infection by *one* of the following criteria:

- Previous negative HIV antibody test within 18 months
- Positive HIV-1 antibody test *in association with* a negative "detuned" HIV antibody assay (which is suggestive of infection within the previous 5-6 months)
- An evolving Western Blot or HIV antibody response

An estimated 150 subjects will be eligible for inclusion. Such individuals will have hade an HIV genotypic test already performed as part of routine clinical care (as per national antiretroviral therapy and resistance testing guidelines). No extra blood specimens will be required. For individuals from whom informed consent has been obtained, the sequences from these genotypes will be further analysed at regions of the virus with different degrees of conservation, which are not those currently associated with drug resistance. The sequences between subjects will then be compared for similarity.

The following information will be linked to each sequence:

- Year and quarter of diagnosis (and of infection if known)
- Information regarding clinical status: CD4 count, viral load, presence or absence of symptoms and signs suggestive of HIV-1 seroconversion illness, presence and nature of concomitant sexually transmitted infections
- Information relating to the subject's sexuality and estimated number of sexual contacts in the 3 months prior to diagnosis. (No other data will be collected, to ensure absolute anonymity is preserved.)

Confidentiality and anonymity will be highly protected, as follows. Individuals will be identified by a unique *study number* and *clinic number* only. These numbers will be irreversibly unlinked. Brighton HIV Services will be unaware of the identity of any of the *study numbers* and the genomic sequence patterns associated with them, or any individual or relationships between them. Conversely, the persons performing the genomic sequence analysis will not be aware of the *clinic number* of each specimen. Specimens will be randomly re-coded into *subject numbers* using a "firewall" system managed by the public health laboratory. All computers are registered and comply with the Data Protection Act.

The individual results will not be able to be given to subjects since it will not be possible to link them (as above). The subject's general practitioner will be informed of their participation in the study, only if permitted. Neither the general practitioner nor Brighton HIV services will be able to be informed of the individual subject results. The overall results of the study will be made available to participants at its conclusion.

Data Analysis

All specimens will have been already collected at routine venesection (as per routine clinical practice) at the Lawson Unit or Elton John Centre in Brighton. Specimens will have been spun and stored at the Microbiology Department in Brighton (as per current routine clinical practice). The relevant specimens will have been batched and sent to the Antiviral Susceptibility Unit in Birmingham where viral sequence analysis will be performed at the accredited laboratory (laboratory protocol available).

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4. Brun-Vesinet F. **BHIVA Resistance Meeting, London, 2002.**
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8. Jacquez JA, Koopman JS, Simon CP, *et al*. **Role of the Primary Infection in epidemics of HIV infection in gay cohorts.** *J Acquir Immune Defic Syndr* 1994; 7: 1169-84
9. Chakraborty H, Sen PK, Helms RW, *et al*. **Viral burden in genital secretions determines male-to-female sexual transmission of HIV: a probabilistic empiric model.** *AIDS* 2001: 15(5); 621-7
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14. Yerly S, Vora S, Rizzardi P, *et al*. **Acute HIV infection: impact on the spread of HIV and transmission of drug resistance.** *AIDS* 2001; 15: 2287-92
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Patient Information Sheet

Study title: **A study to determine the similarity of viral sequences in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

1. What is the purpose of the study?
We are seeing a worrying increase in the number of new cases of HIV infection diagnosed in the UK, and the reasons for this are not clear. The aim of this study is to find out which factors contribute to this increase, and to use this knowledge to help stop more people becoming infected and in turn reduce the growth of the HIV epidemic. What we know so far suggests that patients *recently* infected with HIV (i.e. within 18 months) are much more likely to transmit the virus to another person (when there is a much higher level of HIV in the semen or vaginal secretions). This perhaps begins a chain of infection that grows wider and larger with time. Crucially, most people with early HIV have no indication that they are infected and feel perfectly well, and so will not be aware of the risk of infecting others. Thus, recognising people who may be at risk of early HIV infection may be very useful in preventing the transmission of HIV and thus prevent a much larger number of people being infected.

2. Why have I been chosen?
You are being invited to take part in this study because you were known to have recently acquired HIV at the time you first attended the Lawson Unit.

3. Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. A decision not to take part will not affect in any way the standard of care you receive.

4. What will happen to me if I take part?
No extra clinic visits, blood tests, questionnaires or time will be required from you. We will look at your medical notes for some information, but this information will not reveal your identity.

A single blood specimen, which we took from you on your first visit, will be analysed for this study. The virus contained in this blood was analysed* to identify any potential resistance you may have to anti-HIV drugs, which can help in deciding the best treatment choice for you, if and when you need to start HIV treatment. We would simply like to look for similarities and differences that may exist between different individuals' virus samples. We should have completed the testing of all samples by July 2003.
*known as "genotypic resistance testing"

5. What are the possible disadvantages and risks of taking part?
None.

6. What are the possible benefits of taking part?
Your participation will increase our knowledge of how HIV is transmitted within a population and will help us to develop ways of slowing or halting the epidemic.

7. Will my taking part in this study be kept confidential?
Yes, we can guarantee that you will remain unidentifiable at any time during the study or after study completion. Confidentiality and anonymity will be highly protected, as follows. Your blood sample will be identified by a unique *study number* (known only to the laboratory) and *clinic number* (known only to the clinic). These numbers will be *irreversibly unlinked* (nobody will ever be able to connect the specimen to your clinic number, name or any other marker of your identity). In other words, members of staff at the GUM department will be unaware of the identity of any of the fingerprint patterns for any individual or relationships between them. Conversely, the persons performing the fingerprint analysis in the laboratory will be unaware of the personal identity of any specimen. We do not need to look at or identify "actual" people as such, we just need to look at or identify a pattern or network which can remain completely anonymous. The information we collect from the notes will be minimal and in no way relate to your identity, either directly or indirectly.

8. What will happen to the results of the research study?
The individual results will not be able to be given to you, as it will not be possible to link them (as above). Similarly, neither Brighton HIV services nor the laboratory will be able to be informed of your results, as it will not be possible to link them. The overall results of the study may be published in a medical journal, and will be made available to you at its conclusion.

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CONSENT FORM

Title of Project: **A study to determine the similarity of viral sequences in individuals recently infected with human immunodeficiency virus type 1 (HIV-1)**

Patient Identification Number for this trial:
Name of Researcher: Dr Martin Fisher

1. I confirm that I have read and understand the information sheet dated 27th November 02 (version 1.1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of my medical notes may be looked at by researchers from the Lawson Unit as is relevant to my taking part in research. I understand that my identity will remain unknown to the researchers at all times, up to and including the conclusion of the research. No data collected from the notes will relate either directly or indirectly to my identity. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

RESEARCH PROTOCOL

Title: The use of virological, clinical and epidemiological parameters in understanding the dynamics of HIV-1 transmission in men who have sex with men	
VERSION NUMBER :	1.0
DATE :	08/11/2006

Investigators:
Darshan Sudarshi
Alison Brown
Deenan Pillay
Caroline Sabin
David Pao
Gillian Dean
Noel Gill
Jonathan Clewley
Anne Johnson
David Dunn
Martin Fisher (Chief Investigator)

1. BACKGROUND

1. Why research this area?

A pivotal objective of the National Strategy for Sexual Health and HIV[1]is to reduce ongoing HIV transmission. Whilst most new diagnoses in the UK originate from heterosexual men and women from countries of high endemicity, the majority of transmission within the UK is thought to occur amongst men who have sex with men (MSM). Annual HIV incidence amongst MSM was measured at 3% in England, Wales and Northern Ireland in 2004[2,3]. Data from Brighton shows that from 2002 to 2005, more than half of new diagnoses among MSM were from individuals who had been infected within six months indicating substantial transmission at a local level[4].

2. Factors effecting transmission

Several factors have been postulated to affect the likelihood of onward transmission. As a disease stage, primary HIV infection (PHI) is thought to represent a period of increased infectiousness[5,6] and mathematical models have estimated that between 25 and 47% of new infections may be attributed to PHI[7,8]. It is also well recognised that a large proportion of HIV infection remains undiagnosed (approximately one third of HIV-infected MSM in the UK are thought to be unaware of their infection)[2]. These undiagnosed individuals may represent an important source of new infections[9]. In addition, increasing sexual

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risk behaviours[10], presence of a concomitant sexually transmitted infection (STI)[11], access to antiretroviral therapy (ART)[12], are all thought to influence transmission.

Furthermore, a substantial proportion of newly diagnosed individuals have virus that is already resistant to one or more existing antiretroviral agents (known as transmitted drug resistance)[13,14,15]. It remains undetermined whether these transmissions individuals originate from individuals exposed to ART, or from individuals who have never been exposed to ART.

3. Previous Studies

Phylogenetic methodology (analysing the evolutionary relationships that exist between genetic sequences) has been previously used to investigate HIV transmission. Yerly *et al* used such an approach to demonstrate substantial clustering of viruses between individuals with PHI in a Swiss cohort[16]. In addition, Pao *et al*[17] conducted a phylogenetic analysis among sequences from MSM diagnosed with PHI. Whilst several transmission clusters and clinical correlates were identified, the dataset only comprised MSM diagnosed with PHI, and therefore no comparison was made with other disease stages.

We aim to further this research through obtaining a comprehensive collection of sequences from diagnosed HIV-infected MSM from one geographic locality. Sequences from individuals diagnosed with chronic HIV infection will be obtained

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so the relative importance of PHI in generating onward transmission can be estimated within a broadly realistic context.

4. Importance of research

Better understanding of the factors that influence transmission, will help focus prevention strategies and enable targeting of those groups at highest risk of transmission[1819].

5. Why Brighton?

The HIV/GUM department in Brighton is well placed to undertake the proposed research. This is because it is the single provider of HIV/STI care, (managing over 90% of locally infected HIV individuals[20] in a geographical area) allowing study of a comprehensive sexual network. Trends in both HIV and STI epidemiology in Brighton mirror those of other urban areas in the UK, so results may be generalisable[21].

2. HYPOTHESES:

a. Individuals with primary HIV infection, may be contributing disproportionately to onward transmission
b. Undiagnosed HIV infection (primary and chronic) may be an important source of new infections.
c. ART may impact on transmission
d. Co-infection with an STI may be facilitating transmission
e. A substantial proportion of transmitted drug resistance may originate from drug (ART) naïve individuals who were infected with resistant virus themselves

3. RESEARCH QUESTIONS:

1. Primary research question:

What factors are important in generating HIV transmission among men who have sex with men?

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2. **Secondary research questions:**

1. What impact does disease stage have on transmission?
2. What is the role of undiagnosed infection (primary and chronic) on transmission?
3. How does ART impact on transmission?
4. To what degree is co-infection with an STI associated with transmission?
5. To what extent do individuals infected with resistant viruses (transmitted drug resistance) cluster with other individuals with resistant viruses?

4. METHODS:

1. Study design

- The study will involve a population based approach that combines epidemiology with phylogenetics[22]. We intend to create a database of HIV genetic sequences from HIV-infected men who have sex with men (MSM) attending Brighton HIV/GUM clinic between 2000-2005.

- We will use laboratory algorithms[23] to categorise patients into those who have been recently infected and those that have a chronic infection.

- We will then analyse the evolutionary relationships that exist between HIV DNA/RNA sequences by construction of a phylogenetic tree.

- Through statistical analyses we will assess the impact of various clinical factors on transmission.

2. Inclusion criteria:

MSM diagnosed with HIV-1 infection who are under the care of it HIV clinic and seen at least once between 2000 and 2005. MSM must have been aged at least 16 at diagnosis.

3. Exclusion criteria:

MSM in group 3 who refuse consent (see section 4.4.) will be excluded.

4. Subjects:

To facilitate phylogenetic analysis, HIV-1 polymerase (*Pol*) sequence data will be obtained for all our study subjects (total = 1306). Study subjects will be divided into the following three groups, based on availability of their sequence data:

i. Individuals, from whom we already have *Pol* sequence data available. (n~850)

Since 2000, the British HIV Association (BHIVA) has recommended routine antiretroviral resistance testing on all new diagnoses of HIV infection[24]. Therefore *Pol* sequence data is already available for all of this group.

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ii. **Individuals who have a blood sample stored, from which *Pol* sequence data will be obtained through genotypic testing. (n~50)**

Approximately 50 patients have stored blood samples taken around the time of diagnosis that have not yet been tested for antiretroviral resistance. For these patients, the samples will be sent to the Health Protection Agency for sequencing for routine resistance testing according to BHIVA guidelines[24].

iii. **Individuals who have never had a blood test taken for genotypic testing. (n~400)**

These patients do not have stored blood samples taken around the time of diagnosis. They were generally diagnosed before BHIVA guidelines[24] were put into effect.
For these patients, informed consent will be obtained to take a new blood sample (10ml) for sequence based resistance testing. This group can be further divided into
two sub-groups:

- For patients that have a detectable viral load (VL>1000) blood will be taken from which plasma RNA will be sequenced.

- For patients with an undetectable viral load (VL < 1000), blood will be taken from which proviral DNA will be sequenced.

5. **Procedures and tests:**

For groups 1, 2 and 3:

During each year of the study period (2000-2005) the following information will be collected from the HIV clinical database:

i. ART (Y/N)
ii. If (i) was Y, then classification of their viral load into one of the following categories (VL < 1000, VL>1000, VL > 50000)

iii. Any evidence of STI (one or more of the following: gonorrhoea, chlamydia trachomatis, non-specific urethritis, primary syphilis, primary genital herpes simplex and Trichomoniasis)

iv. Stage of HIV infection:

a. **acute infection within 6 months**:
Defined if any of the following are present:

i. Positive HIV Antibody + previous negative HIV test within 6 months
ii. Negative HIV antibody in association with positive RT-PCR or p24 Ag
iii. Evolving Western Blot or HIV antibody response

iv. Serological testing Algorithm for recent HIV seroconversion (STARHS) assay suggestive of recent infection.

b. **semi-acute infection**
   i. Positive HIV Antibody + previous negative HIV test within 6-12 months + STARHS not suggestive of recent infection.

c. **established infection**
   i. Positive HIV Antibody + (previous negative HIV test >12 months or no previous HIV test) + STARHS not suggestive of recent infection

For group 3 only:
Written, informed consent will be obtained from study subjects in Group 3, by the patients regular clinic doctor.

- The following will be collected or performed:
  - An extra 10 mls of blood

For groups 2 and 3 only:
- Blood will be spun and stored at the Virology Department at Brighton and Sussex University Hospitals (BSUH)
- Blood samples will be sent to the Health Protection Agency for genotypic testing

5. PROPOSED ANALYSIS:

1. Phylogenetic analysis

i. *Pol* sequences from all individuals will be used to construct a phylogenetic tree thus allowing classification by whether or not each appears within a suspected transmission cluster (a group of 2 or more individuals with phylogenetically-related viruses)

ii. The relative contribution of the HIV disease stage, ART, STI (obtained from the clinical data) in phylogenetic clustering will then be assessed.

iii. Any ART resistance found within a cluster will be analysed according to whether the individual concerned is drug experienced or drug naïve.

iv. Utilising estimates of undiagnosed HIV prevalence (from the Brighton Unlinked Anonymous community survey), the potential importance of undiagnosed infection within transmission clusters will be considered.

2. Statistical analysis:

Statistical comparisons of those in a cluster with those not in a cluster will be performed using chi-squared tests, Fisher's exact tests or Mann-Whitney U tests,

as appropriate. Multivariable logistic regression will be used to identify factors independently associated with belonging to clustering. All statistical analyses will be performed using SAS version 8. This will be performed with guidance from Professor Caroline Sabin at the Primary Care and Population Sciences, UCL

6. ETHICAL CONSIDERATIONS:

1. Consent:

Consent is not sought from groups 1 and 2. Samples from group 1 have already been obtained for clinical purposes and sequenced for antiretroviral resistance testing purposes Samples from group 2 have already been taken, and will be sent for sequencing as part of testing for antiretroviral drug resistance [according to BHIVA guidelines[24]].

Consent will be sought from group 3 since stored sera is not available for this group - we will be taking an extra sample of blood (10ml) for research purposes. Written informed consent will be obtained by the patient's regular clinic doctor, and patients will be provided with a patient information leaflet explaining the research. The patient will be given the choice of having the blood taken at the clinic visit or if they require further time to consider it, they can choose to have their blood taken at their next routine clinic visit.

For all three groups, if HIV sequences are found to contain drug resistant mutations, results will be fed back to the patients since this may affect their clinical care.

Our study will use data, which is already present in two existing national databases.
The *Pol* sequence data is collected as part of the UK resistance database
and the clinical data is obtained as part of the UK Collaborative HIV Cohort database. Both of these databases form part of studies, which already have ethical approval and consent was not required (MREC/00/7/47and MREC/01/2/10).

2. Anonymisation:

Data will be anonymised from any patient identifiers before phylogenetic analysis.

The custodian of the data will remove patient identifiers and allocate each record with an anonymised study number. The epidemiological and clinical data will be reconfigured to prevent the recognition of any patient. A minimum cell size of 20 will be employed for each variable both individually and in combination (i.e. at least 20 individuals will fall into any one category). At this stage, the anonymised dataset will be passed on for phylogenetic analysis. No cross-referencing between patient and study ID will be made.

The same anonymisation procedure has been used in a similar study previously conducted by this team[17]. Such methods are consistent with section 28 of the Human Tissue Authority Codes of Practice.

7. PUBLIC ENGAGEMENT WITH SCIENCE:

We have discussed this proposal with providers of HIV prevention (Terrence Higgins Trust, South and London). We have utilised our existing service user representation within the department to ensure that the process of subject recruitment and maintenance of confidentiality will be acceptable to the local patient group. We will include both parties within ongoing steering group discussions.

8. DISSEMINATION OF RESULTS:

Information and results will not only be disseminated through conventional channels (national and international HIV/STI conferences and peer-reviewed journals) but also to other health care professionals and relevant organisations. We have previously disseminated research findings to the local MSM community at both local and national HIV/sexual health organisations (for example, CHAPS initiatives)

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