Diagnostic Validity of Combining HTLV-1 Serology and Immunophenotyping in Adult T-Cell Leukemia

Kazuyuki Sugahara1), Kazuto Tsuruda1), Yasuaki Yamada1,2), Youichi Hirakata1,2), Sunao Atogami3), Hisashi Sohda3), Kunihiro Tsukazaki3), Masao Tomonaga3) and Shimeru Kamihira1,2)

1) Central Diagnostic Laboratory
2) Department of Laboratory Medicine
3) Department of Haematology, Atomic Disease Institute, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852, Japan.

Adult T-cell leukemia (ATL) is heterogeneous and sometimes equivocal to other T-cell neoplasms. Detecting anti-HTLV-1 antibodies is significant for a first screening not only for HTLV-1 infection but also for the HTLV-1-related disorders of ATL and TSP/HAM. The purpose of the present study was to investigate the diagnostic validity of HTLV-1 serology in ATL. The serologic results by a gelatin particle agglutination (PA) assay were highly sensitive (100%) and specific (99.5%) for the results of polymerase chain reaction (PCR) assay in 666 healthy blood donors who live in an area endemic for the HTLV-1 virus. Of 7,536 hospitalized patients, 189 patients with ATL were serologically screened. There were 1,140 patients (15.2%) infected by chance with HTLV-1, showing specificity, sensitivity, positive predictive value (PV), and negative PV of 84.3%, 100%, 14.2%, and 100%, respectively. Since the low positive PV (14.2%) was useless, we tried combining the anti-HTLV-1 assay with the immunophenotyping necessary for the diagnosis of lymphoid neoplasms. This combination gave nearly 100% positive and negative PV, and could prove to be useful in diagnosing ATL with the probability of 98%, especially for epidemiologic studies.

Key words: sensitivity, specificity, predictive value, ATL, HTLV-1, anti-HTLV-1

Introduction

The human T-cell leukemia virus type-1 (HTLV-1) was first identified as the causative agent of adult T-cell leukemia (ATL) in 1980 by the Gallo group4). Since then, several sero-epidemiologic studies4-13) have elucidated the close etiological relationship between the HTLV-1 virus and disorders such as tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) and others of unknown etiology. There are many apparently healthy persons without any clinical symptoms who are infected with HTLV-13,4). Among these carriers, only 1 of 1,000-1,500 develops ATL each year, and the cumulative occurrence rate of this disease is estimated at about 3 to 5% in the carrier's lifetime14). With the development of immunophenotypical diagnosis of lymphoid neoplasms, the discrimination of ATL from T-cell neoplasms without HTLV-1 has recently been proposed by the international lymphoma study group (REAL classification)15). Although they have classified T-cell neoplasms into ATL and non-ATL, it is often difficult to discriminate between ATL and other T-cell neoplasms using routine laboratory methods11,12).

Accordingly, we were prompted to quantitate the validity of the serologic HTLV-1 test as not only a marker of persistent infection but also as a diagnostic tool for ATL. In the present study, using a gelatin particle agglutination (PA) test, we reviewed the clinical HTLV-1 serology of 7,536 patient samples, including 189 ATL patients who were diagnosed by immunophenotyping and molecular analysis. The objectives of this study were to: (1) discriminate ATL immunophenotypically and molecularly from other T-cell neoplasms without HTLV-1 in an area endemic for the virus, (2) quantitatively evaluate the diagnostic validity of antibody detection in diagnosing ATL, and (3) establish objective minimal tests to be used in the diagnosis of ATL using common laboratory assays.

Materials and Methods

Subjects.

Serum samples were collected from 666 healthy blood donors of residents in an area endemic for HTLV-1 and from 7,536 patients who were admitted or referred to our hospital from January 1991 to November 1995. The diagnosis of peripheral T-cell lymphoma/leukemia including ATL was based on a complete hematological battery of May-
Grunwald stained smears, hematoxylin-eosin and immunohistochemistry, and immunophenotyping with flow cytometric techniques. ATL was diagnosed according to the Japan Lymphoma Study Group (JLSG) guidelines. In some of the T-cell neoplasm cases, especially those resembling ATL, the diagnosis of ATL was reached after Southern blot analysis of HTLV-1 monoclonal integration. Cells for immunophenotyping B- or T-lineage were obtained from heparinized peripheral and bone marrow aspirated blood and biopsied lymph nodes.

Detection of anti-HTLV-1 antibody and the provirus

Anti-HTLV-1 antibodies were first screened by a gelatin PA kit (Serodia-HTLV-1, Fujirebio, Tokyo). Some samples, including the indeterminates, were screened again using an ELISA kit (Kyowa Medix, Tokyo) and Western blot kit (Fujirebio) as supplementary confirming tests. All assays were done according to the manufacturer’s instructions and evaluated by the criteria of the kits. To detect HTLV-1 provirus in peripheral blood from the blood donors, a commercially available PCR kit (Amplicor HTLV-1 and -2 Detection kit, Roche, USA) was used.

Molecular analysis of HTLV-1 integration

Determination of the HTLV-1 monoclonal integration in leukemic cells from the blood or lymph node suspensions was performed as previously described. Briefly, high molecular-weight genomic DNA was extracted from peripheral and bone marrow mononuclear cells and lymph node suspension cells in patients with T-cell neoplasm and anti-HTLV-1. Next, 10 μg of DNA was digested with EcoR1 restriction enzyme and were size-fractionated on 0.7% agarose gels. They were then transferred by the Southern blot technique onto a nylon membrane. Hybridization to randomly primed 32P- or digoxigenin-labeled DNA probes of the pX region was performed.

Phenotypical examination of tumor cells

We cytophenotypically and immunophenotypically examined all of the lymphoid neoplasms. ATL cells were cytologically defined by their morphologic features on the blood film stained with May-Grunwald Giemsa, characterized by polymorphic mature cells with convoluted or poly-lobulated nuclei, per the FAB criteria and JLSG guidelines.

Immunophenotyping was performed by standard flow cytometry in a Cytorun (Ortho Diagnostics KK, Tokyo) by using single- and double-labeling with the following monoclonal antibodies (MoAb): CD2, CD3, CD4, CD5, CD7, CD8, HLA-DR, CD25, CD10, CD19, and CD20. A marker was considered positive when it stained more than 30% of the cells above the controls. The immunophenotypes were classified into two main categories: typical (CD4+·CD8-) and unusual (CD4 and/or CD8 antigen abnormalities), based on the criteria of Kamihira et al. The statistical analysis was performed using the FISHER software package (Nakayama Syoten, Tokyo).

Results

1) Anti-HTLV-1 detection in blood donors and patients.

HTLV-1 serologic tests have been routinely conducted in our hospital since 1986 by the PA assay. In the present study, we re-evaluated the PA assay in comparison with the PCR method, and quantitated the diagnostic validity in practical samples from patients with various diseases. As shown in Table 1, only 2 of the 666 samples were discrepant, i.e., seropositive and PCR-negative.

Of the two samples, Western blot analysis revealed a negative finding in one sample (no. 491) and indeterminate in another one (no. 433) which had strong p19 and faint p24 bands (Fig. 1).

The sensitivity and specificity between PA and PCR were calculated as 100% (95% CI, 89.8-100) and 99.5% (95% CI, 98.9-100), respectively. The PA assay actually gave positive results in 1,329 (17.8%) and negative results in 6,307 (82.4%) of the 7,536 routine patient samples tested. We then reviewed the diagnosis of the subject patients from the files of our hospital.

2) T-cell neoplasms and anti-HTLV-1 distribution

Out of the 7,536 patients, 208 were diagnosed as having T-cell neoplasms; 189 patients with ATL and 19 with T-cell malignant lymphoma (T-ML), which included 5 cases of Sezary’s syndrome and mycosis fungoides. All of the 189 ATL, 4 (21.5%) of the 19 T-ML, and 1,136 (15.5%) of the other patients were seropositive for HTLV-1, as shown in Table 2.

One hundred sixty four (86.5%) of ATL had immunophenotypically prototypic helper-T immunophenotype with CD2+, CD3+, CD4+, CD7-, CD8-, CD25+, and HLA-DR+.

Table 1. Serologic (PA) and PCR (Roche) results in 666 healthy blood donors who live in an area endemic for HTLV-1 infection.

<table>
<thead>
<tr>
<th>subjects tests serology</th>
<th>PCR</th>
</tr>
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<tbody>
<tr>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>HTLV-1 HTLV-2</td>
<td>HTLV-1 HTLV-2</td>
</tr>
<tr>
<td>healthy donors</td>
<td>666</td>
</tr>
<tr>
<td>sensitivity</td>
<td>100% (95%CI, 89.8-100)</td>
</tr>
<tr>
<td>specificity</td>
<td>99.5% (95%CI, 89.8-100)</td>
</tr>
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</table>
Fig 1. Western blot analysis of the two samples discrepant between the PA and PCR assays.
Lane 1: positive control
Lane 2: weak positive control
Lane 3: negative control
Lane 4: sample of no. 491 (negative)
Lane 5: sample of no. 433 (indeterminate)
Lane 6: a representative positive sample

Table 2. Serological anti-HTLV-1 Distribution by the PA assay in ATL, peripheral T-cell Lymphomas not associated with HTLV-1, and other disorders.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>anti-HTLV-1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) ATL*</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>(1) proto-typic</td>
<td>164</td>
<td>0</td>
</tr>
<tr>
<td>(2) unusual CD4 DP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>DN</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>b) T-ML without HTLV-1</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>c) Others</td>
<td>1136</td>
<td>6192</td>
</tr>
<tr>
<td>Total</td>
<td>1329</td>
<td>6207</td>
</tr>
</tbody>
</table>
* Diagnosis of equivocal ATL was made molecularly with demonstration of HTLV-1 monoclonal integration. DP, double-positive type; DN, double-negative type

Fig 2. Southern blot analysis of HTLV-1 integration status in patients with seropositive T-cell neoplasm.
Lane 1 to 3: monoclonal integration one band corresponding to ATL
Lane 4: control (pX DNA fragment obtained from the cloned vector)
Lane 5: no demonstrable band corresponding to T-cell neoplasm infected by chance with HTLV-1

abnormalities were observed: CD8-positive type (4; 2.1%), CD4 and CD8-double positive (DP) type (13; 6.9%), and CD4 and CD8-double negative (DN) type (4; 2.1%). All but 1 of the 19 T-ML cases had common helper-T phenotypes with CD3+ and CD4+, similar to that of prototypic ATL. In the ATL cases with diagnostic difficulty, including 25 unusual ATL cases with aberrant phenotype and T-ML infected by chance with HTLV-1, whether or not integration with HTLV-1 had occurred was confirmed by molecular techniques. They were subsequently distributed as shown in Table 2. Figure 2 shows an example of Southern blot analysis on monoclonal integration band in ATL but not in T-ML. The non-monoclonal band corresponds to T-ML infected by chance with HTLV-1 (lane 5).

3) Diagnostic value of combining serology and immunophenotyping in ATL

Based on the raw data of Table 2, specificities, sensitivities, and PVs of the diagnostic tests with Bayes’ rule were computed as shown in Table 3. As a diagnostic tool for ATL, the anti-HTLV-1 antibody test was 100% sensitive, but its positive PV was extremely low (14.2%). Since these findings imply no relevance as a practical diagnostic tool, we then tried combining the serologic test with immunophenotyping, one test of the routine hematologic battery. The combination provided high positive and negative PVs, nearly 100%, with a 95% confidence interval.
previously described"). However, Southern blot analysis, the phenotypically difficult diagnosis, as presently and About 10% of ATL patients admitted to our hospital had phenotypes and unusual morphology of the tumor cells. diverse clinical manifestations and/or aberrant immuno-tests, some ATL cases are difficult to diagnose because of typic ATL can be easily diagnosed by ordinary hematologic correctly screened serologically. In general, althogh proto-

closed very rare ATL cases') and a healthy carrie') with als characterized by an MHC habit. Recent studies dis-

immunological tolerance to the antigens in some individu-

shown') and others'). This may imply a defective virus or have no env-antibodies, by the present authors (data not WB21> could not be excluded as a possible true infection, as “indeterminate” according to the WHO criteria of false positive in the PA. However, the sample designated against the HTLV-1 antigens'). The PA and PCR results were discrepant for only two of our samples, suggesting infection both in ATL and non-ATL cases, and (2) detec-
tion of anti-HTLV-1 has limited diagnostic value for ATL, implication that there is a risk of falsely labeling a patient as having ATL. Accordingly, since the serology in laboratory tests has limited diagnostic value, we need to adopt another objective marker for improving specificity. The clinical diagnosis of lymphoid neoplasms is primarily based on clinical manifestations, cyto- and histo-

morphism, and immunophenotypes. We chose immuno-

phenotyping, one of the most objective and reliable tests for T-cell neoplasms. When combined with serology, both positive and negative PVs became nearly 100%, implying more clinical significance of the two tests for making a correct diagnosis of ATL.

In conclusion, we demonstrated that : (1) HTLV-1 serology by the PA test can sensitively detect HTLV-1 infection both in ATL and non-ATL cases, and (2) detection of anti-HTLV-1 has limited diagnostic value for ATL, but the combination of anti-HTLV-1 testing and immuno-

phenotyping could prove to be useful, with 98% probability. These two tests in combination should be used and evaluated further, particularly for epidemiologic study across the world.

Table 3. Summary of Specificities, Sensitivities, and Predictive Values of HTLV-1 alone Combination of anti-HTLV-1 and T-cell Phenotype in ATL versus non-ATL.

<table>
<thead>
<tr>
<th>Test or testing combination</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Positive PV (95% CI)</th>
<th>Negative PV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-HTLV-1</td>
<td>84.3% (83.5-85.2)</td>
<td>100% (98.8-100)</td>
<td>14.2% (12.3-16.1)</td>
<td>100% (93.5-100)</td>
</tr>
<tr>
<td>combination of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-HTLV-1 and</td>
<td>77.8% (52.3-98.6)</td>
<td>100% (98.8-100)</td>
<td>97.9% (95.9-99.9)</td>
<td>100% (93.5-100)</td>
</tr>
<tr>
<td>T-cell phenotype</td>
<td></td>
<td></td>
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</tbody>
</table>

PV, predictive value : CI, confidence interval

Discussion

HTLV-1 was first identified as the causative agent of ATL. It has since become increasingly evident that the virus is also associated with other disorders such as TSP/HAM, uveitis, arthropathy, infectious dermatitis, and polymyositis[4,11]. Many apparently healthy people are carriers of HTLV-1, and they may develop one of these disorders. Thus, detecting the antibodies against HTLV-1 is of great importance to screen illness due to the HTLV-1 virus, particularly in areas endemic for ATL and TSP/HAM. Considering the cost-and time-benefit and the objectivity of ATL diagnosis, we quantitated the diagnostic validity of the PA serologic test in diagnosing ATL.

Our present results re-confirmed the relevance of the PA assay to detect the viral infection with the highest sensitivity (100%) and specificity (99.5%) comparable to that of PCR results, by testing a sufficient number of serum samples from blood donors who live in an area endemic for the virus. We targeted blood donors to obtain a variety of HTLV-1 infection state, eg. carriers with different sero-status such as seronegative or partially antibody positive against the HTLV-1 antigens[9]. The PA and PCR results were discrepant for only two of our samples, suggesting false positive in the PA. However, the sample designated as “indeterminante” according to the WHO criteria of WB antis[20] could not be excluded as a possible true infection, because about 10% of ATL cases have been reported to have no env-antibodies, by the present authors (data not shown)[20] and others[20]. This may imply a defective virus or immunological tolerance to the antigens in some individu-


Our present data demonstrated that all ATL cases were correctly screened serologically. In general, although prototypic ATL can be easily diagnosed by ordinary hematologic tests, some ATL cases are difficult to diagnose because of diverse clinical manifestations and/or aberrant immunophenotypes and unusual morphology of the tumor cells. About 10% of ATL patients admitted to our hospital had the phenotypically difficult diagnosis, as presently and previously described[1,10]. However, Southern blot analysis, the result of which is a diagnostic “gold standard” of ATL[6] can not be done in all cases because of time and cost constraints. Considering this, the negative PV of 100% presented by the serologic test is very useful in the sero-

logical screening of all cases for ATL at the first clinical step. In contrast, the positive PV of 14% is too low, implying that there is a risk of falsely labeling a patient as having ATL. Accordingly, since the serology in laboratory tests has limited diagnostic value, we need to adopt another objective marker for improving specificity. The clinical diagnosis of lymphoid neoplasms is primarily based on clinical manifestations, cyto- and histo-

morphism, and immunophenotypes. We chose immuno-

phenotyping, one of the most objective and reliable tests for T-cell neoplasms. When combined with serology, both positive and negative PVs became nearly 100%, implying more clinical significance of the two tests for making a correct diagnosis of ATL.

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Reference


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