Forensics in HIV Transmission Crimes

Investigación Forense en Crímenes de Transmisión del VIH

Grazinoli Garrido Rodrigo [1]; Gonçailves da Costa Gabriela [2]

1- *Biomédico; Grad. Segurança Pública; MSc; DSc* Perito Criminal - Diretor do IPPGF/PCERJ Professor Adjunto - PPGD/UCP - FND/UFRJ. ORCID ID https://orcid.org/0000-0002-6666-4008 2- Biomédica

MSc. Rodrigo Grazinoli Garrido - grazinoli.garrido@gmail.com

Recibido: 21-09-2019 Aceptado:15-I-2020

Resumen

La microbiología forense es un área científica que ha surgido con la necesidad de investigar los delitos biológicos, como en el caso de la transmisión intencional del virus de la inmunodeficiencia humana (VIH). Este trabajo exploratorio tuvo como objetivo demostrar cómo la tecnología biomédica, como la filogenética y la cuantificación de la carga viral y los linfocitos T CD4+, puede usarse para producir evidencia técnica que brinde más certeza para determinar la autoría y la materialidad de estas conductas criminales.

Palabras claves

Investigación forense; SIDA, VIH, crimen.
Fuente: DeCS

Abstract

Forensic microbiology is a scientific area that has emerged with the need to investigate biocrimes, as in the case of intentional transmission of the Human Immunodeficiency Virus (HIV). The present exploratory work aimed to demonstrate how biomedical technology, such as phylogenetics and quantification of viral load and CD4+ T lymphocytes, can be used to produce technical evidence that brings more certainty in determining the authorship and materiality of these criminal behaviors.

Key words

Forensics; AIDS; HIV, crime
Source: DeCS

Introduction

With advances in microbiology in recent years, forensic microbiology has been established and strengthened as a new scientific chair for responding to the law at a biological crime event, this is a discipline in which microbiology and forensic science complement each other and are dedicated to tracking and analyzing a biocrime.

The need for the study of microbiological expertise can be applied in biocrimes linked to the transmission of microorganisms intentionally, such as the intentional transmission of Human Immunodeficiency Virus (HIV), a pathogen that causes Acquired Immunodeficiency Syndrome (AIDS).

Many issues arose with this pandemic, one of them was the need on the part of jurists to solve the difficult and complex framework, which affects not only the criminal sphere but also the social and cultural sphere, the conduct of the individual who commits this crime.

The jurisprudence and the doctrine still maintain some disagreement about the possible typifications of crimes involving intentionality in the transmission of the virus. The position of the United Nations Joint Program on HIV / AIDS, to avoid further discrimination against carriers, is to avoid establishing legislation to address the issue or a specific criminal type. Thus, countries such as Brazil seek to frame criminal conduct in broader criminal types.

However, whatever criminal type the conduct may fall under, it is certain that for the perfect characterization of the crime, it is necessary to produce technical evidence based on current biomedical knowledge and technologies. Thus, it would be possible to establish the causal and temporal connection between the conduct and the outcome.

The current research was developed through exploratory and qualitative analysis, based on indirect documentation from national secondary sources. We sought to analyze new technologies applicable to the determination of causality and temporality between certain suspicious behaviors and effective contamination and prognosis of the victim's disease, highlighting the use of viral phylogenetics.

HIV: General Aspects

HIV is grouped into the genus Lentivirus (lentus, from Latin) due to the slow course of infection and thus disease, with a long latency period, persistent viral replication and central nervous system involvement (1). Regarding the family, it is grouped within the Retroviridae family, viruses that have the enzyme Reverse Transcriptase (TR) - responsible for transcribing the RNA genome into complementary DNA (cDNA), being the subfamily Orthoretrovirinae (2). This family of retroviruses has been receiving a lot of attention from scientists in recent decades for causing serious diseases in humans, such as AIDS (3).

HIV is divided into two types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). HIV-1 has worldwide distribution, while HIV-2 is more frequently detected in individuals from African countries (4).

The first clue to the emergence of HIV-2 came in 1986 when a morphologically similar but previously distinct virus was found to cause AIDS in patients in West Africa (4). This new virus, described as HIV-2, was closely related to a virus that caused immunodeficiency in captive monkeys in sub-Saharan Africa. The microorganisms, isolated in these animals, were collectively called Simian Immunodeficiency Virus (SIV), are also grouped with the genus of Lentivirus (4).

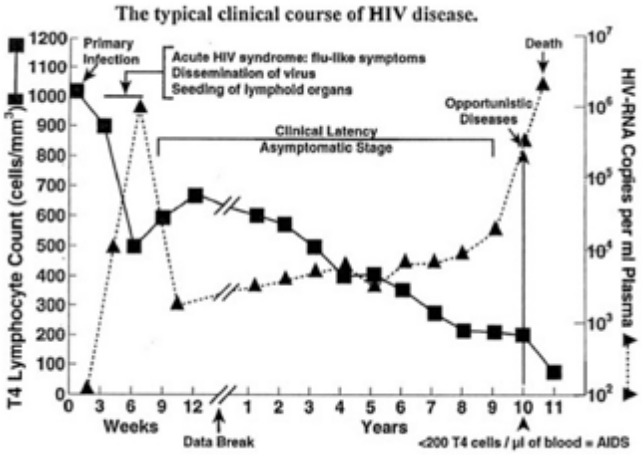
Interestingly and surprisingly, these viruses appeared to be largely non-pathogenic in their natural hosts, the primates, since ape viruses familiar with HIV-1 and HIV-2 were found infecting these animals. These relationships provided the first evidence that AIDS emerged in humans and monkeys as a consequence of infections between different primate species (4). HIV-1 evolved from nonhuman primates, the Central African chimpanzees, which were infected with the Central African Human Immunodeficiency Virus (SIVcpz) and West African mangrove HIV-2 (SIVsm) (5). Thus, it was clear that HIV-1 and HIV-2 were the result of zoonoses from primate-infected virus transfers in Africa (6).

This viral barrier breakdown occurred through hunting activities, which caused humans to acquire SIV (7). However, SIV is a weak virus, typically suppressed by the human immune system within weeks of infection. It is believed that several transmissions, from the simian immunodeficiency virus, from individual to individual in rapid succession have enabled its transformation into HIV over time (8).

HIV infection is said to be chronic or persistent since the infected host is unable to eliminate the infectious agent (9). Most infections by this virus occur through the mucous membranes of the genital or rectal tract during sexual intercourse, but there are other ways of transmission of this pathogen, besides the sexual route, such as parenteral and vertical (10).

After exposure to HIV, if infection occurs, there are approximately ten days called the eclipse phase, before viral RNA is detectable in plasma (11). After this moment, the pathogenic pathway of this infection goes through three main and sequential stages: initial or primary phase, asymptomatic or clinical latency phase, and symptomatic phase (10), as shown in Figure 1.

Figure 1 - Natural history of HIV infection in the absence of antiretroviral therapy (58).



Primary infection, or acute viral syndrome, is defined as the period between the initial infection and the development of the immune response and lasts no longer than two or three weeks (10). In general, at this stage, HIV-infected individuals may develop asymptomatically, presenting with a clinical picture similar to influenza or mononucleosis-like (exhibiting signs and symptoms typical of these conditions - fever, myalgia, malaise, headache, nausea, vomiting, pharyngitis, among others) (12).

The innate immune response established at the focus of the infection attracts an additional amount of T cells, which causes intense viral replication (with viremia values up to 108 copies of RNA / mL of plasma). With the visceral and lymphoid tissue dissemination, which are targets of the action of this pathogen, and because of this, there is a marked decrease in CD4+ T lymphocytes and an absence of immune response on the part of the host, thus ensuring a significant viremia and Antigenemia. These will gradually decrease as the patient develops an immune response to HIV (9, 11).

Importantly, infection with this virus has an immunological window time of approximately 30 days. This phase is characterized by the period between infection and the body's production of antibodies against the viral particle in sufficient quantities to be detected by the tests (13). In this interval, the person may already be infected and still have the antibody test result as negative (14).

After primary infection follows the clinical or asymptomatic latency phase. At this time clinical recovery occurs, with a reduction of viral replication as a result of the immune response, but it is insufficient in magnitude to eradicate the infection. It is at this stage that seroconversion occurs, with the development of antibodies that persist throughout the body (9). Several factors may be implicated in the control of viral

replication, including the presence of neutralizing antibodies and cytotoxic T cells. These exert partial control of infection, but not sufficiently to prevent, in the absence of therapy, the slow and progressive depletion of CD4+ T lymphocytes. and the eventual progression to AIDS (11). In general, it can be said that at this stage a balance is struck between viral replication and host immune response (5).

The asymptomatic phase is therefore characterized by reduced viral loads due to the strong immune response of the host, as there is an additional production of activated CD4+ T lymphocytes that target new infections, and the absence of clinical symptoms and signs of the disease (11). The time between initial infection and the development of clinical disease varies considerably, with an average of ten years (9).

Finally, in the symptomatic phase, there is intense viral replication and decreased immune response due to the gradual decrease of TCD4 lymphocytes throughout the asymptomatic phase. This favors the appearance of neoplasms and opportunistic infections of increasing severity and lethal potential. This period may last a few months or several years (9).

In the advanced stage of HIV infection, infected individuals may progress to AIDS. However, for this to be defined, the patient must have opportunistic infections (such as pneumocystosis, neurotoxoplasmosis, atypical or disseminated pulmonary tuberculosis, cryptococcal meningitis, and cytomegalovirus retinitis) and neoplasms (the most common being Kaposi's sarcoma, non-Hodgkin's lymphoma and cervical cancer in young women). In these situations, the CD4+ T lymphocyte count is below 200 cells/mm^3, most of the time and there are high levels of circulating viruses (15).

As a therapeutic strategy about HIV, there are antiretrovirals (ARVs). These drugs appeared in the mid-1990s to prevent the virus from multiplying in the body. Before the emergence of this type of therapy, clinical management of HIV consisted largely of prophylaxis against common opportunistic pathogens and the management of AIDS-related diseases (16).

ARVs do not destroy the viral particle but prevent its replication, thus helping to prevent the weakening of the immune system. Therefore, its use is fundamental to increase the time and quality of life of those living with HIV / AIDS (14).

There are some classes of ARV drugs, which act at different sites on the viral particle. In general, drugs of two classes are combined to ensure a potential attack on HIV. This strategy aims to prevent resistance to ARVs by the pathogen. The advent of combination therapy, also known as HAART (Highly Active Antiretroviral Therapy) for the treatment of HIV infection, particularly HIV-1, has been seminal in reducing associated morbidity and mortality. HIV infection and AIDS. HAART dramatically suppresses viral replication and reduces HIV plasma viral load below detection limits of the most sensitive clinical trials (<50 RNA copies/ml), resulting in a significant immune system reconstitution as measured by an increase in CD4+ T lymphocytes circulating (16).

The seven major classes of ARVs currently used for HIV treatment are: Fusion Inhibitors (IF), Integrase Inhibitors (II), Protease Inhibitors (PI), Nucleoside Analogs Reverse Transcriptase Inhibitors (NRTI), Reverse Transcriptase Inhibitor (NtRTI) Nucleotides, Non-Reverse Transcriptase Inhibitor (NNRTI) Analogs, and Maturation Inhibitors (MI) (17).

The classes of ARVs that target reverse transcriptase act by inhibiting the action of this enzyme that acts on the synthesis of HIV genetic material. Protease inhibitors impede the processing of viral proteins, leading to the formation of defective viral particles unable to assemble the other complete virus. Besides, there are also virus-cell inhibitors, which fall into two groups: fusion inhibitors and CCR5 inhibitors. For the use of CCR5 inhibitors, it is important to verify if the infection is by viruses that present this surface protein. Integrase inhibitors, on the other hand, prevent viral genetic material from integrating with cell DNA and, finally, maturation inhibitors make the newly formed virus lack infectious capacity (18).

Currently, a relevant issue associated with HIV, which is a means of preventing infection by this virus is Pre-Exposure Prophylaxis (PrEP), which is highly effective and was initiated in Brazil at the end of 2017 (19). Brazil is at the forefront of using PrEP as a means of prevention in Latin America, being the only country in this region where PrEP is available through the public sector. PrEP, also known as “combined

prevention", is a combination of two antiretrovirals (tenofovir/emtricitabine) taken every day by people at high risk for HIV, such as serodiscordant couples and people using injecting drugs. Daily use of this medicine reduces the risk of contracting HIV by sex by over 90% and in people who use injecting drugs by over 70% (20).

However, it is necessary for the individual who uses this method to perform HIV testing every three months (19). It is noteworthy that PrEP does not prevent other sexually transmitted infections (STIs) or pregnancy, so the importance of maintaining the use of other physical means of prevention, such as condoms. Another feature of this prophylactic medium is that its efficacy is greatly reduced by the lack of compliance with the daily dosage (10). PrEP is expensive (although generally free after application to the pharmaceutical program), requires engagement in a health center and frequent follow-up visits (10).

Post-Exposure Prophylaxis (PEP) means taking antiretroviral drugs after being potentially exposed to HIV to prevent infection. OPEP (Occupational Post-Exposure Prophylaxis) was used as a model for the creation of Non-Occupational Post-Exposure Prophylaxis (nPEP), and in many respects they are similar, but for oPEP, in most cases, there is more chances of testing the source of infection (20).

In both oPEP and nPEP, antiretroviral therapy is administered when an individual has been exposed to a suspected or positive HIV secretion of semen, vaginal fluid or blood and seeks medical treatment within 72 hours and continued for 4 weeks. This prophylactic regimen has low side effects and minimal risk of HIV resistance. However, if the patient's source is HIV negative or 72 hours have passed, PEP is not recommended (19).

One fact that should be considered regarding HIV infection is that although cure for this pathogen is not yet a current reality, an important issue is that after a decade of research, there is scientific evidence-based confirmation that the risk of HIV transmission from a person living with HIV / AIDS (PLWHA) who is on antiretroviral therapy (ART) and has achieved an undetectable viral load on blood for at least six months is nonexistent. Being undetectable does not mean that the virus is no longer circulating in the blood, but it is so low that it is not detected by the viral load test (14).

In HIV-related criminal cases, two points should be considered: the amount of virus in the suspect's bloodstream; the immune response of the individual's organism; the nature and efficacy of the therapy performed, considering that it is aware of its seropositive status. Therefore, viral load and CD4+ T lymphocyte count are crucial in this investigation (21).

The CD4+ T lymphocyte count measures the number of this cell line by the flow cytometry technique. Since CD4+ T lymphocytes are the target of HIV, these cells are progressively destroyed and the lower the count, the more the disease progresses and the worse the symptoms. Counting these T cells does not check for HIV, but rather measures the immune system response. Conversely, when the CD4+ T lymphocyte count is high, viral replication is lower. If this occurs over a prolonged time, a long-term nonprogressive individual is characterized. Treatment, risks, and prognosis of seropositive patients will be influenced by CD4+ T cell count (22).

To properly determine viral load, amount of virus in the bloodstream, viral RNA is counted. The quantification of the genetic material of the virus is performed by molecular methods, being a more accurate and direct way of measuring the virus. As already stated, this measure correlates with the response to therapy and can predict the progression of AIDS and how much HIV poses risk to the patient and their sexual partners (22).

It is noteworthy that some HIV-positive individuals may have undetectable levels of the virus; in general, patients with these characteristics are those who use antiretroviral therapy effectively (22). However, even patients considered undetectable in a case of intentional transmission of HIV cannot be excluded from prosecution, even with considerably lower chances of contamination (21). All existing knowledge about HIV should be considered in expertise on a possible crime of HIV transmission.

3. TECHNICAL EVIDENCE PRODUCTION: PHYLOGENETIC ANALYSIS

Forensic science is the key to establishing links between evidence found at a crime scene and suspects linked to it (23), the application of modern techniques to identify or specify evidence in a judicial process is essential (24).

Phylogenetic study has often been used as a forensic technique to investigate whether the relationship between the types of HIV viruses that infect a set of individuals is compatible with the form of transmission between them, thus whether it has occurred directly or indirectly (25).

Molecular phylogenetics, a field of phylogenetics, is a disciplinary study of the evolutionary relationships between organisms using molecular sequences. The methods of analysis used in molecular phylogenetics were originally developed to reveal evolutionary pathways. Even today this area is used in various fields, such as biology and biodiversity (26), molecular epidemiology (27, 28), identification of gene functions (29) and identification of microorganisms in microbiome studies (30, 31).

A phylogenetic tree or phylogeny is a graphical representation of ancestral-descendant relationships between organisms or genetic sequences and should be considered as a hypothesis of an evolutionary relationship between a group of organisms (32). They show evolutionary relationships between a set of Operational Taxonomic Units (OTUs) (33).

The OTU usually represents a species, but can also represent individual organisms in a population, a protein gene or sequence, or a taxon in any taxonomic position (by family, order, class, phylum). The nodes at the tips of the tree are called "outer nodes". They are used to represent OTUs. Another type of node, called "internal nodes," represents a Recent Common Ancestor (RCA). These include the lines, called "branches," used to connect newer and older nodes and show the evolutionary relationships between taxa. A branch that connects two inner nodes is an "inner branch" that shows an old relationship. On the other hand, the branch that joins an inner node to an outer node to show a newer relationship is called an "outer branch." The deepest branch of the tree represents the "root" (33).

Linked to the phylogenetic study is the use of sequencing as an instrument to achieve the final result, the construction of the phylogenetic tree. The classical sequencing technique is based on making many copies of a target DNA, being introduced into the terminator nucleotide polymerization reaction and thus producing fragments of different lengths. Also, the "chain terminator" nucleotides are fluorescently labeled, allowing the ends of the fragments to be determined. Thus, it is possible to organize the various fragments by size and perform sequencing by the terminal bases of each (34).

This technique was described by British biochemist Frederick Sanger and colleagues (35) and is thus called Sanger Sequencing. Currently, however, it is possible to perform individual human sequencing in less than one day, more cheaply (34,35,36). Therefore, Next Generation Sequencing (NGS), in which small sequencing reactions can be conducted in parallel, has been used. Thus, large amounts of DNA can be sequenced, increasing the speed and reducing the costs of the technique and the time to perform it. NGS is a technique that favors high accuracy in results, which is of paramount importance, especially within the forensic application (37).

The defining characteristic of HIV is its exceptional genetic diversity. This high diversity derives from at least four sources: high substitution rates, a very small genome, short generation times and high recombination frequency (38).

Through the progress of this method, it was possible to expand the study of HIV genetic diversity, evolutionary and epidemic processes, improving the characterization of this virus and the possibility of generation of complete virion genomes. In addition to greater sensitivity and accuracy in detecting recombinant viral forms and detecting multiple viral infections in individual hosts (39). Another relevant aspect related to this method is the possibility of detecting HIV variants at a low frequency, about 1%, and it is potentially possible through this detection to specify information about viral particle infection dates,

predicting whether this is a recent infection or not, which in the criminal context may be important to estimate the moment of infection (40,41).

Unlike human DNA, which remains stable for life, RNA, the genetic material of HIV, has a rapid rate of evolution, because TR cannot edit the newly synthesized genome, an activity known as proofreading. Thus, erroneous nucleotide pairings during DNA synthesis are not verified and errors are consequently incorporated into the nascent DNA molecule, thus implying a high rate of incorporation of mutations by this virus. Thus, it gives rise to a high genetic diversity, making this virus a "moving target", which contributes to the host's inability to control and eliminate this pathogen in natural infection (42).

Phylogenetic methods, therefore, are a source for determining patterns of routes that HIV travels in cases of transmission between individuals (46). During transmission, one or a few of these virus variants are passed on and subsequently diverge in newly infected individuals so that epidemiologically linked persons never have the same viral type (47). Due to the enormous genetic variation of the virus, this trait has been successfully used for epidemiological-scale research to understand the evolutionary history of HIV and phylogeography (48), and for local scale to study transmission networks of HIV-positive patients, especially those of HIV-1, because it is the most present in the population (49).

These studies are already used as evidence in court, confirming or refuting the transmission between the defendant and the victim (40). Besides, the phylogenetic study can also be used to develop better public health prevention initiatives for this pathology (49) or to study the transmission of drug resistance (50).

In criminal cases addressing allegations of HIV-1 transmission, the forensic scientific approach may be required to prove the timing and direction of transmission to demonstrate that the defendant infected the victim and that the accused was aware of the diagnosis. HIV positive at the time of the alleged transmission and to confirm that the victim was infected at the time of the events described in the prosecution (40). However, this evidence for individuals infected with HIV-1 is challenging, as each patient has a quasi-species of rapidly evolving viral strains (25). Performing virus differentiation in two epidemiologically linked individuals depends on many factors and cannot yet be reliably predicted (25).

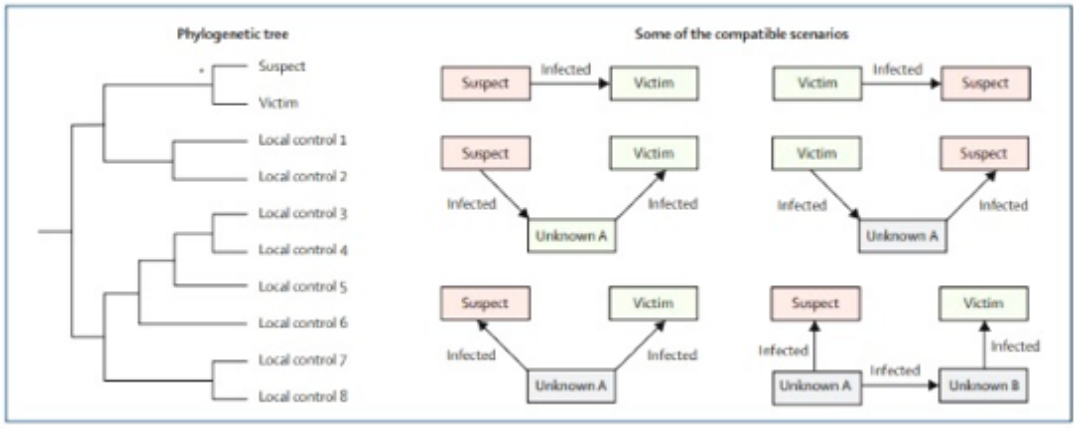
For this reason, there are concerns regarding the use of phylogenetic analysis, particularly whether it may indicate the meaning and timing of HIV transmission and whether intermediate links may be excluded (25, 51). Therefore, the use of this method in courts involves strict care, because it is difficult to know for sure that all the people involved in the transmission network were sampled. Missing calls need to be evaluated through contact tracking, and these depend on testimony from the defendant and victim, and potentially other witnesses. Therefore, in reconstructing a history of transmitting a phylogenetic structure in the context of forensic investigations, one should never assume that all links are known. Victims must remember or be willing to fully disclose all risk contacts (40).

The results of the phylogenetic analysis should be interpreted with caution in a court and need to be placed in the context of other types of evidence (40). A probative combination: forensic evidence related to DNA molecules, evidence of intercourse between parties, and health history of both the accused and the victim should also be analyzed. Phylogenetic inquiry is just one of the many steps required to frame what will be concluded from the phylogenetic tree: that is, to understand whether the tree is consistent with or contradicts the accusations (40).

It is noteworthy that treatment can prevent transmission (52), which is also relevant to determine if the defendant was infectious at the time of the event (40).

- ✓ Therefore, some scenarios (Figure 2) should be taken into consideration:
- ✓ The victim was infected by the defendant, not the other way around;
- ✓ There is a third individual with a similar viral strain, linking the defendant and the victim;
- ✓ Both the victim and the defendant were infected with one or more similar third viral strains;
- ✓ The victim was already HIV positive and was again infected with another strain, by the defendant or a third party.

Figure 2 - Hypothetical phylogenetic tree for investigation of HIV transmission (40).



In a study by Siljic and colleagues in the city of Belgrade, capital of Serbia, in 2011, three individuals were analyzed as a possible source of intentional HIV transmission. The analysis reported one man (individual 1) and two women (individual 2 and individual 3), all diagnosed with HIV serology at the HIV / AIDS Center of the University Hospital for Infectious and Tropical Diseases in August and September of that year, in Belgrade (53).

The initial information regarding these contaminations was as follows:

- ✓ Individual 1 and individual 2 had been married for more than 15 years with two children aged 10 and 14, both considered HIV negative;
- ✓ Individual 3 had been a sexual partner with individual 1. Together, they had several extended stays in Thailand in the years before HIV infection;
- ✓ Individual 1 sues individual 3, alleging conscious contamination by the latter subject. And that individual 3, according to individual 1, knew of his HIV-positive diagnosis and had not revealed it;

Sample analyses were performed using two genes, env, and pol. For the study, thirty-four sequences from these same gene regions of different patients who had HIV-positive serology within two years before and two years after the time of diagnosis of the three questioned patients were included in the phylogenetic analysis, as local controls. Twenty-nine samples of the pol gene sequences of heterosexual, routine hospital subtype B subjects who were resistant to drugs against the viral particle were also added. Because of possible epidemiological linkages with Thailand, 20 subtype B viral sequences in pol and env sampled from those infected in this region were also used, retrieved in the BLASTsearch (Basic Local Alignment Search Tool) tool. The final dataset analyzed consisted of 101 local and 50 foreign sequences as controls for the pol gene and 34 local and 50 foreign sequences as controls for the env gene (53).

As a result, the researchers noted that it was subtype B that infected the three subjects involved in the study. Phylogenetic analyses were very consistent, showing that there was no mix of external control sequences in the transmission cluster. And, the cluster contained no viral sequences from Thailand. As shown in Figures 3 and 4, there was a specific cluster for the three individuals analyzed without mixing of other viral sequences (53).

Figure 3 - Phylogenetic tree based on pol gene sequences obtained in the study. The colored sequences identify the individuals analyzed (53).

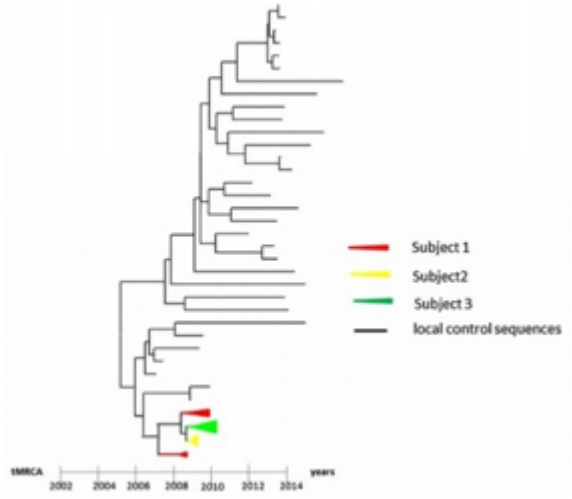
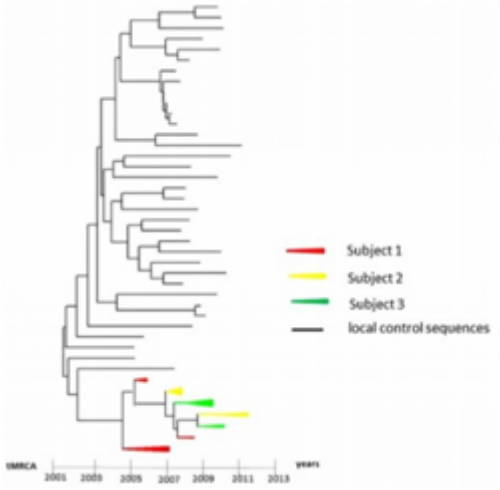


Figure 4 - Phylogenetic tree based on env gene sequences obtained in the study. The colored sequences identify the individuals analyzed (53).



The most recent common ancestor time (tMRCA) provides an estimate for each subject's date of infection, based on a 95% confidence interval of the root branch cluster's tMRCA estimate. For individual 1 the estimated time was between 2003 and 2007 (for the pol gene) and 2006-2008 (under analysis of the env gene), ie 3-8 years before the sampling date (2011). The estimated time for individual 2 was placed between 2007 and 2009 (in) and 2008-2010 (env), 1 to 4 years before the sampling / diagnosis date and for individual 3 between 2007 and 2009 (in) and 2008 -2010 (env), also 1 to 4 years before the diagnostic sampling date, both presented the infection later than the individual 1(53).

This study reinforced the need to use adequate controls so that reliable results could be obtained at the end of the analysis. To reach them, the researchers emphasized the importance of using viral sequences that shared the same risk of transmission, similar geographic location, use of the same gene clades (subtype or recombinant form) and that have proximity in the period of diagnosis of contamination. If sufficient samples are included in the analysis as a control, this may indicate that the persons involved belong to a transmission chain (53).

In conclusion, it was observed that there was a relationship between individuals 2 and 3, and individual 1 is suggestive of being the source of infection for both, due to the estimation of tMRCA. This would refute the a priori hypothesis of HIV transmission from individual 3 to 1, however, there is an epidemiological relationship between the two. However, caution is still needed regarding the interpretation of potential court findings (53).

Given this study, it is observed that the reliability of phylogenetic analysis to "prove" the transmission of HIV between individuals should be approached as detailed as possible (25). Therefore, there is a need for caution in producing technical evidence that should always be analyzed in it is a probative set, as it does not have a superior hierarchical position in the judicial evaluation, but the technical tool enjoys great influence in today's society since it carries the credibility of what is scientific (54).

Phylogenetic analysis is generally performed in research laboratory settings rather than in forensic laboratories. Therefore, it is crucial to maintain the chain of custody, ensuring quality and care when handling the analyzed samples to minimize the possibility of errors such as contamination and labeling and in carrying out the scientific methods involved, which will be reported as an expert report and will be final client to Justice (25, 55).

Another important issue is that if the chosen laboratory has no forensic experience, it is the applicant's task to emphasize the importance of the double-blind test, ie the analyst performing the examination should not be aware of the proposed transmission direction and the other circumstances of the case. Therefore, each person's samples should be tested in two independent laboratories under "blind" conditions, thus eliminating the possibility of laboratory error and investigator bias, thus yielding consistent results (25).

Sample tracking is also essential, so it is emphasized once again that chain of custody maintenance should be given the highest priority, ensuring the authenticity and suitability of expert evidence, thus ensuring evidence tracking from the crime scene to court, strict protocols on evidence being applied (56).

There are many ways to build a phylogenetic tree. Consideration should be given to the reliability of the methods used for this training - including the HIV-specific genes analyzed - as well as the purpose of the tree. It must be as impartial as possible. This includes choosing sufficient and adequate epidemiological controls, analyzing approximately thirty other strains of HIV from individuals who are of the same geographical origin, social context, and potential transmission network as defendant and victim(s) (25).

It has recently become common practice to also include publicly similar sequences selected as database controls using BLAST search. At least ten control sequences must be added to search (40). These should then be compared with the strains being investigated. Using inadequate controls may erroneously emphasize any detected relationship between two viruses as being remarkably unique (25,53).

Also, controls must be collected at the time of the alleged broadcast event. This is crucial in building very complex networks. In most cases, it will be difficult and often impossible to obtain samples from the appropriate controls. As a result, the interpretation of the findings will need to be particularly cautious (25).

It is important to remember that similar strains can be found in more than two individuals if both are part of a broader transmission network, which is very common among individuals with HIV. Consequently, even with controls, phylogenetic analysis cannot "prove" transmission. However, when there is statistical support to link the investigating individual closer to one of the controls rather than the complainant, the technique is reliable enough to exclude the possibility of transmission (25).

It is understandable that in such a case the victim and society want a crime involving HIV infection to be punished, however, no unfair conviction must occur in a court case. Therefore, forensic investigations with phylogenetic and other evidence related to the transmission of this virus are potentially powerful to acquit suspects (25, 57).

The issue of drug resistance due to virus mutations is generally removed from alignments before investigations of HIV transmission in phylogenetic analyzes (25), as viral populations can respond quickly and efficiently to environmental disturbances in which they replicate, offering a wide spectrum of mutations on which natural selection can act (44). Therefore, when there are changes in the environment, for example by administering antiviral drugs, the presence of one or more mutants more able to replicate in this new medium causes the population derived from these mutants to gain resistance and increase their chances of survival. In the case of HIV-1, selective pressure exerted constantly by the immune system results in the virus adapting to new target cells and maintaining a persistent infection (44).

Everyone involved in the criminal justice system must be aware of the limitations of phylogenetic analysis before using such evidence as conclusive or even suggesting HIV transmission between individuals, but all results obtained must be of the highest quality (25, 57). Phylogenetic analysis can and does include some degree of approximation and error (25). However, phylogenetic investigations have proven useful for analyzing HIV transmission in forensic expertise, and many promising advances in research may enable its use in future cases (40).

Conclusions

Forensic microbiology, despite being a new area within forensic science, is of great relevance for the establishment of technical proof of crimes. In investigating the intentional transmission of HIV / AIDS it is

possible to rely on phylogenetic methods, which traces the evolutionary relationship between the viral strains of the victim and the suspect, revealing the path of infection and establishing the causal link between conduct and outcome.

In addition to this correlation, it is also possible to determine the timing of HIV infection in the individuals involved in the case, also establishing the temporality of the crime. Two other issues that should also be considered in these investigations are the quantification of viral load and CD4+ T lymphocytes. With these measures, it is possible to decrease the chances of an individual contributing to transmission in a criminal case.

Therefore, through the use of these tools, it is possible to determine whether there is a crime, and what path the criminal agent takes. Thus, it becomes possible to specify, in this case, authorship and materiality of the fact, generating greater reliability for the State's duty to punish.

References

1. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S. HIV virology and pathogenetic mechanisms of infection: a brief overview. Ann Ist super Sanità. 2013, v. 49, n. 3, p. 255–260.
2. Pa L. Human immunodeficiency viruses and their replication; 3rd. ed. Philadelphia, Lippincott-Raven: [s.n.], 1996.
3. Tenório LZ, Silva FH, Han SW. A Potencialidade dos Lentivetores na Terapia Gênica. Revista Brasileira de Clínica Médica. 2008, p. 260–267.
4. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harbor Perspectives in Medicine. 2011, v. 1, n. 1, p. 1–22.
5. Winslow CY, Kedel FA. Human immunodeficiency virus. Dermatological Manifestations of Kidney Disease. 2015, p. 45–56.
6. Hahn BH, et al. Scientific and Public Health Implications. Science. 2000, v. 287, n. 5453, p. 607–614.
7. Kalish ML, et al. Central African hunters exposed to simian immunodeficiency virus. Emerging Infectious Diseases. 2005, v. 11, n. 12, p. 1928–1930.
8. Marx PA, Alcabes PG, Drucker E. Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa. Philosophical Transactions of the Royal Society B: Biological Sciences. 2001, v. 356, n. 1410, p. 911–920.
9. Loreto S, Azevedo-Pereira JM. A infecção por HIV–importância das fases iniciais e do diagnóstico precoce. Acta Farmacêutica Portuguesa. 2012, v. 1, n. 2, p. 5–17.
10. Huynh K, Gulick PG. HIV Prevention. StatPearls, 2019.
11. Brasil - MS. Brasília-DF 2018 Diagnóstico da Infecção pelo HIV em Adultos e Crianças. 2017, p. 85.
12. Tortora G J, Funke BR, Case CL. Microbiologia. 12ª ed. Pearson, USA, 2016.
13. UNAIDS. Desafio Unaids. 2017.
14. Pereira C, Ferrara AP, Tomio AR. Prevenção, tá combinado? Jovens e Adultos Vivendo com HIV/Aids, Prevenção e Cidadania. 2018.
15. Brasil. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em crianças e adolescentes. 2014. http://www.aids.gov.br/pt-br/tags/publicacoes/protocolo-clinico-e-diretrizes-terapeuticas.
16. Asts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. Cold Spring Harbor Perspectives in Medicine. 2012 Apr;2(4):a007161. doi: 10.1101/cshperspect.a007161.
17. Jota FA. Os Antirretrovirais Através da História, da Descoberta até os Dias Atuais. 2011.
18. Brasil. Medicamentos antirretrovirais. The New England journal of medicine. 2013, p. 1.
19. Zucchi EM, et al. Da evidência à ação: desafios do Sistema Único de Saúde para ofertar a profilaxia pré-exposição sexual (PrEP) ao HIV às pessoas em maior vulnerabilidade. Cadernos de Saúde Pública. 2018, v. 34, n. 7, p. 1–16.

20. CDC. Pre-Expousure Prophylaxis (PrEP). http://www.cdc.gov/hiv/risk/prep/index.html
21. Waldman AE. Exceptions: The Criminal Law's Illogical Approach to HIV-Related Aggravated Assaults. New York Law School, 2011.
22. Furini AAC, et al. HIV/AIDS: relação dos níveis de linfócitos TCD4+ e carga viral com o tempo de diagnóstigo. Arq. Ciênc. Saúde, 2016, v. 23, n. 4, p. 95-98. <http://www.cienciasdasaude.famerp.br/index.php/racs/article/view/491>. doi: https://doi.org/10.17696/2318-3691.23.4.2016.491.
23. Rouxx C, et al. From Forensics to Forensic Science. Current Issues in Criminal Justice. 2012, v. 24, n 1, p.7-24. https://doi.org/10.1080/10345329.2012.12035941
24. Abrantes FEB. Inovações tecnológicas da ciência forense e suas implicações jurídicas. 2014. www.ambito-juridico.com.br/site/?n_link=revista_artigos_leitura&artigo_id=19543&revista_caderno=27.
25. Bernard EJ, et al. HIV forensics: Pitfalls and acceptable standards in the use of phylogenetic analysis as evidence in criminal investigations of HIV transmission. HIV Medicine. 2007, v. 8, n. 6, p. 382–387. doi: 10.1111/j.1468-1293.2007.00486.x
26. Senés-Guerrero C, Schüssler A. A conserved arbuscular mycorrhizal fungal core-species community colonizes potato roots in the Andes. Fungal Diversity. 2016, v. 77, n. 1, p. 317–333. doi: 10.1007/s13225-015-0328-7
27. Kenah E, et al. Molecular Infectious Disease Epidemiology: Survival Analysis and Algorithms Linking Phylogenies to Transmission Trees. PLoS Computational Biology. 2016, v. 12, n. 4, p. 1–29. https://doi.org/10.1371/journal.pcbi.1004869
28. Baele G, et al. Emerging concepts of data integration in pathogen phylodynamics. Systematic Biology. 2017, v. 66, n. 1, p. e47–e65. doi: 10.1093/sysbio/syw054.
29. Chang AB, et al. Phylogeny as a guide to structure and function of membrane transport proteins. Molecular Membrane Biology. 2004, v. 21, n. 3, p. 171–181. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S1409-00152019000200056&lng=en&tlng=es.
30. Carrillo-Araujo M, et al. Phyllostomid bat microbiome composition is associated to host phylogeny and feeding strategies. Frontiers in Microbiology. 2015, v. 6, n. may, p. 1–9.
31. Matsen FA. Phylogenetics and the human microbiome. Systematic Biology, v. 64, n. 1, p. e26–e41, 2015. https://doi.org/10.1093/sysbio/syu053
32. Caldart ET, et al. Phylogenetic Analysis: Basic Concepts and Its Use as a Tool for Virology and Molecular Epidemiology. 2016, v. 44, n. Sep, p. 1392, 2016. https://doi.org/10.22456/1679-9216.81158
33. Zweigerdt R. Molecular Phylogenetics: Concepts for a Newcomer. Advances in Biochemical Engineering/Biotechnology. 2016, p. 1–35. doi: 10.1007/10_2016_49
34. França LTC, et al. A review of DNA sequencing techniques. Quartely Reviews of Biophysics, 2002. https://doi.org/10.1017/S0033583502003797
35. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Biochemistry. 1977, v. 74. DOI: 10.1073/pnas.74.12.5463
36. Alberts B, et al. Biologia molecular da célula. 6. ed. Porto Alegre: Artmed, 2017.
37. Alvarez-Cubero MJ, et al. Next generation sequencing: an application in forensic sciences? Annals of Human Biology. 2017, v. 44, n. 7, p. 581–592. doi: 10.1080/03014460.2017.1375155
38. Castro-Nallar E, et al. The evolution of HIV: Inferences using phylogenetics. 2013, v. 2012, n. 2, p. 777–792. doi: 10.1016/j.ympev.2011.11.019
39. Alves BM, et al. Estimating HIV-1 Genetic Diversity in Brazil Through Next-Generation Sequencing. Frontiers in Microbiology. 2019, v. 10, n. Apr, p. 1–11. https://doi.org/10.3389/fmicb.2019.00749
40. Abecasis AB, Pingarilho M, Vandamme AM. Phylogenetic analysis as a forensic tool in HIV transmission investigations. Aids. 2018, v. 32, n. 5, p. 543–554. doi: 10.1097/QAD.0000000000001728

41. La, C. et al. Viral diversity from next-generation sequencing of HIV-1 samples provides precise estimates of infection recency and time since infection Louisa. "Applied Catalysis A, General". 2019, n. 2010, p. 1–19. doi: 10.1093/infdis/jiz094
42. Sosin DV, et al. Molecular mechanisms oh HIV-1 Genetic Diversity. Molecular Biology (Mosk), 2017, 51(4):547-560. doi: 10.7868/S0026898417030156
43. Cuevas JM, et al. Extremely High Mutation Rate of HIV-1 In Vivo. PLOS Biology, 2015, 16, 13(9):e1002251. doi: 10.1371/journal.pbio.1002251
44. Pinto ME, Struchiner CJ. A diversidade do HIV-1: uma ferramenta para o estudo da pandemia. Cadernos de Saúde Pública. 2006, v. 22, n. 3, p. 473–484. http://dx.doi.org/10.1590/S0102-311X2006000300002
45. Andrews SM, et al. Recent advances in understanding HIV evolution. F1000 Reserch, 2017, 28, 6:597. doi: 10.12688/f1000research
46. Scaduto DI, et al. Source identification in two criminal cases using phylogenetic analysis of HIV-1 DNA sequences. PNAS. 2010, 14, 107(50), p.21242-7. doi: 10.1073/pnas.1015673107
47. Rambaut A, et al. The causes and consequences of HIV evolution. Nature Reviews Genetics. 2004, 5(1), p.52-61. DOI: 10.1038/nrg1246
48. Faria NR, et al. The early spread and epidemic ignition oh HIV-1 in human populations. Science. 2014, 3, 346(6205). p-56-61. doi: 10.1126/science.1256739
49. Bezemer D, et al. Dispersion of the HIV-1: Epidemic in Men Who Have Sex with Men in the Netherlands: A Combined Mathematical Model and Phylogenetic Analysis. PLOS medicine. 2015. https://doi.org/10.1371/journal.pmed.1001898
50. Pineda-Peña AC, et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: perfomance evaluation of the new REGA version 3 and seven other tools. Elsevier. 2013, 19, p.337-48. doi: 10.1016/j.meegid.2013.04.032
51. Romero-Severson EO, et al. Phylogenetically resolving epidemiologic linkage. PNAS. 2016, 113 (10), p.2690-2695. https://doi.org/10.1073/pnas.1522930113
52. Eishleman SH, et al. Analysis of genetic linkage of HIV from couples enrolled in the HIV Prevention Trials Network 052 trial. The Journal of Infectious Diseases. 2011, 15, 204(12), p.1918-26. doi: 10.1093/infdis/jir651
53. Siljic M, et al. Forensic application of phylogenetic analyses – Exploration of suspected HIV-1 transmission case. Forensic Science International: Genetics. 2017, v. 27, p. 100–105. doi: 10.1016/j.fsigen.2016
54. Puerari DN, PORTES MAA, GARRIDO, RG. Autonomia Da Prova Técnica No Processo. Colloquium Humanarum, v. 11, n. Especial, p. 459–464, 2015.
55. Giovanelli A, Garrido, RG. A perícia criminal no Brasil como instância legitimadora de práticas policiais inquisitoriais. Revista LEVS/UNESP. 2011, v. 7, p. 5–24. http://revistas.marilia.unesp.br/index.php/levs/article/view/1672
56. Garrido RG, Giovanelli A. Ciência Forense Uma Introdução à Criminalística. 2. ed. Rio de Janeiro: Projeto Cultural, 2015. v. 1. 214p.
57. Learn GH, Mullins JI. The microbial forensic use of HIV sequences. HIV Sequence Compendium 2003, p. 22–37. https://www.hiv.lanl.gov/content/sequence/HIV/COMPENDIUM/2003/partI/Learn.pdf
58. Landi A, et al. Modelling and control of HIV dynamics. 2007, v. 9, p. 162–168. DOI:10.1016/j.cmpb.2007.08.003