Cytomegalovirus Detection in Transplant Patients: Comparison of Different Methods in a Prospective Survey

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In a prospective survey, all transplant patients at the hospital of the University of Zürich were screened for cytomegalovirus (CMV) infection. CMV infections were detected in a total of 40 of 104 transplant recipients; 31 could be diagnosed by CMV immunoglobulin M serology, 27 could be diagnosed by viremia, 11 could be diagnosed by antigenemia, and 13 could be diagnosed by the start of virus secretion. Combined application of serology and the detection of viremia showed the highest sensitivity (39 of 40 cases). Of the patients with severe clinical symptoms, six of seven had primary CMV infections caused by a positive transplant. Therefore, it is strongly indicated that patients with known risk factors should be regularly surveyed by a combination of methods.

After transplantation, many patients develop cytomegalovirus (CMV) infections regardless of the type of transplantation. The transplanted organ or, less often, transfused blood are known causes of primary infection (1, 8). Because of the increasing number of transplantations, rapid CMV diagnostic tests have become important. Virus detection methods have been considerably improved, and new techniques have been published (2, 9, 11). To develop a strategy for the detection of CMV infections in our laboratory, different detection methods were compared and a correlation of diagnosed CMV infections with CMV-specific clinical symptoms was attempted.

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All organ recipients transplanted between May 1988 and August 1989 were examined for CMV infection on a weekly basis during hospitalization. Serum samples were taken for the detection of CMV immunoglobulin G (IgG) and IgM. The detection of CMV IgG was done by an indirect enzyme-linked immunosorbent assay as described previously (4, 5). IgM was tested with an enzyme-linked immunosorbent assay using labelled antigen (Medac AG, Hamburg, Germany). Leukocytes were isolated from heparinized blood with dextran (11). They were tested by the shell vial assay (12) or by conventional cell culture. From the same preparation, leukocytes were used for the detection of antigenemia by the method described by van der Bij et al. (11). Detection of virus in specimens other than leukocytes was done as described before (12).

The characteristics of the patients observed are listed in Table 1. The correlation of CMV infection with clinical symptoms is discussed below. A total of 104 patients were followed up, including 44 kidney, 9 renal pancreas, 33 heart, 7 liver, and 11 bone marrow transplant recipients. The results of the detection of CMV infection by different methods are presented in Table 2. Of 40 infected patients, CMV infection could be identified in 31 of them by CMV IgM serology (only the first positive result after the previously negative sample was counted). In 27 infected patients, viremia could be found by the shell vial assay. In general, this viremia lasted only for a short period except for two cases in which it could be detected for 2 weeks. Detection of antigenemia was less efficient, with only 11 cases of CMV infection being identified by this technique. The classical cell culture method detected six cases of CMV infection. Because of the work load, only 56 patients were surveyed by using this technique. In 13 infected patients, the start of virus secretion could be observed. In the present survey, the efficiency of the detection of antigenemia was lower than that described in the previous study (12) and by other authors (2, 10, 11). One reason for this might be that patients were selected on the basis of clinical symptoms and, therefore, had a higher degree of antigenemia. In addition, other important factors influenced antigenemia, e.g., the percentage of antibody-negative recipients of an organ from antibody-positive donors was higher in our first study (12). It is also possible that the treatment with hyperimmunoglobulins or antiviral agents had an influence on the degree of viremia. However, this effect was not systematically investigated.

From the results presented (Table 2), it was obvious that none of the methods used was able to detect all cases of CMV infection. However, by a combination of different methods, this goal could almost be achieved. IgM detection, detection of viremia by shell vial assay, or both methods together identified 39 of 40 cases (Table 3), whereas serology, detection of antigenemia, or the two combined identified 33 cases. Only 28 cases of CMV infection were revealed by the detection of viremia, antigenemia, or both. Under clinical conditions, it is not possible to follow up all patients for the same length of time or with exactly the same frequency. Therefore, some positive samples might have been missed. The other tests used were much less efficient and therefore were not included in this comparison.

It is important to recognize a CMV infection as early as possible. Because of immunosuppression, viremia may be detectable before IgM serology (2, 3, 7). Detection of antigenemia or viremia became positive on day 35 and day 39, respectively. This was similar to the results published by van den Berg et al. (11). On average, IgM seroconversion became positive on day 55 and virus secretion became positive on day 60. These mean values, however, do not reflect the
large individual variation (result not shown) which made it impossible to predict which parameter becomes positive first. The results in Table 3 reflect this situation to a certain degree. By using a combination of two different techniques, the likelihood of recognizing CMV infection could be increased.

It would be important to predict under which conditions a CMV infection could lead to severe clinical symptoms. To analyze the data, patients were classified. Patients were determined to have CMV infections if IgM could be found after a previously negative result, if viremia was detected by one or more methods, if virus started to be secreted, or a combination of any of these factors. Patients with positive laboratory parameters without clinical symptoms were considered to have asymptomatic infections. Patients were considered to have mild to moderate CMV infections if they had positive laboratory parameters in combination with the following clinical symptoms: unexplained fever for 3 or more days, arthralgia myalgia, or positive hematological signs of a virus infection in the absence of other infectious disease. Patients were considered to have severe CMV infections if they had positive laboratory parameters in combination with pneumonia, neurological symptoms, superinfections, graft reactions, or wasting disease. Of the 104 transplant patients, 51 remained uninfected (Table 1). Forty transplant patients showed infection with positive laboratory parameters; 13 were asymptomatic without positive parameters. Of the 40 cases, 16 were asymptomatic and 24 demonstrated symptoms of differing degrees of severity. Seventeen of the

infected patients showed mild to moderate symptoms, and seven showed severe symptoms. Six of the patients with severe symptoms were seronegative recipients of an organ from a seropositive donor.

In published studies, the incidence of primary infection varies considerably (6, 10). Of the cases of CMV infection, various percentages have been reported to lead to severe clinical symptoms (30 and 78% in references 6 and 10, respectively). In our study, the incidence of a primary infection leading to a severe clinical symptom was 67%. In virus recurrence, the incidence of severe clinical symptoms was much lower (6, 10). This was also the case in the present study (1 of 31 infected patients). Therefore, it is indicated that patients with risk factors should be more strictly surveyed than others.

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REFERENCES


