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High viral load is necessary to detect human immunodeficiency virus by polymerase chain reaction in blood-soiled needles

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The estimated risk of contamination through percutaneous injuries involving HIV-contaminated sharp objects is 0.32%. It is well known that a high viral load is one of the conditions that increase the risk of HIV-1 transmission in occupational accidents. In order to estimate the level of viral load that could be necessary for HIV-1 transmission to health care workers undergoing occupational accidents with contaminated needles, blood-soiled needles were obtained from HIV-positive patients just after drawing blood to determine HIV-1 viral load by NASBA. Detection of HIV-1 genome was performed by reverse-transcription polymerase chain reaction (RT-PCR) after RNA extraction directly wash-outs collected from needles immediately after blood drawing. Samples collected from needles used on 102 HIV-1-positive patients were RT-PCR tested but only 7 were HIV-1-positive. Each patient sample was compared to its respective viral load. All 7 RT-PCR-positive samples were from patients with viral loads higher than 160,000 RNA copies/mm^3. Since the needle samples tested here could be considered to be of very high risk of HIV-1 transmission following an occupational accident, RT-PCR detection of HIV-1 genome was only possible in patients with high viral load. These results indicate that, among all risk factors, high HIV-1 viral load probably is the most important risk factor for HIV transmission to health care workers during occupational exposure.

Key words: HIV, viral load, polymerase chain reaction (PCR), RNA, occupational accidents.

INTRODUCTION

Since HIV/AIDS was first identified 30 years ago, more than 60 million people have been infected and approximately 30 million people have died (Jiang et al., 2014). The HIV/AIDS has become one of the greatest

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Table 1. Characteristics of 102 patients enrolled in the study.

Variable	Patient (%)
Median Age (years)	37.5 (10-66)
Male	65 (63.7)
Female	37 (36.3)
CD4+ cells/mm3 (median)	327.5
CD4 < 200 cells/mm3	34 (33.3)
Viral load (median, log10)	4.46
Undetectable viral load	29 (28.4)
≤ 150,000 RNA copies/mL	58 (56.8)
≥150,000 RNA copies/mL	15 (14.7)

problems of public health worldwide (Kopfer and McGovern, 1993) and occupational accidents with blood or body fluids may pose a high risk to health care workers (HCWs). Both blood-borne hepatitis viruses (hepatitis B [HBV] and hepatitis C [HCV] viruses) and the human immunodeficiency virus (HIV) represent the vast majority of cases of occupational infection (Garcia and Facchini, 2009). Prüss-Üstün et al. (2005) reported that the total number of infections attributable to injuries with sharp instruments, due to occupational exposure to percutaneous injuries, was 16,000 HCV, 66,000 HBV and 1,000 HIV infections among HCWs. After percutaneous occupational exposure of infected blood, the highest risk for HIV infection is 0.3% and after mucosal exposure is 0.09% (Ippolito, 1993). However, among all risk factors, the ones associated with the highest risk are exposure to a large quantity of blood from the source patient, as indicated by a device (e.g., a needle) visibly contaminated with the patient's blood; a procedure involving a needle placed directly in a large vein or artery; and a deep injury (Carpenter, 1997). However, the exact amount of HIV-1 remaining in these needles has not been measured.

The detection of HIV-1 RNA copies and their use as a viral load is just a surrogate measure of the viral titer, assessing only the amount of cell-free virus in the peripheral blood, but it has been assumed that a low viral load (e.g., < 1,500 RNA copies/mL) or one that is below the limit of detection is probably associated with a lower risk of HIV transmission, but it does not rule out the possibility of viral infection (CDC, 2005). Measurements of HIV-1 viral loads may play an important role in assessing the infectivity of the source patient in the context of an occupational exposure.

Based on this assumption and the high sensitivity of reverse-transcription polymerase chain reaction (RT-PCR), we investigated the ability of RT-PCR to detect HIV-1 genome in the whole blood retained in needles used to collect blood for HIV-1 viral load measurements, since this approach mimics the occupational exposures associated with the highest risk for HIV-1 infection. We also compared the RT-PCR results with the viral load of these patients, aiming at the definition of which viral load level posed the highest risk to HCWs after an occupational exposure.

MATERIALS AND METHODS

We analyzed blood-soiled needles used on HIV-1 positive patients, just after blood drawing to determine the HIV-1 viral load. Viral load was measured by the NASBA assay according to manufacturer's protocol, and reported as the number of HIV-1 RNA copies/mL. Patients were enrolled at the Blood Collection Center of the Hospital das Clínicas da Faculdade de Medicina de RibeirãoPreto, São Paulo State, Brazil, after signing a written consent form.

We collected blood samples, one by one, directly from the veins of the patients using sterile, previously unused needles (25×8 mm; Becton Dickinson, USA) after blood drawing, washed with 140 µl phosphate-buffered saline (PBS), and then properly discarded. PBS washings were transported to the virology laboratory under refrigeration where the RNA was extracted from the samples. RNA extraction was done with the QIAamp RNA viral kit (QIAGEN, Inc., Valencia, California, USA) according to the manufacturer's instructtions. The first cDNA strand was synthesized in a 20 µL final volume containing 0.1mMdNTP (Pharmacia, Piscataway, NJ), 7.5 U of Superscript™ (Invitrogen, Carlsbad, CA), 4 µL of 5X reaction buffer (Invitrogen, Carlsbad, CA), 25 pmol of random primers (pdN6; Pharmacia, Piscataway, NJ) and 11 µL (in average, 2.4 µg) of total RNA. The mixture was then incubated at 42°C for 1 h, and then for 1 min at 70°C to denature the reverse transcriptase. Amplification of HIV-1 was performed with two sets of primers in a two-step nested RT-PCR strategy using the primers described by Rezende et al. (2002). In our laboratory, this RT-PCR protocol had an analytical sensitivity of 500 RNA copies/mL (data not shown).

The study was conducted from December 2000 to May 2001, and it was approved by the Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina de RibeirãoPreto. Clinical data of the patients were blindly obtained by chart review and correlated with the PCR results.

RESULTS

Samples of 102 patients were studied, 63.7% male and median age of 37.5 years. Most recent examination showed median CD4 count of 327.5 cells/mm^3, undetectable viral load in 29 patients and median viral load of 4.46 log10 in the rest of them (Table 1). Most patients had antecedent opportunistic infection (66%), CD4 nadir below 200 cells/mm3 (73%) and two of them had been diagnosed recently. Zidovudine, lamivudine and indinavir were the antiretroviral drugs most commonly used.

Out of 102 PBS washings, RT-PCR detected only 7 samples positive for HIV-1, and in these samples, the viral load ranged from 160,000 to 870,000 HIV-1 RNA copies/mL (Table 2). RT-PCR negative results included 29 patients with undetectable viral load (below 50 RNA copies/mL), 58 patients with viral load below 150,000 copies/mL, and 8 patients with viral load higher than 150,000 copies/mL (Table 1). All patients with viral load higher than 150,000 copies/mL were classified in the CDC category C3.

DISCUSSION

Our results show that most high viral load is considered a

Table 2. Viral load measurements on HIV-positive samples by RT-PCR.

Patient	Viral load (copies/mL)
1	160,000
2	210,000
3	220,000
4	300,000
5	530,000
6	620,000
7	870,000

risk factor for HIV infection on occupational exposures thus, we have investigated the presence of HIV-1 remaining on needle-retained blood samples of 102 HIV-positive patients through the ability of PCR to detect HIV-1 genome. This approach mimics an occupational exposure to a hollow-bore needle that had been used for accessing a large blood vessel, and where there would still be blood present on needles. Garcia and Facchini (2009) found that percutaneous exposures occur more often than the mucocutaneous exposures, and also needles were usually more frequently involved in percutaneous exposures. While preventing exposures to blood and body fluids is the primary means of preventing occupationally HIV infection, appropriate post-exposure management is an important element of workplace safety (CDC, 2005), and knowing the viral load at the moment of the occupational accident might give us an idea of the risk of HIV-1 transmission involved in the accident.

To reach our goal, we investigated which levels of HIV-1 viral load, according to NASBA values, would be detected by a RT-PCR protocol used in our laboratory that readily detects RNA copies as low as 500 copies of HIV-1 RNA/mL. NASBA values were used for comparison due to the fact that it is the technique elected by the Brazilian Government to measure the HIV-1 viral loads of patients followed at the AIDS clinics. The rational for this study is that both molecular assays are readily available in our hospital, and RT-PCR could be used to assess the risk for a possible HIV-1 contamination during occupational exposures. Our results show that RT-PCR results showed a good correlation with the viral load obtained on the same sample, since RT-PCR was positive only in patients with high viral loads, ranging from 160,000 to 870,000 HIV-1 RNA copies/mL. The fact that more than half of the samples with high viral load were RT-PCR negative is probably due to the amount of blood that was present on the needles.

The size of the inoculum is also an important factor in the assessment of a possible risk of infection by a microorganism. The HIV-1 inoculum associated with occupational exposure is yet to be determined. Based on the facts that the lowest HIV-1 viral load detected by RT-PCR was 160,000 HIV-1 RNA copies/mL and that in a needle stick injury, the volume of the inoculum ranges from 10 to 100 µL (Kopfer and McGovern, 1993) the data acquired in this study show that the viral titer in the patients' needles ranged from 1,600 to 16,000 virus/mL. This finding is important because it suggests that viral load as low as 1,600 virus/mL may be implicated in HIV-1 transmission after occupational exposures.

Heimer et al. (1992) demonstrated that PCR is a suitable technique to detect HIV-1 proviral DNA in needles used in injecting drug users since they were able to detect the HIV-1 proviral DNA, especially when there was visible blood in the returned needle. In this study, instead of detecting the proviral DNA, we tried to detect the HIV-1 RNA genome on the blood that was still present on the needles used for collecting blood from HIV-1 patients. Thus, the data presented here differ from those previously published and provides a rough estimate of what happens in a real occupational accident. Even though our study may be criticized due to the fact that it did not use the RT-PCR to produce a quantitative data on the HIV-1 levels contaminating the needles used to draw blood from our patients, the correlation with NASBA measurements agrees with other studies showing that the higher the source of patient's viral load, the higher the risk of acquiring HIV-1 after a percutaneous accident. Prüss-Üstün et al. (2005) reported that 4.4% of HIV infections in HCWs globally were due to sharps injuries. Precise worldwide data are not available on the annual number of needle sticks and other percutaneous injuries among HCWs. However, estimates indicate that 600,000 to 800,000 of such injuries occur annually in the United States (NIOSH, 1999), but only a few cases of HIV-1 infection following occupational accidents have been described, probably due to the amount of virus present in the needles involved in the accident. According to the CDC (2001), 23,951 cases of AIDS among HCWs were reported, representing 5% of the 469,850 adults or adolescents with AIDS. In 57 HCWs with documented occupationally acquired HIV infection, most (86%) were exposed to blood and most (88%) had percutaneous injuries, but the amount of virus in the device involved in the accident has never been investigated as it was done on this study. In this study, HIV-1-specific RT-PCR was positive only in patients with high viral loads, suggesting that this low RT-PCR positivity could be related to a low amount of virus retained in the needles and this could explain why there are few cases of HIV-1 infection following the accidents, since the immune response could more easily manage to control the infection resulting from a small amount of viruses. The small amount of viruses would also be more readily eliminated with specific anti-retroviral treatment. Furthermore, our data presents a cut-off (160,000 HIV-1 RNA copies/mL) that can be used in future studies set out to determine the risk of HIV-1 infection following occupational exposures, and from the these data, it can also be inferred that easy access to Highly Active Antiretroviral Therapy (HAART) and stimulating patient's good adherence to treatment will result in lower viral load and will make it more difficult for HIV-1 transmission to occur following an occupational exposure.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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