DIAGNOSTIC VIROLOGY – EXCESSIVE EXPENDITURE OR ESSENTIAL CONTRIBUTION TO HEALTH CARE?

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Diagnostic virology – excessive expenditure or essential contribution to health care?

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Wolfgang Preiser was born in Frankfurt am Main, Germany, in 1965. He studied medicine at the Johann Wolfgang Goethe University in his home town and at University College London. After graduating in 1991 he completed a diploma in Tropical Medicine and Hygiene at the London School of Hygiene and Tropical Medicine in 1992 and then commenced his specialist training in medical virology under Prof. Hans Wilhelm Doerr at the Institute for Medical Virology in Frankfurt. From 1995 to 1999 he continued his specialist training as honorary senior registrar and lecturer under Prof. Richard S. Tedder at the Department of Virology, University College London Medical School. During this time he came to South Africa for the first time to spend several weeks at the University of Cape Town and the then National Institute for Virology. After qualifying as Member of the Royal College of Pathologists (MRCPath), London, in 2000 and having been awarded his first doctoral degree (Dr. med.), he subsequently returned to Frankfurt as consultant virologist to complete his second doctoral degree (Habilitation, Dr. med. habil.), which was awarded in 2005.

His principal research interests are the development, improvement and evaluation of novel methods for virological laboratory diagnosis, particularly in HIV-infected patients. He works on quantitative viral genome detection, the measurement of virus-specific cellular immunity and the detection of antiviral resistance-associated mutations, as well as operational and epidemiological problems.

He also has a keen interest in tropical and emerging viral diseases, having done research on the diagnosis and epidemiology of tropical, zoonotic and arthropod-borne viruses, such as SARS, dengue and yellow fever. He served as a temporary advisor to the World Health Organization on SARS in China during 2003 and led the Frankfurt group who, together with colleagues from Hamburg, was among the first to isolate the SARS-associated coronavirus in spring 2003.

Having come back to South Africa many times in the years following his first visit in 1998, both for pleasure and for work, he applied for and gladly accepted the position as professor and head of the Division of Medical Virology, Faculty of Health Sciences, Stellenbosch University / National Health Laboratory Service on the Tygerberg campus in 2005.

But it is not only the country itself he has fallen in love with: Wolfgang married Rika (a Suid-Afrikaanse meisie) in 2007 and the family is about to grow ...
I dedicate this paper to my family, friends and colleagues who have accompanied and helped my professional development and who have tolerated me for all these years.

The colleagues without whose help I would not be delivering my inaugural lecture today are too numerous to be listed individually, but they will know who they are when they read this!

I particularly want to honour my two mentors, Professor Hans Wilhelm Doerr and Professor Richard S. Tedder. I am realising more and more how much they have influenced me (and how right they were with most things they said, even if at the time that did not seem so!). I am continuing the practice of clinical virology in their footsteps and have taken their 'schools' into the next century and onto another continent.

I arrived in Tygerberg a bit more than four years ago. Since then the Faculty has become my professional home. We have had some tough times in between, but by and large this is an exciting and stimulating environment. To my colleagues in the Division of Medical Virology I would like to say "baie dankie" – it is a privilege working with you! You deserve special recognition for your great support and dedication to our common goal. I look forward to many more years of enjoying virology while working with you to make a meaningful contribution to health care in South Africa and beyond!

Last but definitely not least, I dedicate this paper to my wife Rika, who endures me (and soon not only me): Ich liebe Dich!
INTRODUCTION

Despite the title, this is not a health economics paper – it is a virological one. More precisely, it is a paper on clinical virology, a much misunderstood discipline at the interface between virology as a basic science and laboratory medicine. As with all diagnostic pathology, its purpose is to assist clinical medical colleagues in managing their patients.

The concept of ‘virus’ (Latin “poison”) as submicroscopically small infectious agents was developed at the end of the 19th century. The developments of electron microscopy in 1929 (Krüger et al., 2000), virus isolation in cell culture (Enders et al., 2009), polymerase chain reaction (PCR) for the amplification of genome sequences and many more discoveries all contributed to the modern discipline of diagnostic virology (Preiser et al., 2002).

We are – and this is true not only for South Africa, but also for much of the world – facing financial constraints and must make important decisions as to how available health care funding should be spent. Using concrete examples, I will endeavour to make the case for a balanced, rational and well-informed approach to virological laboratory testing. I will try to argue that although laboratory testing may appear ‘unproductive’ when compared to expenditure on drugs, for example, it can, if used prudently, make an important contribution to patient care and be cost-effective. I will also make the case for maintaining a state-of-the-art diagnostic service for individual patients at least in part of the health sector; information gained from this may, if used wisely, allow making inferences of importance far beyond the individual case.

THE CASE OF EMERGING VIRAL INFECTIONS

Not too long ago it was widely believed that modern medicine had conquered the microbial world and that infectious diseases would no longer pose a serious challenge to humankind. The age of antibiotics and vaccines seemed to herald the end of an ancient scourge. Sir Frank Macfarlane Burnet, 1960 Nobel Laureate in Medicine, said two years later “… one can think of the middle of the twentieth century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as a significant factor in social life”. A few years later, Surgeon General William Steward told the United States Congress: “The time has come to close the book on infectious diseases”.

How wrong they were! In 1981, the American Centers for Disease Control and Prevention published a report on an unusual cluster of cases of a rare type of lung infection in young men (Centers for Disease Control and Prevention, 1981). This was the first published report on what was soon termed “acquired immunodeficiency syndrome”, or AIDS. Two years later, virologists at the Institut Pasteur in Paris had identified the causative agent, a retrovirus first termed lymphoadenopathy-associated virus (LAV) (Barré-Sinoussi, 2009; Montagnier, 2009). Today this virus is known as the human immunodeficiency virus (HIV), and globally an estimated 33 million people were alive and infected with HIV in 2007 (Joint United Nations Programme on HIV/Aids, 2008).

HIV may be the most important, but it is not the only emerging viral disease. A variety of factors contribute to the emergence of novel or the re-emergence of ‘old’ viruses, many of them man-made and many of them at an increasing rate (Cleaveland et al., 2007; Ludwig et al., 2003). Three of them are discussed here:

Severe acute respiratory syndrome (SARS)

In late 2002, a new, serious and transmissible respiratory illness emerged in southern China. In February 2003 a physician from the affected area who had been exposed when treating patients travelled to Hong Kong. During his stay there, before he succumbed to the disease himself, he unwittingly transmitted the disease to local residents and other travellers, who became ill and transmitted the disease to others when they returned to their home countries. Classic epidemiological methods helped to trace these cases back to the one in Hong Kong, and it became clear that the world was facing an outbreak of a hitherto unknown infectious disease, which was called severe acute respiratory syndrome or SARS (Breiman et al., 2003).

On 15 March 2003, a medical doctor and his two travel companions were admitted to the isolation unit at the Frankfurt university hospital for highly contagious infectious diseases. He had treated patients with what
was later recognised as SARS in Singapore and had subsequently embarked on his journey. While in New York he developed a highly febrile pulmonary infection that did not respond to antibiotics. He decided to return home. Only at this stage was it recognised that he was probably suffering from SARS himself, and during the stopover in Frankfurt he and his accompanying family members were taken to hospital.

Based on the travel history, the scant information available at the time and in view of the potential clinical implications, it was decided to consider even remote differential diagnoses. Therefore, serum samples from the three patients were tested for antibodies against parainfluenza 1, 2 and 3, the dengue, respiratory syncytial, hanta-, adeno-, influenza A and B and measles virus as well as Coxiella burnetii and Mycoplasma pneumoniae. In addition, respiratory and other specimens were tested for the presence of parainfluenza 1 to 4, the human metapneumo-, respiratory syncytial, influenza A and B, adeno-, entero-, herpes-, nipa- and hendra virus as well as Mycoplasma pneumoniae and Legionella pneumophila by means of antigen and/or nucleic acid tests. Within 24 hours, all these tests had been conducted and had yielded negative or unremarkable results, which strengthened the case for a new, unknown infectious agent (Rickerts et al., 2003).

To attempt to recognise a novel viral agent, respiratory samples were studied by transmission electron microscopy. Indeed particles resembling paramyxoviruses were seen in a throat swab from the Singaporean doctor. In parallel, a variety of different cell cultures were inoculated with a sputum sample. After four days' incubation, a clear cytopathic effect had become visible in the Vero cell culture. Electron microscopy of the cell culture supernatant revealed particles resembling coronaviruses – the first hint at the nature of the new agent (Figure 1).

Its further characterisation was achieved by molecular testing at the Bernhard Nocht Institute in Hamburg. Reverse transcription PCR on cell culture supernatant, using several 'degenerate' primer pairs, obtained 20 different DNA fragments. Two of the genetic sequences obtained showed homology to known coronavirus sequences, but they were distinct from any previously known human or animal coronaviruses: A new coronavirus had been discovered (Drosten, Günther, Preiser, Van der Werf, et al., 2003).

The above findings could, however, not rule out that the new virus might just be an 'innocent bystander': a previously unrecognised human virus, possibly of low virulence and perhaps now found by chance in the course of thorough studies?

Vero cells infected with the Frankfurt coronavirus as antigen and a fluorescence-conjugated goat anti-human antibody were used to test acute and convalescent serum samples from the second Frankfurt SARS patient for the presence of specific antibodies. By demonstrating seroconversion, these serological tests proved that she had undergone acute infection with the new coronavirus during the course of her illness. While not being able to fulfil Koch's postulates and therefore formally proving the coronavirus as the aetiologic agent for SARS (once this had been done, the World Health Organization (WHO) officially declared the new coronavirus the cause for SARS in mid-April 2003), this finding further strengthened the causal relationship between the agent and the disease.

Figure 1: Left: Vero cell culture four days after inoculation with sputum from the Frankfurt SARS index patient, clearly showing a cytopathic effect; right: coronavirus-like particle from cell culture supernatant visualised by electron microscopy.
The SARS experience highlights the power of having an extensive repertoire of virological laboratory tests available for dealing with outbreaks of novel infectious diseases (Drosten, Preiser et al., 2003). Being able to rule out other causes quickly assisted with the clinical management and infection control of the SARS patients. By using the full spectrum of available routine diagnostic resources in an imaginative way, the Frankfurt-Hamburg group became one of three research groups within a WHO-coordinated international collaborative effort to independently and simultaneously identify the putative causative agent (World Health Organization, 2003). This breakthrough formed the basis for further research projects on improving the diagnosis, understanding the pathogenesis and developing antiviral agents and vaccines against the new disease (Drosten et al., 2004; ter Meulen et al., 2004, 2006; Van den Brink et al., 2005).

However, it has to be stated that laboratory testing did not contribute much to bringing the SARS outbreak under control; this was achieved through the rigorous application of ‘traditional’ epidemiological measures, such as contact tracing and isolation (Berger et al., 2004).

Poliomyelitis in Namibia
Poliomyelitis is actually the opposite of an emerging viral disease: After plaguing humankind for millennia it will hopefully soon be a thing of the past. Improved hygiene during the 20th century had paradoxically worsened the polio problem in industrialised countries: by moving from an endemic situation (in which nearly everyone is exposed in early life, when the chance of developing paralytic illness is small, and subjects are later protected by the ensuing life-long immunity) to one of seasonal epidemics (with an overall reduction in the number of infections but an increased number of paralytic cases, as the average age at acquisition of infection shifted upwards and the manifestation rate increases with age). Consequently, huge efforts were undertaken to develop poliovirus vaccines. In 1954, Jonas Salk produced the first successful poliovirus vaccine consisting of formalin-inactivated viruses. In 1961, the live attenuated oral poliovirus vaccine developed by Albert Sabin became available.

The vaccines were so successful in stemming polio that in 1988 a worldwide eradication programme was embarked upon, to make polio the second infectious disease ever to be eradicated (after smallpox). The polio eradication strategy is based on sustained high-routine infant immunisation coverage, supplemented by mass polio immunisation campaigns targeting all children younger than five years, irrespective of their immunisation history. The third component is surveillance for cases of acute flaccid paralysis (AFP). The idea is to target the ‘tip of the iceberg’, in other words the small percentage of infected individuals who suffer from ‘classic’ paralytic polio disease, as evidence of continued virus circulation. To ensure adequate surveillance, the target is notification of at least one case of AFP (due to any non-traumatic cause) per 100 000 children up to 15 years of age a year. Of these AFP cases, at least 80% must have two stool samples tested for poliovirus within two weeks of onset. If these standards are fulfilled, AFP surveillance is functioning adequately and will be able to detect polio cases if they were to occur.

ProMED-mail (Program for Monitoring Emerging Diseases), an internet-based reporting system dedicated to rapid global dissemination of information on outbreaks of infectious diseases, reported on 2 June 2006 that “panic is sweeping through suburbs north of Katutura” (near the Namibian capital Windhoek) after three people had died and nineteen others were hospitalised with an unidentified paralytic disease (ProMED-mail, 2006a). Polio was thought to be unlikely, as Namibia had been polio-free since 1999 and those affected were young adults rather than children.

In what appeared to be an unlinked event, a 39-year-old farmer from eastern Namibia, who had undergone a cholecystectomy at a private hospital in Windhoek, developed a severe paralytic illness in mid-May due to which he had to be ventilated and later died. A stool sample collected from this patient on 15 May 2006 was referred to the Division of Medical Virology at Tygerberg National Health Laboratory Service (NHLS). A type 1 poliovirus was isolated from this sample and characterised by the National Institute of Communicable Diseases (NICD) in Johannesburg (ProMED-mail, 2006b) as a wild-type poliovirus type 1 of the South Asia genotype, circulating in India and introduced to Angola in 2005.

This unexpected finding caused considerable upheaval and forced a somewhat reluctant reconsideration of the paralytic disease outbreak due to its temporal and geographic relationship. And indeed a renewed diagnostic effort identified the same virus as the cause of the ongoing outbreak. A national immunisation campaign was organised as a matter of urgency and succeeded in terminating poliovirus circulation in Namibia.

An unusual feature of the Namibian outbreak was that mostly adults were affected. This is possibly
explained by the fact these age cohorts had not been immunised as children and, due to a lack of natural exposure in sparsely populated Namibia prior to the vaccination era, had not been infected with wild polioviruses.

So what are the lessons from this experience? Namibia’s performance with respect to routine polio immunisation coverage (around 80% receive three doses of oral poliovirus) and AFP surveillance (annual notification rate 2/100 000, stool collection rate 88%) had met WHO targets. Nevertheless, polio was able to cause an outbreak and remain undetected for several weeks until the virus was identified in the index case – who was most likely not the ‘first’ case in the outbreak, but somebody with access to private health care and therefore state-of-the-art laboratory diagnosis. This should serve as a reminder that each and every case of AFP is important and must be diagnosed appropriately (Taljaard et al., 2006).

Influenza virus

The world is currently experiencing the first influenza pandemic in 40 years. Since 2003, virologists and public health authorities around the world had been watching the highly pathogenic avian influenza virus A(H5N1) as it spread from South-East Asia to affect domestic and wild birds across wide parts of Eurasia and Africa. Fortunately, despite causing a massive panzootic with serious consequences for poultry farming, this virus still needs to acquire one essential quality that would allow it to cause a pandemic: It lacks easy transmissibility between human beings. As of 1 July 2009, there have been 436 confirmed human cases, including 262 deaths worldwide, almost all of which were the result of (mostly massive) exposure of a human directly to infected birds.

Influenza A viruses are noted for their ability to undergo changes termed antigenic ‘drift’ and ‘shift’. The underlying mechanism of antigenic drift is mutations (the replication of influenza, like all RNA viruses, is highly error-prone) leading to more or less subtle changes in the virus’s surface antigens, haemagglutinin (HA, H) and neuraminidase (NA, N). Such modified surface structures may be less well recognised by the host population’s pre-existing immune responses (resulting from earlier influenza infection or immunisation), thereby conferring an evolutive advantage on the mutant virus as it is better able to spread through that population.

This ongoing antigenic drift of the influenza viruses circulating in the human population necessitates continuous adjustments of the components of the influenza vaccine (Korsman, 2006). The WHO Global Influenza Surveillance Network consists of laboratories around the globe that obtain influenza viruses, isolate and characterise them. On the basis of these results, the WHO tries to anticipate the strains that will most likely circulate during the subsequent season and issues recommendations to vaccine manufacturers. These ongoing influenza surveillance efforts require the involvement of sentinel practices and clinics that submit respiratory samples from patients with influenza-like illness to a laboratory nearby. If an influenza virus is isolated, this is then referred to the National Influenza Centre for further analysis, the results of which feed into the annual recommendations for vaccine composition.

While these ongoing efforts are somewhat unspectacular, they are of utmost importance. Without them, vaccine effectiveness would be left to chance. The viral isolates obtained may also yield important additional information; for example, a large proportion of the circulating A(H1N1) viruses during the 2007 and 2008 influenza seasons were resistant to the antiviral drug oseltamivir, with obvious implications for the treatment of patients (Besselaar et al., 2008). It is to be feared that with the decreasing availability of virus isolation – in other words facilities for handling cell cultures, a method increasingly abandoned in favour of molecular techniques – influenza surveillance will become difficult to maintain at its present high standard.

In contrast to antigenic drift, antigenic shift refers to massive changes through the switch of one type of HA and/or NA to another. There are 16 recognised subtypes of HA and 9 of NA. They all circulate in the influenza viruses’ natural host species, aquatic birds, but only some are found in other species. An H1N1 virus newly appeared in human beings in 1918, causing the worst ever recorded influenza pandemic. It was replaced in 1957 by an H2N2 virus. This in turn contributed its N2 segment to the H3N2 virus, which caused the 1968 pandemic, and has since persisted in the human population. In 1977, the H1N1 virus re-appeared and has since co-circulated with the H3N2 virus, both undergoing continuous antigenic drift.

This situation only changed when in March 2009, an unusual increase in influenza-like illness cases was noted in parts of Mexico (Schoub, 2009). Shortly thereafter, several human infections with a novel influenza A(H1N1) virus were reported from the USA. Although the gene segments of this virus seemed closely related to segments previously seen in influenza viruses isolated from pigs, their combination was unique. Based on the steadily growing number of available genome sequences

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of the new virus, scientists have been able to elucidate its origin. It seems to combine three gene segments from the classical swine influenza lineage, two from the Eurasian swine influenza lineage and three from the swine triple reassortant lineage (one of which probably entered the swine population only around 1998 from human beings) (Michaelis et al., 2009).

It is thought that the gene segments making up the 2009 A(H1N1) virus have been circulating undetected for an extended period, as they are rather divergent from their nearest relatives. The virus has a low genetic diversity, suggesting that the introduction into humans was a single event or multiple events of similar viruses. No known molecular markers predictive of adaptation to humans are currently present in 2009 A(H1N1) viruses, suggesting that previously unrecognised molecular determinants could be responsible for the transmission among humans.

The name ‘swine flu’ is not correct, as the virus has only ever been isolated from pigs that had been infected by a human being during the ongoing outbreak. Traditionally, pandemic viruses were named after their presumed geographic area of origin; so should it rather be the ‘Mexican’ or the ‘North American’ flu? To refer to it as ‘influenza A(H1N1)’ is virologically correct but confusing; after all, from 1918 until 1957 and again since 1977 we have had a circulating ‘human’ strain with the same subtype. It is therefore probably best to talk of the ‘novel’, ‘new’ or ‘pandemic’ influenza A(H1N1) virus.

What is the contribution of medical virology in the context of this pandemic? First of all, specific tests had to be developed and set up for the new virus strain. This was facilitated through the WHO: Public health service laboratories around the world could request the Center for Disease Control and Prevention’s real-time RT-PCR kit allowing the detection of any strain of influenza A virus, the new A(H1N1) strain specifically and a ‘housekeeping’ gene of human origin to control for adequacy of specimen and test procedure. Other organisations also made their in-house PCR protocols available and supplied positive control material. Laboratories such as Tygerberg Virology were therefore able to prepare themselves for the first suspected cases of the new disease.

Molecular diagnostics are the method of choice for detecting the pandemic influenza A(H1N1) virus. The virus can be isolated using the same cell line as for seasonal influenza viruses. However, there are no specific antibodies available yet that would allow identification of the new strain; therefore, for further investigation of influenza A virus isolates – which may be seasonal A(H1N1), seasonal A(H3N2) or pandemic A(H1N1) – it is currently best to use the specific PCR (World Health Organization, 2009a). Although a fourfold or greater rise in specific pandemic influenza A(H1N1) virus antibody titres in haemagglutination inhibition or microneutralisation tests indicates recent infection with the virus, antibody serology is by its very nature retrospective and therefore of little use for acute diagnosis (Allwinn et al., 2002).

Virological testing will be needed to provide critical information throughout the course of a pandemic; however, the requirements will differ depending on the point in time and location and have to be generated by different components of surveillance activities (World Health Organization, 2009b).

Component 1, early detection and investigation, aims to detect and investigate the first evidence of sustained human-to-human transmission of an influenza virus with pandemic potential. Once the initial investigation indicates that sustained human-to-human transmission is occurring, component 2 ensues: a comprehensive assessment of the 100 earliest (recognised) laboratory-confirmed cases for each country to characterise the new disease, its epidemiological and virological features, clinical characteristics and risk factors. This information will help determine the composition of a pandemic vaccine and inform assessments of the severity of the pandemic.

During this phase, every single suspect case should not only be laboratory-tested but clinical information collected using the WHO Pandemic Influenza Case Summary Form, which comprises patient demographic and exposure information, pre-existing conditions, clinical presentation and course, outcome (death, recovery, etcetera), clinical samples obtained and test results. All virus isolates are submitted to a WHO Collaborating Centre for further characterisation.

Finally, component 3, pandemic monitoring, aims to gather information to monitor the course of the pandemic, its activity, geographic spread, trend, intensity and impact. During this component, there is no longer a need to try to obtain a sample from each and every suspect case. Oftentimes, pandemic activity will be so widespread and intense that this would be impossible anyway. At this stage testing efforts are focused on confirming infection in new areas, testing severe cases (to confirm the diagnosis and to identify a possible increase in virulence) and monitoring the co-circulation of the pandemic with seasonal influenza.
viruses (World Health Organization, 2009c). During an ongoing pandemic, the main objective of laboratory surveillance is to monitor the evolution of the virus to detect any genetic drift or re-assortment that may affect pathogenicity, identify emerging drug resistance, ensure the specificity and sensitivity of current diagnostic assays and inform vaccine development. After the cessation of routine laboratory testing of all suspected pandemic influenza cases, unusual events, such as clusters of cases of severe or fatal H1N1 infection, clusters of respiratory illness requiring hospitalisation, or unexplained or unusual clinical patterns associated with serious or fatal cases will still need to be monitored closely.

In terms of individual patient management, routine laboratory testing of all cases of influenza-like illness is no longer recommended at this stage (National Institute for Communicable Diseases, 2009). Laboratory capacity is overstretched, and a test result is not needed for the clinical management of most individual patients. With a high incidence of pandemic influenza in the population, the positive predictive value of a diagnosis based on clinical criteria alone is very high (in other words, most patients who seem to be suffering from influenza are indeed). During this stage, testing is only recommended for patients with severe infections, where a laboratory diagnosis will assist in patient management, or for patients hospitalised due to a lower respiratory tract infection in whom influenza is one of the differential diagnosis. Testing can also be done for symptomatic patients with co-morbidity who are at risk of serious complications if it will guide clinical management. Other reasons to test are case clusters where the cause of the outbreak must be found, and an individual who has died from suspected pandemic influenza A(H1N1).

It is crucial to ensure that antiviral drug therapy must never be deferred until a test result is available; because neuraminidase inhibitors must be commenced as soon as possible after the onset of symptoms, even a same-day testing service would mean an unacceptable delay. Commercially available rapid (also called near-patient, bed-side or point-of-care) tests to detect the influenza virus antigen in respiratory specimens offer no solution: An evaluation found low sensitivities between 40% and 69%, which means negative test results do not rule out pandemic influenza A(H1N1) infection (Centers for Disease Control and Prevention, 2009). It is far better to treat patients empirically based on clinical suspicion, severity of illness and risk of complications.

MANAGING VIRAL INFECTION

With the continuously increasing repertoire of antiviral drugs, clinical virology is more and more required to assist with identifying patients in need of therapy and with monitoring those receiving antiviral treatment. HIV provides a prime example to briefly describe virology’s contribution at various stages.

Diagnosing infection

Since the mid-1980s, tests for the detection of HIV-specific antibodies have been available commercially. Good HIV antibody screening tests reach unsurpassed levels of sensitivity and specificity (Preiser & Korsman, 2007). Nevertheless, problems continue to be encountered, with laboratory-based assays (Preiser, Brink et al., 2000) but even more so with so-called rapid/simple test devices, which form the basis for diagnosing infection in the worst-affected countries. It is a challenge to ensure that users with little or no laboratory background perform the tests correctly (Moodley et al., 2008), but the tests themselves may display poor performance under certain conditions (Claassen et al., 2006; Preiser et al., 2009).

For infants born to HIV-infected mothers, the detection of viral genome, for example by PCR, replaces antibody testing for the diagnosis of infection, due to the presence of transplacentally acquired maternal antibodies. This method, too, like all laboratory tests, generates some false-negative and some false-positive test results; patients should therefore ideally be re-tested. Where budgetary constraints do not permit re-testing at least of positive results, it can be argued that the baseline HIV viral load test performed before initiation of antiretroviral therapy (ART) serves as a confirmatory test.

Monitoring patients on antiretroviral treatment

Once a patient is on ART, its effectiveness must be monitored. Therapy failure may be due to a number of causes, including poor adherence, pharmacokinetic factors, changes in drug metabolism, for example through co-medication, and others. Not only does therapy failure mean the patient’s HIV disease will progress; if left unaddressed, therapy failure of any cause will in addition give rise to drug failure due to the emergence of antiretroviral drug-resistant HIV strains.

The classic view on ART failure is of a sequence of events: first, virological failure, evident from an increase in HIV viral load (ART should ideally suppress viral
replication, resulting in an 'undetectable' viral load); second, immunological failure, evident from a decline in the number of CD4-positive T-lymphocytes in the patient’s blood (as a marker of deteriorating immunological function); and finally, clinical failure, that is the occurrence of clinical conditions such as opportunistic infections as a consequence of progressive HIV-related immunodeficiency.

Since it was recognised that for HIV, like probably most chronic viral infections, the extent of viral replication correlates with disease activity and progression, viral load assays (to quantify the concentration of viral genome in the patient’s blood) have become important prognostic and monitoring tools (Berger et al., 2001; Berger and Preiser, 2002; Preiser, Elzinger and Brink, 2000). The viral load is the result of viral replication and therefore allows assessment of its activity, which in turn has important clinical implications. Considerable efforts have gone into developing and continuously improving assays (Berger, Scherzed et al., 2002; Berger et al., 2005; Claassen et al., 2008); several such tests are available commercially and used routinely and frequently for monitoring of ART, where resources are available.

The problem is that laboratory testing is costly and not universally available. While ART guidelines in industrialised countries make extensive use of both CD4 count and viral load testing, this is different for the public health approach to scaling up access to ART: The WHO recommends that if laboratory capacity is unavailable, initiation of ART be based on clinical staging and that patients on ART be monitored clinically both in terms of drug-related toxicity and treatment failure (World Health Organization, 2006; World Health Organization, 2008).

This approach is supported by a modelling study that predicted that the proportion of African patients who will survive for five years on ART will be 83% for HIV viral load monitoring and 82% each for CD4 monitoring and for clinical monitoring on its own (Phillips et al., 2008). Therefore, HIV viral load monitoring was found not to be cost-effective.

However, this view has come under strong criticism. There is a lively debate on the optimal way to monitor patients on ART in resource-limited settings. A number of studies have shown that monitoring neither by clinical criteria alone nor based on CD4 counts is sufficient (Bagchi et al., 2007; Kantor et al., 2009; Mee et al., 2008). The risk lies not only in late recognition of ART failure, which can lead to the emergence of antiretroviral drug resistance, but also in misclassifying patients as failing and switching them unnecessarily to second-line treatment regimens that are more expensive, less well tolerated and often the last option available (Sawe & McIntyre, 2009).

Increasingly the issue is rephrased as “Viral load testing: How much is necessary? How little is possible?” A number of authors argue for including HIV viral load monitoring into ART programmes following the public health approach and are likening the present policy to ‘running with scissors’ (Bendavid et al., 2008; Calmy et al., 2007; Lawn et al., 2008; Moore & Mermin, 2008; Schooley 2007; Smith & Schooley, 2008).

Sawe and McIntyre et al. (2008:464) argue that “the time has come to work toward the progressive introduction of appropriate viral load monitoring technology in these programs with the same sense of urgency and commitment as the world approached ART access. To do less is to abandon the global success of ART to an early collapse”. To achieve this goal, HIV viral load assays need to be developed and evaluated that are simpler, cheaper and as good or better than currently available ones (Cheng et al., 2008; Rouet & Rouzioux, 2007). While a number of in-house assays have been developed that fulfil these criteria (Drosten et al., 2006; Rouet et al., 2008; Preiser et al., 2006), these still require a sophisticated laboratory and skilled personnel. Ultimately, a point-of-care, maybe just semi-quantitative, HIV viral load test would be desirable.

Fortunately, the South African antiretroviral roll-out programme provides for both CD4 counts and HIV viral load testing, albeit at relatively low frequencies and with conservative thresholds to trigger action (National Department of Health, 2004). Apart from being beneficial for the individual patient, summary data on such testing may also be useful. The NHLS provides all laboratory tests for patients within the South African public health service. Summary statistics on numbers of tests done and the proportion of tests with a certain result (for example with an HIV viral load below the level of detectability as a marker for successful ART) are a valuable tool to continuously assess the success of the ART programme (National Institute for Communicable Diseases, 2008).

**Antiretroviral drug resistance**

The latest addition to the repertoire of tests used routinely for ART monitoring in ‘rich’ countries is genotypic characterisation to test for drug resistance-associated mutations. Again, technology has developed rapidly (Stürmer et al., 2004a; Stürmer et al., 2004b;
Sührmer et al., 2005), but at this stage is still dependent on a highly specialised laboratory and consequently very expensive.

Rather than making resistance testing part of routine ART monitoring, as guidelines for Europe for example do (European Aids Clinical Society, 2008), the approach for developing countries must at this stage be one of assessing the natural history of antiretroviral drug resistance in typical cohorts (Van Zyl et al., 2008; Van Zyl et al., 2009). Because the public health approach to ART ensures minimal inter-patient variation, this approach will yield important insights to help improve ART programmes. Instead of using resistance testing for individual patient diagnosis, surveillance on a population level will for the foreseeable future be all that can be attained in resource-constrained settings.

CONCLUSIONS AND OUTLOOK

The purpose of this paper was to put forward the concept of virological laboratory diagnosis as more than just a tool to help with the management of individual patients. Even outside structured surveillance programmes, relevant information can be gained as a ‘side-product’ of routine laboratory testing. While this is already being done to some degree, there can be little doubt that the yield could be improved by a more systematic approach, better organisation and structuring. I have given some examples to highlight the value of state-of-the-art virological diagnosis not only to optimally manage the patient tested but also to gain data of public health importance. More research is needed to determine how much and what type of diagnostic testing is necessary, and how little is sustainable without compromising the quality of patient care and, at least in the medium to long term, also programme viability and population health.
REFERENCES


