New Diagnostic Techniques for The Detection of Opportunistic Pathogens Involved in Pneumonia in Immunocompromised Hosts

The development of rapid and sensitive diagnostic tools is an urgent need in the management of opportunistic serious infections in immunocompromised patients with or without human immunodeficiency virus (HIV) infection. During the 1990’s molecular biological techniques have been applied for the rapid detection of infectious agents: nucleic acid hybridization techniques and the polymerase chain reaction (PCR).

*Mycobacterium tuberculosis* (*M. tuberculosis*) is one of the most important pathogens which is frequently associated with HIV infection. The World Health Organization (WHO) estimates that 1.7 billion inhabitants of the earth are infected with *M. tuberculosis*, of whom 8 million develop active disease and 3 million die annually (1) and that the global incidence of tuberculosis will increase by 57% between 1990 and 2005. The main reason for this increase is the world-wide spread of HIV infection, because dual infection with HIV and *M. tuberculosis* results in a greatly increased risk of tuberculosis (2).

Amplification of target mycobacterial DNA using PCR offers the possibility of rapid and sensitive detection of mycobacterial infection. This method has already been used by several groups (3–6), and has been shown to facilitate rapid detection of positive samples. One study showed that PCR is highly specific in sputum samples (3). However, most proven tuberculosis samples were smear positive, and the sensitivity in smear-negative, culture-positive patients could not be deduced from this study.

The PCR approach does have drawbacks (7). First, the procedure is costly and laborious. Second, the test is susceptible to false-positive results. In particular, the contamination of samples with mycobacterial DNA or amplification products from prior tests (“carryover”) remains an important obstacle to routine use.

*Pneumocystis carinii* (*P. carinii*) is another important pathogen which is also frequently involved in pneumonia in the immunocompromised host. Especially in AIDS patients, the incidence and seriousness are very high. Direct detection of *P. carinii* by use of toluidine blue O, other simple staining techniques or by fluorescent monoclonal antibodies was found to be very sensitive, specific and rapid with the use of induced sputum or bronchoalveolar lavage fluid (8).

A PCR technique has been applied for the detection of *P. carinii* using respiratory secretion and blood as the specimens (9). A simple and rapid 2-step PCR has also been described for the detection of this microorganism (10). These new PCR techniques are likely to be the most useful in the monitoring of sputum for breakthrough infection in patients on prophylactic therapy (11).

Cytomegalovirus (CMV) is a ubiquitous virus and possesses widely variable propensity to cause invasive disease in different host settings. Manifestations of active CMV infection range from asymptomatic viral shedding to retinitis, pneumonia, gastrointestinal ulceration, and disseminated disease (12).

Several studies have demonstrated that viremia of high grade correlates with invasive disease (13, 14). But there still remains uncertainty because not all viremic individuals develop disease. Therefore, the diagnosis of active CMV infection still depends mainly on traditional techniques such as viral isolation and shell vial centrifuge culture assay by staining with monoclonal antibody to the CMV immediate-early antigen. Recently, rapid detection of CMV by PCR is widely investigated and PCR has been described to be more sensitive than culture in the detection of the virus in various types of clinical specimens (15, 16). Ishigaki et al (17) proposed that serum PCR positivity is correlated with CMV pneumonia in bone marrow recipients.

CMV and *P. carinii* are common opportunistic pathogens and often are concurrently involved in pneumonia in immunocompromised hosts. We attempted the simultaneous detection of CMV-DNA and *P. carinii*-DNA using rapid cycle DNA amplification in capillary PCR testing (18) and concluded that this method is useful for the rapid and sensitive simultaneous detection of CMV-specific and *P. carinii*-specific DNA fragments, in order to pursue early initiation of treatment for these infections. Shimomoto et al (19) applied PCR on induced sputum for making the diagnosis of the initial stage of acute pulmonary infections due to three major pathogens, *P. carinii*, *M. tuberculosis* and CMV, in hemophilia patients infected with HIV. They conducted PCR 111 times in 29 patients. Prophylactics against *P. carinii* were given to all the patients whose CD4+ count was less than 200×10⁶/l. As a result, *P. carinii* DNA was not detected in all of the induced sputum samples. *M. tuberculosis* – DNA and CMV-DNA, however, were detected in one and four patients, respectively. The results of this study suggest the necessity for prophylactics in the prevention of *P. carinii* pneumonia in seriously immunocompromised patients and also the need to attempt to simultaneously detect the other two pathogens, *M. tuberculosis* and CMV, in the same specimen.
References