Eur J Clin Microbiol Infect Dis (2000) 19:557–560

Note

False-Positive IgM Antibody Tests for Cytomegalovirus in Patients with Acute Epstein-Barr Virus Infection

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Abstract The diagnosis of acute cytomegalovirus (CMV) infection is frequently based on a positive IgM result. False-positive reactions due to interfering infections may exist. Between August 1998 and May 1999, 62 patients were found to be IgM positive and IgG negative with the Axsym assay (Abbott, Germany). Serological testing for Epstein-Barr virus (EBV) was performed in these patients to detect any cross-reactivity due to acute mononucleosis. Additionally, the results of the CMV Axsym was evaluated in 40 patients with acute EBV infection. The results suggest that the CMV-IgM Axsym assay shows a lack of specificity due to acute EBV infection. Precautions must be taken when CMV-IgM Axsym results are interpreted. It seems necessary to confirm equivocal results with another technique and to take into account other clinical and biological observations.

Introduction

The normal immune response to a primary cytomegalovirus (CMV) infection is the appearance of initial transient IgM antibodies followed by persistent IgG antibodies. Serological diagnosis of primary CMV infection is easy in cases of seroconversion. The discovery of anti-CMV IgM antibodies in a single serum sample, however, does not allow the diagnosis of a current primary infection. IgM antibodies can persist for months after primary infection [1] or reappear during recurrences of CMV infection [2]. The appearance of specific IgM may also be due to a heterotypical immune response caused by intercurrent infections [3–6]. Reactivation of a latent viral infection due to transient suppression of cellular immune functions or to polyclonal stimulation during acute Epstein-Barr virus (EBV) infection is a well-known phenomenon [7–9]. Antigenic cross-reactivity among the herpes viruses may also account for false-positive serological results [10–13].

When using the microparticle enzyme immunoassay (MEIA) (Axsym; Abbott, Germany),we observed a considerable number of positive or equivocal CMV-IgM results in the absence of IgG. Some of the patients in whom these results were obtained were subsequently monitored, and no IgG seroconversion was observed, suggesting that the IgM result was falsely positive. The aim of the study was to confirm this hypothesis and to propose an explanation for possible false-positive test results.

Materials and Methods

Sera were tested for anti-CMV IgG and IgM by commercial MEIAs (Axsym; Abbott, Germany). The CMV antigens used in the Axsym IgM indirect assay were recombinant antigens (pp150/UL32, pp52/UL44, pp65/UL83 and pp38/80a). For the IgM test, the procedure and the interpretation of results were performed as recommended. The result was negative (index value ≤ 0.399), equivocal (index value >0.400 and ≤ 0.499), or positive (index value ≥ 0.500). Sera were also tested for anti-CMV IgM by a capture enzyme immunoassay (EIA) (Eticytok; DiaSorin, Italy). The antigen used in this assay was the CMV strain AD169. The procedure and interpretation of the results were performed as recommended. The result was negative (OD < cut-off value) or positive (OD > cut-off value).

Sera were tested for anti-EBV IgG and IgM by indirect EIAs (Enzygnost; Behring, Germany). In both assays the target antigens were EBV-infected cells. The procedure (including removal of rheumatoid factor for the IgM determination) and interpretation of the results were performed as recommended. EBV IgG was quantified in arbitrary units according to a one-point quantification method (alpha-method, Behring). For the IgM test, the result was negative (OD < 0.1), equivocal (OD $\geq$ 0.1 and $\leq$ 0.2), or positive (OD > 0.2). Anti-EBNA IgG was tested by an indirect EIA using a recombinant (EBNA)-1 protein (Biotest, Germany). The short incubation protocol was used to differentiate between recent and past EBV infection. The procedure and interpretation of the results (positive or negative) were as recommended. Heterophile antibodies were determined by the Clear-

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view test (Unipath, UK). Serological criteria for acute EBV infection were a seroconversion (appearance of specific IgM and IgG in a previously seronegative patient) or a positive EBV-IgM result in the absence anti-EBNA IgG.

Denaturation techniques for distinguishing primary infection (with low-avidity IgG antibody) from secondary infection (with high-avidity IgG antibody) have been established for a variety of viruses. Sera were tested by the 8 M urea denaturation procedure [14, 15]. The standard EBV- and CMV-IgG EIAs (Enzygnost) were modified as follows. The patient's serum was added to wells coated with EBV or CMV antigens (EBV- or CMV-infected cells). All of the following steps were carried out in an automated fashion using the Behring Elisa Processor III (BEP III). After 1 h of incubation at 37 °C, the wells were rinsed with 200 µl of 8 M urea (or with 200 µl wash buffer in the reference well) and soaked in this solution for 5 min to remove low-avidity antibodies. The method was then continued according to the manufacturer's recommendations. For each serum, the OD of the reference well was compared with the OD obtained by the urea procedure. An avidity index was calculated and expressed as a percentage: avidity index = (OD urea/OD reference) × 100. As described for CMV, an avidity index of ≤ 50% is highly suggestive of a recent infection (within the past 3 months) and an avidity index of ≥ 65% is highly suggestive of a past infection (more than 3 months previously). An avidity index of between 50 and 65% was considered a grey area and does not allow the date of the infection to be determined [14].

Group 1 included all 62 patients (36 females and 26 males), among whom were seven immunocompromised patients (5 kidney and 2 liver transplant patients who attended our institution). All these patients were CMV-IgM positive or equivocal and IgG negative by MEIA between August 1998 and May 1999. If not performed at the time of sampling, serological testing for EBV was performed retrospectively. In each case, a further sample was required to confirm or exclude a CMV seroconversion.

Group 2 included 40 additional immunocompetent patients (25 females and 15 males) selected on the basis of an acute EBV infection according to the serological results (seroconversion or IgM positive, anti-EBNA negative, and low-avidity IgG). If not performed at the time of sampling, serological testing for CMV serology was carried out retrospectively.

None of the patients in groups 1 and 2 were congenitally infected.

Results and Discussion

In group 1, 47 of 62 patients had a positive CMV IgM-MEIA result in the absence of IgG, and 15 patients had an equivocal result. These patients represented 2% of all CMV IgG-negative patients tested during this period. The mean age in this group was 22 years (range, 1–66 years). Serological results for EBV and CMV follow-up in group 1 patients are summarized in Table 1.

Of these 62 patients, 8 were IgM positive and 54 IgM negative when tested with the capture EIA. CMV follow-up samples were obtained from 30 patients, among whom 5 seroconversions were observed. In three of these five patients, the first serum sample was also IgM positive by the capture EIA.

Table 1 Results of CMV follow-up and serological testing for EBV in group 1 patients

	Anti-CMV Axsym IgG negative/IgM positive or equivocal ($n = 62$)		
	Anti-CMV IgM capture EIA positive ($n = 8$)	Anti-CMV IgM capture EIA negative ($n = 54$)	Percent of total
Results of CMV follow-up			
Seroconversion	3	2	8.1
No seroconversion	1[a]	24[b]	40.3
No follow-up	4[a]	28[c]	51.6
Serological result for EBV			
Primary infection	2	15	27.4
Past infection	5[d]	29[e]	54.9
Seronegative	1	10[e]	17.7

[a] Includes 1 acute infection with EBV
[b] Includes 7 acute infections with EBV
[c] Includes 8 acute infections with EBV
[d] Includes 3 CMV seroconversions
[e] Includes 1 CMV seroconversions

All patients were tested for EBV. Seventeen (27.4%) had a primary EBV infection (mean, 18 years; range, 1–46 years). In four patients seroconversion to EBV positivity was observed. In the other 13 patients the serological diagnosis was based on the following criteria: all were anti-EBV-IgM positive and anti-EBNA-IgG negative, and 9 were also positive for heterophile antibodies. The IgG avidity index measured in 12 of them was low (≤ 50%), as expected in patients with a recent infection (range, 2–41%; mean and median, 20%). Clinical and biological data available in most cases suggested acute mononucleosis (pharyngitis, non-A, non-B, non-C CMV hepatitis, activated lymphocytes, elevated liver enzyme activities). No evidence of dual EBV and CMV primary infection was observed in group 1. However, this possibility cannot be ruled out because samples for serological follow-up were not available in all cases.

In the 40 patients without any evidence of EBV or CMV primary infection, serological testing for herpes 6 IgM was performed. Four patients were IgM positive, which suggests an additional role of herpes 6 primary infection in patients with false-positive CMV IgM-MEIA results.

The existence of EBV/CMV cross-reaction as the reason for false-positive CMV IgM reactions was further confirmed by testing retrospectively 40 patients (mean age, 16 years; range, 2–62 years) with acute EBV infection diagnosed on the basis of serological data (group 2). All of these patients were anti-EBV-IgM positive and anti-EBNA-IgG negative, and 31 of 37 were positive for heterophile antibodies. The IgG avidity index measured in 38 of them was low, as

Table 2 Results of the Axsym anti-CMV assay in the 40 patients with primary EBV infection (group 2)

	Asxym CMV result			
	IgG negative ($n=22$, 55%)		IgG positive ($n=18$, 45%)	
	IgM negative	IgM positive or equivocal	IgM negative	IgM positive or equivocal
Total no.	13	9	2	16[a]
Percent IgG negative	59.1	40.9	–	–
Percent IgG positive	–	–	11.1	88.9

[a] Two of these patients demonstrated an anti-CMV-IgG low avidity (9% and 15%)

expected in patients with a recent infection (range, 4–50%; mean, 18%; median, 16%). Clinical and biological data, available in most cases, confirmed the diagnosis.

CMV IgG and IgM MEIA results in these group 2 patients are detailed in Table 2. Twenty-two patients were CMV-IgG negative, and nine of them were IgM positive or equivocal. All were CMV-IgM negative by the capture EIA. Since these sera were selected retrospectively, no samples for follow-up testing were available to exclude a CMV seroconversion.

Eighteen patients in group 2 were CMV-IgG positive and 16 were IgM positive. In 3 of these 16 patients, a primary CMV infection was excluded; indeed, previous serological testing performed in our laboratory indicated that they were infected with CMV for more than 1 year. In the remaining 13 patients, the CMV-IgM capture EIA and the CMV-IgG avidity index were evaluated to clarify the serological results. Among the eight patients who were also IgM positive by the capture EIA, six demonstrated a high CMV-IgG avidity index (95%, 95%, 81%, 79%, 76%, and 65%, respectively) and two a low CMV-IgG avidity index (9% and 15%, respectively). Of the five patients who were negative in the capture EIA, four had a high CMV-IgG avidity index (100%, 99%, 82%, and 77%, respectively). In one case the IgG titer was too low and the avidity index could not be measured. The higher rate of false-positive IgM results in CMV-IgG-positive patients indicates that polyclonal activation, a well-known phenomenon in acute EBV infection [7–9], could play an additional role. Indeed, among the patients with acute EBV infection, 18 were CMV-IgG positive, 16 of whom were positive for IgM in the MEIA. In two cases a low CMV-IgG avidity index strongly suggested acute coinfection with both EBV and CMV. In the 14 remaining cases, acute CMV infection was excluded. In this population of CMV- and EBV-seropositive patients, the measurement of the IgG avidity index appears to be the parameter of first choice to discriminate between past and recent infections [14, 15].

To our knowledge, no evaluation of the Axsym MEIA for CMV has been published previously. Extensive evaluation should be performed to determine the clinical sensitivity and specificity of this assay. Our results indicate that the CMV-IgM Axsym assay shows a lack of specificity, possibly due to cross-reaction and polyclonal activation during acute EBV infection. Consequently, precautions must be taken when CMV-IgM Axsym results are interpreted. It seems necessary to confirm equivocal results with another technique and to take into account other clinical and biological observations. When the EBV status of the patient is not known, serological testing for EBV, if not requested at the time of sampling, should be proposed.

Acknowledgement The technical assistance of M. Broes, G. Deridder, D. Goffaux, M. Peerts and A. Varga is gratefully acknowledged.

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