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High-Level Sensitivity of Quantitative pp65 Cytomegalovirus (CMV) Antigenemia Assay for Diagnosis of CMV Disease in AIDS Patients and Follow-Up

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Cytomegalovirus (CMV) antigenemia was evaluated in 174 patients positive for human immunodeficiency virus. Antigenemia could be detected in 96.7% of patients with CMV disease, 76.9% of patients suffering from a relapse of the disease, and 11.4% of asymptomatic patients with CD4 levels of <100 cells per μl. No antigenemia was detected in patients with CD4 levels of 250 to 500 cells per μl. Specificity and the positive predictive value for CMV disease were increased only if more than 5 positive cells per slide were considered. However, CMV disease may also occur in patients with low-grade antigenemia.

More than 25% of AIDS patients experience cytomegalovirus (CMV) disease during the progression of immunodeficiency (2), and this trend is increasing (13). Autopsy studies indicate that CMV disease may occur in 50 to 90% of AIDS patients (7, 12). Several studies of the CMV antigenemia (AG) assay for human immunodeficiency virus (HIV)-positive patients have been reported (3–6, 8–11), but only a few studies evaluated the quantitative aspects of the assay (3, 5, 6, 8).

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In a cross-sectional study, 174 HIV-positive adults (160 male and 14 female) were tested for CMV AG. The mean age was 40 years (range, 23 to 83 years). The risks for HIV infection were homo- or bisexual activity (74.2%), intravenous drug use (7.5%), heterosexual activity (5.8%), use of blood products (1.2%), and unknown (11.5%). CMV-seropositive patients were included in the study and were divided into the following groups: CD4 counts of 250 to 500 cells per μl, asymptomatic (group 1 [$n = 30$]); CD4 counts of <100 cells per μl, asymptomatic (group 2 [$n = 35$]); CD4 counts of <100 cells per μl, suspected CMV disease (group 3 [$n = 57$]); CD4 counts of <100 cells per μl, suspected relapse of disease (group 4 [$n = 9$]); confirmed CMV disease (group 5 [$n = 30$]); and confirmed relapse of disease (group 6 [$n = 13$]). Thirty HIV-negative healthy blood donors served as controls (15 CMV seropositive and 15 CMV seronegative). Confirmed CMV disease or relapse of disease was defined as (i) typical inflammation of the retina that improved with therapy or (ii) both typical inclusion bodies and positive immunostaining in tissue biopsy specimens. Suspected CMV disease or relapse was defined as signs or symptoms highly suggestive of CMV disease or relapse (i.e., gastrointestinal ulcera or unexplained diarrhea, pancreatitis, cholangitis, demonstration of CMV in the bronchoalveolar lavage, and unexplained neurological symptoms) in patients who had no biopsy or whose biopsies had revealed either inclusion bodies or positive immunostaining but not both. All blood specimens were tested by the immunofluorescence technique in duplicate, with 2×10^5 peripheral blood leukocytes for the preparation of the slides and acetone fixation being used as described previously (1). For each slide, the number of positive cells was determined by two independent observers. The AG assay was defined as positive if at least 1 cell per duplicate slide was typically stained. Indefinite results or atypical staining was considered negative.

Different results for positive or negative by the two independent observers occurred for only 6 of 340 slides (1.8%). For these slides, the number of positive cells was low (1 to 6 cells per slide). Discrepant results (i.e., positive and negative) for duplicate testing of the same sample were seen for 22 of 340 pairs (6.4%), with the positive slides also having low numbers of positive cells (1 to 6 cells per slide).

The occurrences of CMV AG in the different groups are shown in Table 1. Sensitivity, specificity, and predictive values are shown in Table 2. Among all 101 patients with CD4 counts of <100 cells per μl who did not have documented CMV disease (i.e., groups 2 to 4), 35 (35%) were AG positive at any level.

Follow-up evaluation of the 101 patients with CD4 levels of <100 cells per μl but without proven CMV disease (groups 2 to 4) revealed CMV disease in 8 of 35 (23%) who were AG positive and 2 of 66 (3%) who were AG negative ($P = 0.003$). In cases in which CMV disease or relapse was suspected clinically (groups 3 and 4), CMV disease was subsequently documented in 5 of 31 patients (16%) who were AG positive and did not receive antiviral treatment (mean, 3.4 months later; range, 1 to 5 months) and 2 of 35 AG-negative patients (6%) (6 and 7 months later) ($P = 0.24$). The four asymptomatic patients with CD4 counts of <100 cells per μl (group 2) had low-grade AG (1 to 5 positive cells per slide); all four patients developed CMV disease (1, 5, 7, and 15 months later).

Patients presenting with retinitis tended to have lower CD4 levels than patients with gastrointestinal disease (75 and 55%, respectively, with fewer than 20 positive cells per slide). Forty-

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TABLE 1. Percentages of positive test results and mean numbers of antigen-positive cells per slide by group

Group[a]	No. of patients tested	No. of positive patients (%)	No. of positive cells per slide			Distribution of quantitative AG (% of patients) by no. of positive cells per slide			
			Mean (SD)	Median	Range	1–5[b]	6–19	20–99	100–2,000
HIV-negative controls									
CMV negative	15	0							
CMV positive	15	0							
1	30	0							
2	35	4 (11.4)	3 (2)	3	1–5	100			
3	57	27 (47.4)	34 (51)	8	1–203	41	26	18	15
4	9	4 (44.4)	135 (168)	90	2–461	25	25		50
5	30	29 (96.7)	119 (312)	21	1–1,967	24	21	28	28
6	13	10 (76.9)	260 (448)	15	1–1,440	30	20	10	40

[a] See the text for definitions of the groups.
[b] For the frequencies of low-grade AG, $P = 0.003$ for group 2 versus groups 5 and 6, $P = 0.07$ for groups 2 to 4 versus groups 6 and 7, and $P = 0.24$ for groups 3 and 4 versus groups 5 and 6.

two percent of the antigenemic patients with retinitis showed only low-level AG (1 to 5 positive cells per slide). Five of seven patients with both retinitis and gastrointestinal disease exhibited more than 100 positive cells per slide.

We have shown that the CMV pp65 AG assay is a sensitive method for the diagnosis of CMV disease in AIDS patients. In a cross-sectional analysis, the test had a sensitivity of 97% for patients with primary disease and 77% for patients with relapse, resulting in an overall sensitivity of 91%. The negative predictive value of the AG assay was 96%. These results are in agreement with reports by other investigators (3, 4, 6, 9). The specificity reported by Mazzulli et al. (11) may be somewhat higher than that reported here because of the more strict definition of CMV disease used in the present study. The lower sensitivity of the CMV AG assay for patients with relapse may result from a lower viral load during antiviral maintenance therapy or from a lower sensitivity of the assay for CMV retinitis which may reflect localized rather than disseminated disease, especially during relapse.

A clear correlation between high-level AG and symptomatic disease was apparent in the present study (Table 1). The quantification of the AG increased both the specificity and the positive predictive value of the test for the diagnosis of CMV disease. However, the sensitivity for disease was decreased when only high-grade AG was considered (Table 2). CMV disease, especially retinitis, may also occur in patients with low-grade AG. In the present study, retinitis was associated with AG of fewer than 5 positive cells per slide for 42% of the patients. The presence of retinitis in patients with low systemic viral loads has been reported in other studies (3, 5, 8). However, other investigators have not described such an association (4, 6).

In conclusion, the pp65 AG assay is a sensitive and rapid method for the diagnosis of CMV disease in AIDS patients. Our data suggest that the assay may also be useful for the diagnosis of CMV disease in clinical situations in which CMV is suspected but the diagnosis is difficult to confirm. In these cases, a negative AG assay appears to be useful in ruling out primary CMV disease. High levels of AG appear to be highly suggestive of CMV disease. Patients with high levels of AG should be carefully examined. However, low levels of AG do not exclude disease, since low-level AG may also occur in patients with confirmed CMV disease, especially retinitis. Thus, an ophthalmologic exam of at-risk patients with low-grade AG (i.e., CD4 levels of <100 cells per μl) should be performed. If disease has been excluded, both antigenemic and nonantigenemic patients are at continued risk for the subsequent development of CMV disease if the CD4 count is less than 100 cells per μl. A prospective longitudinal study of high-

TABLE 2. Sensitivity, specificity, and predictive values of the AG assay for proven CMV disease or relapse

Patient group	No. of patients with CMV disease or relapse		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	AG positive	AG negative				
All individuals[a]						
CMV positive	39	35	91	78	53	97
CMV negative	4	126				
All HIV-positive patients						
CMV positive	39	35	91	73	53	96
CMV negative	4	96				
>5 positive cells per slide	29	17	67	87	63	89
<5 positive cells per slide	14	114				
HIV-positive patients with CD4 levels of <100 cells per μl						
CMV positive	39	35	91	65	47	95
CMV negative	4	66				
>5 positive cells per slide	29	17	67	81	63	84
<5 positive cells per slide	14	74				

[a] Including HIV-negative control patients.

risk patients is needed to determine the temporal pattern of AG relative to the onset of disease.

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