Rapid Diagnosis of Cytomegalovirus Interstitial Pneumonitis by the Polymerase Chain Reaction Method —Comparison of Four Diagnostic Procedures—

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Summary

The authors conducted a comparative study on the rapid diagnosis of cytomegalovirus (CMV) interstitial pneumonitis by 4 different methods. Among them, the polymerase chain reaction (PCR) method was found to achieve the most rapid diagnosis of CMV infection. The clinical usefulness of making a diagnosis from the serum antibody titer and viral isolation was questionable as compared with the PCR method. Therefore, early diagnosis of CMV infection by PCR may be promising especially for the compromised patients with hematological diseases.

Introduction

Interstitial pneumonitis caused by cytomegaloviruses (CMV-IP) is one of the lethal complications in immunocompromised patients with leukemia and/or after bone marrow transplantation (BMT)1). Successful treatment of patients with the symptoms of CMV-IP is sometimes difficult because a rapid and reliable routine diagnostic procedure has not been established. Reduction of the mortality rate can be expected because the anti-CMV drug ganciclovir (DHPG) is available.

In this study, an attempt to diagnose CMV-IP was made in 7 immunocompromised patients by 4 different procedures.

The rapidity and reliability of these procedures are discussed.

Patients and Methods

[Patients and Specimens]

The subjects were 7 patients diagnosed clinically as having CMV-IP on the bases of a pneumonia shadow on chest X-ray, fever and hypoxemia (Table 1). These included 2 cases of adult T-cell leukemia (ATL), 1 each of multiple myeloma (MM) and acute lymphocytic leukemia (ALL), and 3 cases of chronic myelogenous leukemia (CML). Specimens of blood, urine and bronchoalveolar lavage (BAL) fluid were collected from the patients during the acute stage of CMV-IP.

[Polymerase chain reaction (PCR)]
The DNA for PCR was prepared by using IsoQuick nucleic acid extraction kit (MicroProbe, Garden Grove, U.S.A.) from the clinical specimen. The PCR primers were 5'-TTGCAGGCCACGAACAACGT-3' and 5'-GTCTACGGATTGCTGACGTC-3' and the PCR product was 305 bp in length. The condition for the PCR was the same as described previously. After agarose gel electrophoresis, the PCR product (the 305-bp band) was examined by ethidium bromide staining.

Anti-IEA human monoclonal antibody, HRP-C7, was used for DIP as described previously.

Both IgG and IgM against CMV were measured by commercially available ELISAs (enzyme-linked immunosorbent assay, cytomegalo Ig-G-EIA, Denkaseiken, Tokyo, Japan).

Virus isolation was carried out as described previously. After inoculation of urine from the patients into human embryonic lung fibroblasts, the cultures were examined daily for development of cytopathic effects (CPE) characteristic of CMV.

**Results**

By the PCR method, the BAL, blood and urine samples were positive in 100% (5/5), 85.7% (6/7), and 57% (4/7) respectively. The results were obtained within about 5—6 hours after collection of specimens. By the DIP method, positive BALs were found in 2 of 4 cases (50%) and peripheral blood samples in the 3 cases examined were negative. These results became manifest after about 16 hours. Serum CMV-IgG was positive in all 7 cases, indicating a past history of the infection. CMV-IgM was positive in only 1 case. The results became manifest within 7 days. The virus was isolated from the urine in 2 of the 7 cases (Table 2). The results were obtained after 3 weeks. Administration of DHPG resulted in improvement of the pneumonia in 4 of the 7 cases, and 3 patients including 2 ATL patients were died from respiratory insufficiency due to CMV-IP.

**Discussion**

The conventional diagnostic procedures for CMV infections depend to a large extent on virus isolation and the serum antibody titer. These methods require several days for the final diagnosis and a delay in the diagnosis and treatment usually causes a fatal outcome. If diagnosis can be early owing to a rapid diagnostic method, and if DHPG is administered, then it can be anticipated that the prognosis will be favorable. Therefore, a rapid diagnostic procedure has been expected as the number of compromised hosts is increasing.

In this study, we compared 4 diagnostic methods for CMV infections (Table 2), and found that it was
Rapid diagnosis for CMV-IP by PCR

Table 2  Results of CMV detection by 4 diagnostic procedures

<table>
<thead>
<tr>
<th>Diagnostic procedure</th>
<th>PCR</th>
<th>DIP</th>
<th>ELISA IgG</th>
<th>Virus isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>BAL</td>
<td>PB</td>
<td>Ur</td>
<td>BAL</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
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<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>+</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
</tbody>
</table>

+: positive, -: negative, NT: not tested, BAL: bronchoalveolar lavage, PB: peripheral blood, Ur: Urine

Table 3  Therapy guidelines for CMV infection

<table>
<thead>
<tr>
<th>PCR method</th>
<th>Clinical signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>PB</td>
</tr>
<tr>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>(+)</td>
<td>(+/-)</td>
</tr>
<tr>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>ND</td>
<td>(+)</td>
</tr>
<tr>
<td>ND</td>
<td>(-)</td>
</tr>
<tr>
<td>Observation group</td>
<td>None</td>
</tr>
<tr>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

(+)•1: Fever, dry cough, leukopenia, diarrhea, hypoxemia and abnormal shadow on chest X-ray are the major clinical signs

revealed the diagnosis by PCR was superior to the other methods in regard to the accuracy and rapidity. The positive rate was higher with PCR than with DIP in cases of CMV-IP. If CMV-IP is clinically documented especially in association with leukemia or post-BMT immunodeficiency, more than 90% of patients will die. Four out of 7 such patients were cured by early supportive care including administration of DHPG, since early diagnosis was made by PCR. Thus, PCR is reliable and useful in making an early diagnosis of CMV infection.

In our institution, the following strategy (Table 3) for the treatment of CMV infections was employed. All the patients with BAL fluid positive for CMV were given DHPG at a dose of 5 mg/kg every 12 hours for 1 or 2 weeks. When BAL was not performed, DHPG therapy was started only in case of the presence of both clinical signs and a positive finding of CMV in the peripheral blood and/or from urine by PCR. Patients with negative BAL and with no clinical symptoms were observed without being given DHPG.

Furthermore, it is still controversial whether or not treatment should be given to BAL-positive patients without pneumonia detected by chest X-ray. We considered that BAL positive is equivalent to CMV disease. Therefore, DHPG was administered according to the treatment scheme shown in Table 3. With regard to prophylactic use of DHPG, we believe that prompt diagnosis by PCR and early treatment of CMV disease is preferable to prophylactic DHPG.

References

1) Schmidt GM, Horak DA, Niland JC, Dunkan SR, Forman SJ, Zaia JA and the CITY of HOPE-STANFORD-SYNTEx


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サイトメガロウイルス肺炎の迅速診断—4種類の検査法の比較

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要 旨
臨床的に診断したサイトメガロウイルスによる間質性肺炎(CMV-IP)を4種類の検査法で迅速性と信頼性について比較検討した。PCR法でのDNA診断が最も迅速で，信頼性が高かった。血清診断やウイルス分離は迅速性でPCR法よりも有用性が低かった。したがって，血液疾患におけるcompromised hostでのサイトメガロウイルス感染症の早期診断はPCR法によるDAN診断が有用であった。