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Evaluation of the COBAS AMPLICOR CMV MONITOR Test for Detection of Viral DNA in Specimens Taken from Patients after Liver Transplantation

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Detection of cytomegalovirus (CMV) DNA in blood by PCR is a sensitive method for the detection of infection in patients posttransplantation. The test, however, has low specificity for the identification of overt CMV disease. Quantitative CMV PCR has been shown to overcome this shortcoming. The COBAS AMPLICOR CMV MONITOR test was evaluated by using consecutive serum and peripheral blood mononuclear cell (PBMN) samples from liver transplant patients. Twenty-five patients had CMV viremia (by shell vial cell culture assay) and/or tissue-invasive disease (by biopsy); 20 had no active infection. A total of 262 serum and 62 PBMN specimens were tested. Of 159 serum specimens from patients with overt CMV infection, the COBAS assay detected CMV DNA in 21 patients (sensitivity, 84%). Only 1 of 103 samples from patients with no evidence of active infection had detectable CMV DNA (341 copies/ml). By comparison of 62 matching serum and PBMN samples by the same assay, 12 PBMN samples were exclusively positive, whereas only 2 serum samples were exclusively positive ($P < 0.05$). At the time of clinical CMV infection, viral copy numbers were higher in PBMNs than serum from four of five patients. The COBAS AMPLICOR CMV MONITOR test is a sensitive and specific test for the quantitative detection of CMV DNA in blood. Clinical applications of the assay will require further validation with samples from a larger population of transplant patients.

Despite the remarkable success of human organ transplantation in recent years, infection with cytomegalovirus (CMV) and its sequelae continue to cause considerable morbidity posttransplantation, therefore limiting the effectiveness of organ transplantation in the treatment of end-stage organ disease (19, 22, 24). Disease caused by this virus occurs in 20 to 60% of solid-organ transplant recipients (5, 12, 22). In addition, the introduction of newer and potentially more potent immunosuppressive agents is likely to change the natural history of CMV disease. Because of this, there has been a steady increase in the use of antiviral agents for prophylaxis against CMV disease in the organ transplant population (8). Concurrently, the search for a diagnostic assay that can accurately identify patients with the highest risk for CMV disease and that would allow timely medical intervention has been under way in many laboratories (1, 9, 23).

Available diagnostic tools enable the determination of past exposure to CMV (e.g., serology), CMV surveillance after organ transplantation (e.g., antigenemia test and PCR), and the identification of CMV by viral isolation techniques (e.g., conventional tube and shell vial cell cultures) at the time of clinical disease. The clinical utility of these techniques, however, is dependent upon their specific applications with transplant patients. Compared to viral isolation, the quantitative antigenemia test has a better sensitivity at detecting CMV viremia (6, 7). Overall, both procedures provide very high sensitivities (83 to 100%) and relatively acceptable specificities (86%) for the diagnosis of CMV disease (17). Nonetheless, the predictive values for the diagnosis of CMV tissue-invasive disease, the most severe form of the illness, range only from 50 to 60% (21). Thus, in many patients, organ involvement may be present, despite negative results by culture of blood for the virus. Alternatively, CMV viremia may be present without overt symptomatology or organ involvement. These limitations have prompted the search for a laboratory assay that is more predictive of symptomatic CMV infection before the onset of clinical disease. PCR-based qualitative detection of CMV DNA in peripheral blood samples has provided 100% sensitivity for the diagnosis of CMV infection; however, the specificity has generally been 50% or less as an indicator of CMV disease (17, 18). To overcome this shortcoming, the applicability of quantitative measurement of the CMV load by PCR has been investigated (13, 15). Results from earlier studies indicated a positive correlation between high CMV DNA loads and CMV disease, coincident with an increase in the sensitivity and specificity of quantitative PCR for the diagnosis of CMV infection. However, these assays are home-brewed and lack standardization, and the results are often not reproducible between laboratories. Additionally, such home-brewed PCRs can be tedious and require long turnaround times. To achieve a comparability of quantitative PCR among laboratories, there is a need for a commercial assay for the rapid detection of CMV DNA in clinical samples. The COBAS AMPLICOR (CA) CMV MONITOR test (Roche Diagnostics, Branchburg, N.J.) is an automated system developed for PCR amplification, detection, and quantitation of CMV DNA from bodily fluids (3, 10).

The purpose of the present study was to evaluate the performance of the quantitative CA CMV MONITOR test with stored serial specimens of serum and peripheral blood leukocytes from liver transplant patients. This study was designed to be a premarket evaluation of the CA CMV MONITOR test.

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TABLE 1. Clinical status, IgG serology (donors and recipients), and PCR results for liver transplant recipients with CMV infection

Patient	Presence of clinical symptoms	Pretransplant donor, recipient IgG serology[a]	Specimen no.	Shell vial cell culture and histologic detection of CMV		PCR (CA CMV MONITOR test [quantitative assay]) result	
				Shell vial assay result	Histology result	No. of copies/ml of serum	No. of copies/2 × 10^6 PBMNs
1	Yes	D+, R−	1	−		ND[b]	ND
			2	−		ND	ND
			3	−		ND	549
			4[c]	**+**		**ND**	**1,480**
			5	−		2,550	555
			6	−		960	ND
2	Yes	D+, R−	1	−		ND	ND
			2	−		ND	ND
			3	−		ND	ND
			4	**+**	Hepatitis	**ND**	**7,230**
			5	−		2,210	3,080
			6	−		2,170	2,350
3	Yes	D+, R−	1	−		ND	ND
			2	−		ND	ND
			3	−		ND	ND
			4	**+**		**363**	**10,900**
			5	−		675	9,400
			6	−		2,140	NA[d]
4	Yes	D+, R−	1	−		ND	ND
			2	−		ND	NA
			3	−		ND	413
			4	**+**	Hepatitis	**699**	**9,550**
			5	−		3,410	NA
			6	−		18,600	7,560
5	Yes	D+, R+	1	−		ND	ND
			2	−		ND	NA
			3	−		ND	ND
			4	−		996	NA
			5	−		2,130	6,940
			6	**+**		**83,000**	ND
6	Yes	D−, R+	1	−		ND	ND
			2	−		ND	ND
			3	**+**	Hepatitis	**ND**	**ND**
			4	−		1,530	NA
			5	−		41,000	NA
			6	−		26,600	NA
7	Yes	D+, R+	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	−		345	
			5	**+**		**ND**	
			6	−		ND	
8	Yes	D+, R+	1	−		ND	
			2	−		ND	
			3	+	Hepatitis	ND	
			4	−		**ND**	
			5	−		ND	
			6	−		ND	
9	Yes	D+, R−	1	−		ND[b]	
			2	−		ND	
			3	−		ND	
			4	−		ND	
			5	−		ND	
			6	−		ND	
			7	−		978	

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TABLE 1—*Continued*

Patient	Presence of clinical symptoms	Pretransplant donor, recipient IgG serology[a]	Specimen no.	Shell vial cell culture and histologic detection of CMV		PCR (CA CMV MONITOR test [quantitative assay]) result	
				Shell vial assay result	Histology result	No. of copies/ml of serum	No. of copies/2 × 10^6 PBMNs
			8	−		708	
			9	−		ND	
			10	−		NA[c]	
			11[d]	**+**	Gastritis	**ND**	
			12	−		ND	
10	Yes	D+, R−	1	−		ND	
			2	−		ND	
			3	**+**	Hepatitis	**1,520**	
			4	−		12,500	
			5	−		NA	
			6	−		6,130	
11	Yes	D+, R−	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	−		ND	
			5	−		ND	
			6	**+**	Hepatitis	**6,220**	
			7	−		7,850	
			8	−		5,030	
			9	−		3,810	
			10	−		813	
12	Yes	D+, R−	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	−		ND	
			5	**+**		**3,250**	
			6	−		9,490	
13	No	D+, R−	1	−		ND	ND
			2	−		ND	NA
			3	−		ND	17,000
			4	−		1,050	32,300
			5	+		5,350	76,700
			6	−		17,900	NA
14	No	D+, R+	1	−		ND	ND
			2	−		ND	ND
			3	−		ND	530
			4	+		ND	9,140
			5	−		571	69,500
			6	−		1,090	NA
15	No	D+, R+	1	−		ND	ND
			2	−		ND	ND
			3	−		ND	ND
			4	+		ND	ND
			5	−		ND	ND
			6	−		ND	ND
16	No	D+, R+	1	−		ND	2,160
			2	−		ND	ND
			3	−		ND	2,150
			4	+		ND	3,580
			5	−		532	21,100
			6	−		ND	NA
17	No	D+, R−	1	−		ND	ND
			2	+		ND	3,190
			3	−		827	NA
			4	−		742	1,180

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TABLE 1—*Continued*

Patient	Presence of clinical symptoms	Pretransplant donor, recipient IgG serology[a]	Specimen no.	Shell vial cell culture and histologic detection of CMV		PCR (CA CMV MONITOR test [quantitative assay]) result	
				Shell vial assay result	Histology result	No. of copies/ml of serum	No. of copies/2 × 10^6 PBMNs
			5	−		ND	1,220
			6	−		ND	NA
18	No	D+,R+	1	−		ND	ND
			2	−		ND	ND
			3	−		ND	NA
			4	−		552	23,400
			5	+		6,880	38,200
			6	−		19,200	210,000
19	No	D+,R+	1	−		ND	NA
			2	−		ND	ND
			3	−		981	534
			4	−		18,500	NA
			5	+		8,400	78,600
			6	−		NA	1,430
20	No	D+,R+	1	−		ND	NA
			2	−		ND	NA
			3	−		ND	ND
			4	−		ND	NA
			5	−		1,920	1,320
			6	+		82,300	102,000
21	No	D+,R+	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	−		ND	
			5	+		ND	
			6	−		ND	
22	No	D+,R+	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	−		ND	
			5	+		5,160	
			6	−		6,370	
23	No	D+,R−	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	+		ND	
			5	−		232	
			6	−		589	
24	No	D+,R+	1	−		ND	
			2	−		ND	
			3	−		ND	
			4	+		ND	
			5	−		ND	
			6	−		ND	
25	No	D+,R+	1	+		1,970	
			2	−		ND	
			3	−		564	
			4	−		NA	
			5	−		ND	
			6	−		ND	

[a] D, donor; R, recipient; +, CMV IgG positive; −, CMV IgG negative.
[b] ND, not detectable (lower limit of detection, 288 copies/ml).
[c] Boldface indicates that the patient was symptomatic.
[d] NA, sample not available.

TABLE 2. Detection of CMV DNA in serum by CA CMV MONITOR test (quantitative assay)

CMV infection	Specimens				Patients			
	Total no.	No. positive	No. negative	% Positive	Total no.	No. positive	No. negative	% Positive
Yes	159	51	108	31.1	25	21	4	84
No	103	1	102	0.97	20	1	19	5

MATERIALS AND METHODS

Subjects, samples, and definitions. Forty-five patients who had received liver allografts at the Mayo Clinic, Rochester, Minn., from December 1993 to January 1997 were selected on the basis of the availability of serially stored specimens. CMV viremia was defined as detection of CMV in peripheral blood by shell vial cell culture (16, 20). CMV infection was considered asymptomatic when viremia occurred in the absence of clinical symptoms. Patients were considered to have CMV disease if a positive shell vial culture result with blood was associated with symptomatology such as fever and unexplained fatigue, leukopenia, thrombocytopenia, abnormal liver function tests, and tissue-invasive disease as evidenced by virus isolation from tissue in cell cultures and/or a typical histopathology with identification of viral antigens with special stains. CMV-seropositive patients who had no positive CMV culture results were considered to have latent CMV infection.

All patients received conventional immunosuppression consisting of azathioprine, cyclosporine, and prednisone. Organ rejection episodes were confirmed by tissue biopsy, and patients with such episodes were treated with intravenous methylprednisolone. Individuals with steroid-resistant rejection received OKT3, at which time concurrent intravenous ganciclovir was administered. Otherwise, none of the patients received antiviral drugs primarily for CMV prophylaxis. Once CMV was detected, either by blood culture or by histopathology, patients were treated with intravenous ganciclovir at 5 mg/kg of body weight twice daily for 2 weeks.

Samples. Sera and peripheral blood mononuclear cells (PBMNs) were collected at weekly intervals for the first 6 weeks from liver transplant recipients and were stored at −70°C. PBMNs were isolated by using a Ficoll-Paque solution (Pharmacia Biotech, Piscataway, N.J.), counted, and aliquoted prior to freezing. At the time of PCR testing, samples containing PBMNs were resuspended in 200 μl of phosphate-buffered saline to a concentration of 4×10^5 cells, from which nucleic acid was extracted.

CA CMV MONITOR test. For quantitation of CMV DNA, the CA CMV MONITOR test (Roche Diagnostics) was used with both serum and leukocyte samples. Two hundred microliters of each serum sample was added to 600 μl of guanidinium thiocyanate lysis reagent, to which dextran blue and an internal quantitation standard (QS) had been added. The QS is plasmid DNA with primer binding regions identical to those of the target sequence, but with a modified probe binding site to enable the differentiation of the QS-specific amplicon from the target amplicon. For this study, the QS had a DNA copy number of 288 copies/ml. DNA was then precipitated with 800 μl of isopropanol by centrifugation, washed once with 1 ml of 70% ethanol, and resuspended in 400 μl of specimen diluent. Three controls with values that span the dynamic range of the test (negative, low-positive, and high-positive values) were included with each batch of specimens. Assay results for the CMV low-positive control were between 1.2×10^3 and 1.9×10^4 DNA copies/ml; the CMV high-positive control had between 5.0×10^4 and 4.4×10^5 DNA copies/ml. A negative result by the CA CMV MONITOR test indicated the absence of detectable CMV DNA; thus, this may mean either the lack of viral DNA or the presence of CMV below the lower limit of detection, as indicated by the QS copy number.

Following specimen preparation, 50 μl of each processed specimen or control was added to 50 μl of the master mixture, which was contained in amplification tubes specifically designed for use with the CA CMV MONITOR analyzer. The master mixture for CMV detection contained deoxynucleoside triphosphates, *Taq* DNA polymerase, the enzyme cofactor magnesium, uracil-*N*-glycosylase, biotinylated CMV-specific primers (primers LC383 and LC342c) and salts. Immediately after on-system amplification, the CA CMV MONITOR instrument automatically added denaturation reagent into each amplification tube to chemically denature the amplicons and form single-stranded DNA. Sevenfold serial dilutions of the denatured products were created by the addition of amplicon diluent, allowing the measurement of the target and the internal QS over a dynamic range. The biotin-labeled amplified products were captured with a suspension of magnetic microparticles (Dynal AS, Oslo, Norway) coated with amplicon-specific oligonucleotide probes specific for CMV and QS. Following hybridization, the CA CMV MONITOR instrument proceeded to wash away unbound materials, and the biotinylated amplicon was detected by avidin-horseradish peroxidase-tetramethylbenzidine-hydrogen peroxide colorimetric reaction. The reaction resulted in a blue complex, the intensity of which was measured by the CA CMV MONITOR instrument at a wavelength of 660 nm. The intensity of the absorbance was recorded for each sample. The measure of the CMV DNA concentration was based on a comparison to the QS present in each amplification reaction mixture. The CA CMV MONITOR test low-positive control was targeted at 1.6×10^3 copies of CMV DNA/ml; the CA CMV MONITOR test high-positive control was targeted at 2.5×10^4 copies of CMV DNA/ml. However, the acceptable range of each of these controls was lot specific and was defined by the ranges included in each CA CMV MONITOR control kit.

The dynamic range of the CA CMV MONITOR test was between 4×10^2 copies of CMV DNA per ml (10 copies of CMV DNA/PCR mixture) and 1.0×10^5 copies of CMV DNA per ml. The linearity of the assay was determined by testing four replicates of each of eight samples adjusted to contain levels of between 4×10^2 copies of CMV DNA per ml (10 copies of CMV DNA/PCR mixture) and 1.0×10^5 copies of CMV DNA per ml (2.5×10^3 copies of CMV DNA/PCR mixture). Final test results were reported as a numerical concentration in number of DNA copies per milliliter.

The same procedure for specimen preparation was followed for PBMN samples. DNA was extracted from 4×10^5 cells that had been resuspended in 200 μl of phosphate-buffered saline. From this material, 50 μl of processed specimen, that is, the amount equivalent to approximately 5×10^4 cells input into the PCR mixture, was loaded into the CA CMV MONITOR instrument. Nucleic acid amplification and detection proceeded in an automated fashion. The results of the PCR assay were adjusted to a dilution factor of 40 and were reported as number of DNA copies/2×10^6 cells. Clinical data for each patient were collected and analyzed.

Statistics. Paired two-by-two frequency tables were prepared for the evaluation of the quantitative PCR test with serum and leukocyte specimens for yields of CMV DNA.

RESULTS

A total of 262 consecutive serum samples from 45 liver transplant recipients were analyzed. Twenty-five patients (55%) had overt CMV infection diagnosed by either a positive viral blood culture result or a characteristic tissue biopsy result for CMV, and 20 (45%) had no evidence of active CMV replication (i.e., they were latently infected and noninfected) (Table 1). Among the 25 patients with active infection, 13 had asymptomatic CMV viremia and 12 had CMV disease (5 had symptomatic viremia, 6 had hepatitis, and 1 had gastritis). Subjects were further categorized by donor and recipient CMV immunoglobulin G (IgG) serostatus, as follows: donor positive and recipient positive, 25 patients; donor positive and recipient negative, 12 patients; donor negative and recipient positive, 7 patients; donor negative and recipient negative, 1 patient.

CA CMV MONITOR test (quantitative assay). CA CMV MONITOR test runs were remarkably consistent in that the negative control had no detectable viral DNA and the positive controls yielded viral DNA at copy numbers within the specified range. Therefore, it was not necessary to repeat any test run throughout the duration of the study. Each of the total 385 specimens (sera and cells) from both CMV-viremic and nonviremic individuals had optical density values within the accepted range. Of the 103 serum samples from 20 patients without CMV viremia, one specimen was positive by the CA CMV MONITOR test with a DNA copy number of 341 (Table 2). Conversely, all PBMN samples taken from patients without active CMV infection had no detectable CMV DNA by this assay.

From the same 262 serum samples, CA CMV MONITOR test positivity was detected for 51 specimens from 21 of 25 patients (sensitivity, 84%) in the CMV-positive group. Results for 62 serum samples were matched to those for cell (PBMN) specimens assayed by the CA CMV MONITOR test. For 12 specimens the cellular fraction of blood was exclusively posi-

TABLE 3. Comparison of detection of CMV DNA by CA MONITOR test from serum and PBMNs[a]

Result for serum	No. of PBMN samples with the following result:		
	Positive	Negative	Total
Positive	21	2	23
Negative	12	27	39
Total	33	29	62

[a] $P < 0.05$.

tive, whereas for 2 specimens serum was exclusively positive (Table 3) ($P < 0.05$). Of the serum specimens from 19 patients positive by the CA CMV MONITOR test, 14 (66.7%) were positive before ($n = 7$) or at the same time as ($n = 7$) shell vial cell culture positivity.

The CA CMV MONITOR test detected CMV DNA in sera from symptomatic (11 of 12; 91.7%) and asymptomatic (10 of 13; 76.9%) patients infected with this virus (Table 4). Concurrent PBMN specimens from 14 of these patients were tested by the CA CMV MONITOR test. CMV DNA was detected in five of six (83.3%) symptomatic patients and seven of eight (87.5%) asymptomatic patients. For paired serum and PBMN specimens from four of five (80%) symptomatic patients, higher viral copy numbers were found in PBMNs at the time of diagnosis of CMV infection. Conversely, the serum of one patient had a viral load of 83,000 copies/ml, but no CMV was detectable in the concurrent PBMN sample.

DISCUSSION

Prospective studies have highlighted the limitations of conventional virologic methods such as serology, tube and shell vial cell cultures, and antigenemia assays for the surveillance and diagnosis of CMV infection (22, 25). Alternatively, CMV DNA can be detected by PCR with 100% sensitivity in serial blood specimens obtained from patients posttransplantation (2, 17). However, because of the high sensitivity of this nucleic acid amplification technique, the significance of the presence of CMV DNA in asymptomatic patients is not clear. For example, qualitative PCR results do not discriminate between those patients who have symptomatic CMV infection and those who do not. Quantitative PCR formats may yield more clinically relevant results than qualitative PCR formats for detection of CMV DNA. For example, with a cohort of 43 liver transplant patients, we found that a cutoff of 7,000 copies of DNA, determined by a semiquantitative assay of blood leukocyte samples, increased the specificity and positive predictive value of the PCR for the diagnosis of established CMV disease from 33 to 89% and from 54 to 82%, respectively, without reducing the 100% sensitivity and negative predictive value of the test (13).

TABLE 4. Laboratory diagnosis of CMV infection in liver transplant patients by cell culture or histology compared with PCR

Clinical status	No. of patients	No. of patients positive by conventional means of detection in blood or tissue	CA CMV MONITOR test (quantitative assay) result (no. of patients positive/ no. of patients tested)	
			Serum	Cells
Symptomatic	12	12	11/12[a]	5/6[b]
Asymptomatic	13	13	10/13	7/8

[a] Four patients had both CMV viremia and organ involvement, and four had CMV organ involvement alone.

[b] Two patients had both CMV viremia and organ involvement, and two had CMV organ involvement alone.

Importantly, home-brewed PCR methods are hampered by a number of technical limitations. These PCR assays have highly customized protocols (the specimen type, nucleic acid extraction method, DNA polymerase enzyme, primers, cycling conditions, and amplicon detection formats used). Additionally, quantitative tests may be semiquantitative (dilutions), or results are obtained by coamplification of an internal target construct along with the viral DNA in the sample. Substantial effort is continually required to maintain optimal performance of the PCR assay. Nevertheless, these individualized home-brewed tests do not yield equivalent results; thus, reproduction of the tests and comparison of data among medical institutions are not meaningful (4, 14).

Because of these clinical practice concerns, our goal was to evaluate the commercial, automated, and quantitative CA CMV MONITOR test for its ability to detect CMV DNA in blood samples from liver transplant patients who had not received prophylactic treatment for CMV infection. By testing serial samples obtained from each patient weekly, the CA CMV MONITOR test (quantitative assay) detected CMV DNA in 84% of the patients in the CMV-positive group, i.e., those with a positive shell vial cell culture assay result and/or histopathology characteristic of CMV infection. Only 1 of 20 patients (1 of 206 samples) without cell culture evidence of CMV infection had a positive test result by the CA CMV MONITOR test (quantitative assay) (Table 2).

As expected from previous work in our laboratory (13, 14), PBMNs were more effective than corresponding serum samples in yielding CMV DNA. For this study, serum and PBMN samples from only five patients (patients 1 to 5, Table 1) with symptomatic CMV disease were taken at the same time for comparison. At the time of symptomatology, CMV DNA copy levels were higher in PBMN fractions than in sera for four of five patients. (It should be noted, however, that the volume of sample used for each specimen, i.e., 200 μl of serum and 4×10^5 cells, may not yield equivalent amounts of extracted nucleic acid.) A serum specimen from patient 5 yielded unexpected results (83,000 copies/ml), but CMV DNA was undetectable in the corresponding PBMN sample. Unfortunately, additional samples from this patient were not available for repeat analysis. This database needs to be expanded to determine clinically significant threshold levels of CMV DNA.

Our results strongly support the need for additional studies to validate the clinical use of the CA CMV MONITOR test as a quantitative test for the sensitive and specific detection of CMV DNA in blood samples of immunocompromised organ transplant patients. The manifold goals are to have a standardized commercial test that allows the detection of CMV prior to the onset of clinical disease and for the discrimination of symptomatic from asymptomatic CMV infection. In addition, a commercial test that predicts disease progression, that assesses the risk of relapsing infection after antiviral therapy, and that acts as a surrogate marker for drug-resistant CMV strains is important for the appropriate management of these patients. Only then can timely medical intervention be possible and the morbidity associated with CMV infection be curtailed.

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