Rapid Diagnosis of Cytomegalovirus and *Pneumocystis carinii* Pneumonia by Using the Capillary Polymerase Chain Reaction

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**Key words:** CMV, *P. carinii*, PCR, capillary

**Summary**

We attempted to detect cytomegalovirus DNA (CMV-DNA) and *Pneumocystis carinii* DNA (*P. carinii*-DNA) in sputum samples of 18 hematological neoplasm patients with pneumonia, using rapid cycle DNA amplification. A thermal cycler based on recirculating hot air was used for rapid temperature control of 10-μl samples in this glass capillary tubes. After a total amplification time of 15 min, the amplified products were electrophoresed on agarose gels and visualized with ethidium bromide.

In three cases, CMV-DNA was detected at about the time the pneumonia occurred. These patients were successfully treated with ganciclovir in the early stages of infection and CMV was not detected by the virus culture method. In four other cases, *P. carinii*-DNA was detected in their sputum samples but not detected by Grocott staining. These four cases of *P. carinii* were successfully treated with sulfamethoxazole-trimethoprim. For detection of CMV-DNA and *P. carinii*-DNA using the capillary polymerase chain reaction (PCR), a different temperature setting base on the primer difference was not necessary. Therefore, capillary PCR was performed at the same time for detection of CMV and *P. carinii*.

We conclude that capillary PCR amplification is a valuable tool for rapid diagnosis and early treatment of pneumonia due to CMV and *P. carinii*.

**Introduction**

Opportunistic infections are one of the major factors that affect the outcome for immunocompromised patients. Of these, interstitial pneumonia caused by the cytomegalovirus and *Pneumocystis carinii* pneumonia can sometimes be fatal. Recently, effective agents for the treatment of these disorders, ganciclovir for cytomegalovirus, and sulfamethoxazole-trimethoprim for *P. carinii*, have become available. Therefore, if early treatment is implemented on the basis of early definite diagnosis, we can anticipate cures of these pneumonia disorders.

The polymerase chain reaction (PCR) method developed by Saiki et al. can be used for the diagnosis of infectious diseases because it enables the detection of DNA of pathogens in very small quantities in specimens in a short time. On the other hand, a large number of pathogens are possibly involved in opportunistic infections. If we perform a conventional PCR for the detection of at least several of the most suspicious pathogens, it takes much time before making a precise diagnosis.

For this reason, we used rapid cycle DNA amplification using Capillary PCR, and attempted to detect *P. carinii* and the cytomegalovirus simultaneously with the goal of reducing the test time to a large extent.
Table 1 Results of DNA amplification of CMV and P. carinii

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (Sex)</th>
<th>Underlying disease*</th>
<th>Results of capillary PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29 (M)</td>
<td>MM</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>31 (F)</td>
<td>ALL</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>33 (F)</td>
<td>MDS</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>35 (F)</td>
<td>ATL</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>36 (F)</td>
<td>CML</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>39 (M)</td>
<td>ATL</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>40 (M)</td>
<td>AMoL</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>43 (M)</td>
<td>ML</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>44 (M)</td>
<td>ALL</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>48 (F)</td>
<td>ALL</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>50 (M)</td>
<td>ATL</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>55 (F)</td>
<td>MH</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>56 (F)</td>
<td>AMoL</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>59 (M)</td>
<td>ATL</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>60 (F)</td>
<td>CML</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>63 (M)</td>
<td>ALL</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>65 (F)</td>
<td>ML</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>66 (M)</td>
<td>AML</td>
<td>+</td>
</tr>
</tbody>
</table>

*M: MM, multiple myeloma; ALL, acute lymphocytic leukemia; CML, chronic myelocytic leukemia; MDS, myelodysplastic syndrome; ATL, adult T cell leukemia; AMoL, acute monoblastic leukemia; ML, malignant lymphoma; MH, malignant histiocytosis; AML, acute lymphocytic leukemia

Material and Methods

We used sputum of hematological neoplasm patients with coincident pneumonia (Table 1).

Preparation of sputum: The sputum was treated with Sputazyme (Kobayashi Pharmaceutical Co.) at 37°C for 10 min. All the samples were centrifuged at 1600 × g, for 15 min, then the sediments were resuspended in a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5), and treated with Proteinase-K (80 μg/ml) at 56°C for 60 min.

PCR: For the detection of cytomegalovirus DNA, the primers to amplify the 159 bases in the region coding immediate early antigen: IE1 (5'-CCACCCGTGGTCCAGCTCC-3') and IE2 (5'-CCCCTCCTCC-CTGAGGCACC-3') were synthesized with a DNA synthesizer (Milli Gen/Biosearch), while for the detection of P. carinii, the primers PAZ102-E (5'GATGGCTGTTTCCAAGCACA-3') and PAZ102-H (5'-GATGGCTGTTTCCAAGCACA-3'), amplifying a 346-bp sequence, were used as reported by Wakefield, et al. DNA amplification for capillary PCR was performed in 50 mM Tris, pH 8.5, 3 mM MgCl2, 20 mM KCl, 500 μg of bovine serum albumin per ml, 0.5 μmol each of the primer IE1, IE2 or PAZ102-E, PAZ102-H), 0.5 mmol of each deoxynucleotide triphoshatate (dNTP), 1 μl of DNA sample and 0.4 units of taq polymerase (Promega Co. USA) per 10 μl unless specified otherwise. Samples (10 μl) were placed in the center of 10.8-cm lengths of microcapillary tubing (Idaho Technology, USA), and the ends were sealed with a gas lighter. A 1- to 2-cm column of air in each side of the sample allowed easy sealing of the tubes. Thirty-five cycles of DNA amplification were performed in a hot air thermal cycler (Air Thermos-Cycler 1605, Idaho Technology, USA). The thermal profile is as follows: (1) denaturation, 94°C 3 seconds. (2) annealing, 55°C 3 seconds. (3) extension 72°C 12 seconds. The amplifications were subjected to electrophoresis in agarose gels, which were tested for the presence of bands by visualization with ultraviolet light after ethydium bromide
Diagnosis of CMV and *P. carinii* pneumonia by capillary PCR

1. Capillary PCR

   Fig. 1 shows the results of the agarose gel electrophoresis following PCR for both cytomegalovirus and *P. carinii*. PCR was performed at the same time for cytomegalovirus and the *P. carinii*. PCR was performed at the same time for cytomegalovirus and the *P. carinii*, and it was possible to obtain all the results in about two hours. Amplified DNA fragments of cytomegalovirus and *P. carinii* were each demonstrated as one clear band. Moreover, the capillary PCR was 100- to 1000-fold more sensitive than the conventional PCR (data not shown).

2. Results of capillary PCR in patient’s sputum

   As shown in Table 1, the PCR was positive for cytomegalovirus in three of the 18 patients. In these three patients (cases 4, 8, 13), CMV was not detected by the virus culture methods. In four other patients (cases 3, 9, 15, 18), *P. carinii* was positive by capillary PCR in sputum but was not detected by Grocott staining. In cases 4, 8, and 13, treatment with ganciclovir was begun immediately after the positive PCR result for CMV was obtained, resulting in negative conversion on about the sixth day after treatment, and the pneumonia disappeared in two weeks after the treatment. In case 3, in which PCR was positive for *P. carinii* treatment with sulfamethoxazole-trimethoprim by drip infusion was begun on the day when the positive result was obtained. The PCR reading turned negative on the tenth day of the treatment, and the pneumonia completely disappeared one month later. In addition, in three other cases (cases 9, 15, and 18), early treatment of *P. carinii* pneumonia with sulfamethoxazole-trimethoprim was successful.

Discussion

The PCR method has contributed significantly to progress in genetic molecular biology and has been applied in many different biological science fields. In this study, we used it specifically for genetic diagnoses of cytomegalovirus and *P. carinii* pneumonia involved in opportunistic infections.

We were able to detect cytomegalovirus DNA by the capillary PCR method in three of 18 patients with hematological malignancy. Diagnosis of cytomegalovirus pneumonia by the PCR method has been
frequently reported. Eisele et al.9) studied 18 cases following bone marrow transplantation. They described good indicators for ganciclovir treatment for the results of PCR tests on blood. Also, Ishigaki et al.10) investigated immunocompromised patients with cytomegalovirus pneumonia. They found a relationship between the amount of cytomegalovirus DNA in the serum and the progress of pneumonia.

The number of patients who showed a positive PCR for CMV in this study was too small to draw a definite conclusion. However, it seems unlikely that a diagnosis of CMV pneumonia could not be made only on the basis of a positive PCR in blood. Although the data were not shown, we found that the blood of about 70% of these patients who were seropositive for CMV and had been treated with a steroid for a long time was CMV-DNA positive by the PCR. For confirming a diagnosis of cytomegalovirus pneumonia, it is believed that proof of positive DNA from local materials, such as sputum, a transbronchial lung biopsy specimen and bronchoalveolar lavage fluid are essential.

The diagnosis of *P. carinii* pneumonia is as difficult as that of CMV pneumonia. Until now, the only way to confirm the diagnosis of *P. carinii* was limited to histological verification of the presence of the *P. carinii* protozoa. Therefore, there was very little chance for antemortem diagnosis of *P. carinii* pneumonia. With this PCR method, however, presence of the *P. carinii* protozoa was demonstrated at an early stage in the sputum in four of 18 patients. Moreover, it was possible to improve and cure this *P. carinii* pneumonia by treatment with sulfamethoxazole-trimethoprim by intravenous drip infusion. *P. carinii* pneumonia develops at a high incidence11) in patients with severely impaired immune system such as those with AIDA. However, in the hematological neoplasm patients in this study, impairment of the immune system is not so extreme. Therefore, we believe it is necessary to realize that *P. carinii* pneumonia is not a rare and unique kind of pneumonia. *P. carinii* pneumonia must be considered for those cases in which a lowering of the immune system function is possible to even a slight degree.

Further, in the present PCR using capillaries, we could use a smaller (10 µl) amount of the reaction mixture. As the capillary is made of thin glass, the heat conduction is excellent, and the reactant liquid can be quickly brought to the desired temperature. This reduces the time required for the denaturation, annealing, and elongation steps of the PCR and prevents unspecified reactions of the primer. Thus, temperature settings based on the primer differences become unnecessary. In conclusion, the capillary PCR method is found to be extremely useful for early diagnosis of opportunistic infections, particularly those due to cytomegalovirus and *P. carinii*, and for starting early treatment. However, conventional biological methods (virus culture, serological tests, histopathological tests, etc.) must be performed because the PCR method has problems of false-positivity or negativity.

We believe that this capillary PCR is a test method which should be actively practiced and could become the most cost-effective method of diagnosing CMV and *P. carinii* pneumonia.

References

Diagnosis of CMV and *P. carinii* pneumonia by capillary PCR


キャピラリーPCRを用いたサイトメガロウイルス肺炎と

カリニー肺炎の早期診断

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（平成5年３月1日受付）
（平成5年6月23日受理）

要旨

キャピラリーPCRを用いて、肺炎を合併した血液悪性疾患患者18例の喀痰からサイトメガロウイルスDNAとカリニーDNAの検出を試みた。

ホットエアーや再循環を利用したサーマルサイクライナーを使用し、キャピラリー管の中で10μLのサンプルをDNA増幅した。15分の增幅後、アガロース電気泳動で検出した。

3例にCMV-DNAが検出され、ウイルス培養では陰性であった。これらの症例は、ガンジクロビルの早期投与により肺炎症状は消失した。4例にカリニーDNAが検出され、ST合剤の投与により肺炎を治癒させることができた。また、これらの症例はグルコット染色陰性であった。

キャピラリーPCRにおいて、CMV、*P. carinii*検出のための検出のための温度設定を同一にすることが可能であり、同時にPCRを施行することができた。

CMV、*P. carinii*肺炎の早期治療を目的とした早期診断において、キャピラリーPCRは有用な検査法であった。

平成5年10月20日