

Evaluation of quantification method of HTLV-1 proviral load in white blood cells using whole blood

Sato T^{1,2}, Yagishita N¹, Araya N¹, Yamauchi J¹, Takahashi K¹, Kunitomo Y¹, Hasegawa Y¹, Higashikuse Y³, Miyachi K³, Yamano Y^{1,2}

¹ Institute of Medical Science, St. Marianna University School of Medicine.

² Division of Neurology, Department of Internal Medicine, St. Marianna University School of Medicine.

³ Minaris Medical Co., Ltd.

Background:

HTLV-1 proviral load (PVL) is an important biomarker for risk assessment of developing adult T-cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in asymptomatic carriers. Therefore, robust and reliable PVL data is important. However, the current method for determining PVL in peripheral blood mononuclear cells (PBMCs) has a problem. The PVL decreases due to neutrophil contamination if it takes a few days before PBMC isolation. Special blood collection tubes and reagents that overcome this problem are available in the market, but they are costly. Therefore, we investigated the feasibility of quantification method of PVL in white blood cells (WBCs) using whole blood without isolating PBMCs.

Methods:

1) Both “EDTA whole blood” and “heparin whole blood” were collected from 7 HTLV-1-infected individuals. They were stored at -20°C, 4°C, and room temperature for 0, 1, 2, and 3 days. DNA extraction was performed, and the PVLs were measured and compared with the value on Day 0. 2) Both “EDTA whole blood” and “PBMCs isolated from the Vacutainer CPT blood collection tube[®] within two hours” were collected from 31 HTLV-1-infected patients at a medical institution, and their specimens were frozen and collected via air transportation. From the extracted DNAs, the PVLs were quantified. The PVLs in WBCs were compared with that in PBMCs. For comparison, PVL in WBCs was corrected using 40% or the proportion of the mononuclear cell fraction. 3) Both “EDTA whole blood” and “PBMCs isolated in a clinical test company” were collected from 37 HTLV-1-infected patients at some medical institutions in Japan and the same methods as in 2) were performed.

Results:

1) PVL data obtained from EDTA whole blood had less variation than those from heparin whole blood. The most stable PVL data were obtained from EDTA whole blood stored at -20°C. 2) The PVLs in WBCs corrected by the proportion of the mononuclear cell fraction were highly correlated with the PVLs in PBMCs ($r = 0.967$). 3) Even when collected from all over Japan, the PVLs in WBCs corrected by the proportion of the mononuclear cell fraction were highly correlated with those in PBMCs ($r = 0.958$). It was suggested that PVLs from WBCs and PBMCs are convertible.

Conclusion:

By quantifying PVL in WBCs using whole blood without separating PBMCs, robust and reliable PVL data can be obtained regardless of the location of the sample

collection. In addition, by using the conversion formula, it is assumed that the PVL data in PBMCs accumulated so far can be utilized.

Disclosure of Interest Statement:

The authors declare no conflict of interest.