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# Evaluation of Four Methods for Cytomegalovirus Antibody Detection for Use by a Bone Marrow Transplantation Service

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**Four methods, latex agglutination, indirect fluorescent antibody, enzyme immunoassay, and complement fixation, were compared for cytomegalovirus antibody screening and for pre- and posttransplant determinations on bone marrow transplant recipients. Latex agglutination was most sensitive (98%) and specific (97%) for screening and pretransplant determinations and was quickest and easiest to perform. In posttransplant sera from allogeneic bone marrow transplant recipients, all methods except complement fixation detected cytomegalovirus antibody from therapeutically administered globulin preparations; this made it difficult to determine the significance of changes in cytomegalovirus antibody titer.**

Cytomegalovirus (CMV) is the most common infectious cause of death in allogeneic bone marrow transplant (BMT) recipients (10). CMV antibody-positive recipients may be infected via reactivation, and CMV antibody-negative recipients may acquire primary CMV infections via virus-containing blood products or marrow. Therapy with CMV antibody-negative blood products is effective in preventing CMV infection (4). In CMV-infected BMT recipients who show seroconversion or rising antibody to CMV, survival time is longer and mortality is decreased (10), while survival is decreased in those whose CMV antibody titer decreases or fails to rise (14, 17). CMV antibody testing is important in screening blood and blood products and in determination of pre- and posttransplant CMV antibody levels in BMT recipients. Although there are many reports comparing CMV antibody-screening methods (1, 3, 5, 7–9, 12), few have emphasized evaluation of changes in antibody level and none has specifically addressed BMT-related CMV serology.

Eighty-three serum samples (17 from blood donors, 38 random samples from hospitalized patients, and 28 collected pretransplant from BMT patients) were tested by latex agglutination (LA), indirect immunofluorescence (IFA), enzyme immunoassay (EIA), complement fixation (CF), and anticomplement immunofluorescence (ACIF). Twofold serial dilutions of sera, 1:8 to 1:1,024 for IFA (Virgo reagents; Electro-Nucleonics, Inc., Columbia, Md.), 1:1 through endpoint of agglutination for LA (CMVScan Card Test; BBL Microbiology Systems, Cockeysville, Md.), 1:8 to 1:2,048 for CF (traditional microtiter method [15], using CMV antigen purchased from Whittaker M.A. Bioproducts, Walkersville, Md.), and a 1:50 dilution for EIA (CMV RQ Bio-Enzabead kit; Organon Teknika, Charleston, S.C.), were tested, and results were interpreted by using the respective manufacturer's directions. Serial dilutions, 1:8 to 1:4,096, were tested by a modification of the ACIF method of Reynolds et al. (13). Antigen smears were from Virgo CMV IFA kits (Electro-Nucleonics), guinea pig complement was from Whittaker M.A. Bioproducts, and fluorescein isothiocyanate-conjugated immunoglobulin G (IgG) fraction goat anti-guinea pig C3 was from Organon Teknika, Malvern, Pa. The ACIF result was accepted as the true value.

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The initial and final sensitivities and specificities of the four methods in the testing of 83 serum samples are shown in Table 1. LA showed the highest sensitivity (97.9%) and high (97.1%) final specificity. Initial LA testing yielded four falsely positive results, all with agglutination in undiluted sera only. Low-titer (≤1:2) false-positive results with LA in screening of blood and blood products have been reported. These authors concluded, as we have in this study, that the significance of agglutination at low titers is difficult to assess (3) but is not a significant problem in testing blood and blood products (1). EIA, IFA, and CF were all less sensitive than LA, and all, except CF, were less specific.

Twenty-eight pretransplant serum samples (included in the 83 samples from Table 1) were evaluated. Of 15 patients who were CMV antibody positive by ACIF, 9 (60%) were identified by all methods, 5 (33%) were falsely negative by CF alone, and 1 (6.6%) was falsely negative by CF and EIA. Of 13 patients who were CMV antibody negative by ACIF, 10 (77%) were identified by all methods, 2 (15%) were falsely positive by EIA, and 1 (7.7%) was falsely positive by LA. The emphasis in pretransplant serology is on specificity because this evaluation determines whether the BMT recipient will receive CMV antibody-screened blood products. The CMV CF method showed 100% specificity. However, during the course of this study, 6 of 15 patients who were CMV antibody positive by ACIF, LA, IFA, and EIA were falsely identified as CMV antibody negative by CF. All six of these individuals needlessly received CMV antibody-negative products. CMV CF antibody has been shown to decrease and disappear with time, making CF of questionable value for CMV antibody status determinations (16).

The LA method correctly identified 13 of 13 CMV antibody-positive BMT recipients and 14 of 15 CMV antibody-negative recipients in pretransplant determinations. The single false-positive sample showed agglutination in undiluted serum only. Due to the critical nature of pretransplant CMV determinations, it is our recommendation that follow-up testing be performed on any pretransplant sample demonstrating low-titer (undiluted sera only) LA results. We recommend ACIF for this testing. EIA and IFA showed 91.4 and 85.7% final specificity, respectively, in pretransplant testing; neither showed any advantage over LA.

A total of 183 sequential serum samples collected bi-

TABLE 1. Sensitivity and specificity of four methods for CMV antibody detection in testing of 83 serum samples

| Method | % Sensitivity[a] | | % Specificity[a] | |
|---|---|---|---|---|
| | Initial[b] | Final[c] | Initial | Final |
| LA (CMVScan; Becton Dickinson) | 97.9 | 97.9 | 88.6 | 97.1 |
| IFA (Virgo; Electro-Nucleonics) | 83.3 | 91.7 | 80.0 | 85.7 |
| EIA (CMV RQ Bio-Enzabead; Organon Teknika) | 87.5 | 89.6 | 91.4 | 91.4 |
| CF | 72.9 | 72.9 | 100.0 | 100.0 |

[a] ACIF result was accepted as the true value.
[b] Calculation based on single initial determination.
[c] Calculation based on repeat testing of discrepant samples.

weekly for the first 3 months posttransplant and monthly thereafter from 24 allogeneic and 4 autologous BMT recipients were also tested by LA, IFA, EIA, CF, and ACIF and for CMV IgM by an IFA method. For CMV IgM IFA, sera were pretreated by passage through a DEAE-dextran Sephadex column (Isolab, Inc., Akron, Ohio), and the eluted IgM fraction was tested per the manufacturer's directions, using the Virgo CMV IgM IFA kit (Electro-Nucleonics). Urine, stool, peripheral blood, and throat swab samples for virus isolation were collected at the intervals given above for sequential sera.

Significant changes in CMV antibody titer (Table 2) were detected by all four methods in three patients who were CMV antibody positive pretransplant. CMV was isolated from one of these patients; the other two had no evidence of CMV infection, and none had CMV IgM. Ten patients who were CMV antibody negative pretransplant, eight who had no evidence of CMV infection and two from whom CMV was eventually isolated, seroconverted within 1 to 2 weeks posttransplant by all serologic methods except CF; none had CMV IgM. All 10 patients were allogeneic BMT recipients who received a pooled IgG preparation, Sandoglobulin (Sandoz, Inc., E. Hanover, N.J.). CMV antibody was detected in this globulin (LA, 1:128; IFA, 1:512; EIA, 1:1,000 to 1:2,000; and CF 1:32), which was administered from 8 days pretransplant through 100 days posttransplant. Only two of these patients were followed for CMV antibody beyond 100 days posttransplant, and their CMV antibody levels declined.

No significant changes in CMV antibody level were detected in 12 recipients who were CMV antibody positive pretransplant (Table 2). CMV was isolated or confirmed in four of these, only one had CMV IgM, and eight showed no evidence of CMV infection. Seven of the eight were allogeneic BMT recipients who received Sandoglobulin; inconsistent twofold fluctuations in CMV antibody titer were detected in their sera. Such fluctuations were not observed in the sequential samples from one CMV antibody-positive autologous transplant recipient who did not receive Sandoglobulin. Three autologous BMT recipients were CMV antibody negative pretransplant and remained so during the course of the study.

Sandoglobulin contains antibody against many infectious agents, including adenovirus, coxsackieviruses A and B, Epstein-Barr virus, herpesvirus, hepatitis viruses A and B, influenza viruses A and B, measles virus, mumps virus, parainfluenza virus, poliovirus, rubella virus, *Bordetella pertussis*, and various pneumococci, as well as many others (Sandoglobulin Immune Globulin Intravenous, Pharmacology and Clinical Studies; Sandoz, Inc., 1984). These antibodies, like CMV antibodies, may affect laboratory assays. Excellent communication between physicians and laboratories is requisite to prevent misinterpretation of laboratory test values which may be affected by globulin administration.

For measurement of CMV antibody in BMT recipients, the LA method was sensitive, specific, and simple to perform. The EIA used here produced satisfactory results in acceptable runs. However, innumerable problems, both mechanical and technical, unresolvable by technologists or representatives of the manufacturer, caused frequent repetition of test runs and unacceptable delays in reporting of results. These problems remained unresolved for a period of 18 months and caused this EIA system to be rejected for use in this laboratory. The CF method demonstrated poor (72%) sensitivity and was too cumbersome to be of value as a screening method. The IFA yielded both falsely positive and falsely negative results, and test interpretation was complicated by nonspecific binding. In this study, as in previous

TABLE 2. CMV profiles of 28 BMT recipients posttransplant

| CMV antibody status pretransplant | No. of patients | Confirmation of CMV infection | CMV antibody changes by LA, IFA, EIA, and CF |
|---|---|---|---|
| Significant change | | | |
| Postive | 1 | Buffy coat isolate | Fourfold increase by all methods |
| Positive | 2 | No CMV infection confirmed | Fourfold increase by all methods |
| Negative | 2 | CMV isolated at or near time of death or patient lost to follow-up | No increase following CMV isolation; initial increase consistent temporally with that observed in 8 CMV antibody-negative recipients receiving globulin therapy |
| Negative | 8 | No CMV infection confirmed | Increase 1–3 wk posttransplant by all methods except CF; consistent with globulin administration |
| No significant change | | | |
| Positive | 3 | Isolation/histologic confirmation at or near time of death | No increase by any method |
| Positive | 1 | Urine isolate | No increase by any method during 1-yr post-virus isolation |
| Positive | 8 | None | Twofold fluctuations in titer for 7 allogeneic transplant recipients who received globulin |
| Negative | 3 | None | Autologous transplant recipients; no globulin therapy |

evaluations of CMV serologic techniques (5, 12), the difficulty of interpretation of IFA results caused this method to be rejected.

CMV infection was not diagnosed serologically in six of seven patients by using a column separation method with IFA for CMV IgM detection or by measurement of significant changes in CMV antibody by five methods. These results suggest that CMV serology methods that rely on changes in total antibody level may be ineffective with patients such as BMT recipients, whose antibody response may be weak or absent due to both severe immunosuppression and underlying disease. Mirolo et al. (11) and Ashley et al. (2), using immunoblotting techniques, demonstrated that total antibody levels may remain constant, although reactivity to specific viral polypeptides may change during infection. Landini et al. (6) identified two polypeptides that are constantly and preferentially reactive with CMV IgM, and Mirolo et al. (11) profiled the reactivity of CMV IgG antibody with intermediate-molecular-weight polypeptides to differentiate the CMV IgG antibody responses of acute CMV hepatitis, reactivation CMV in renal transplant recipients, and CMV infection in pregnant women. Use of new technology, rather than traditional methods, may be requisite if CMV infection in populations such as BMT recipients is to be evaluated serologically.

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