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Lavori originali

Original articles

Hepatitis B virus DNA quantitation and detection of core promoter, precore and polymerase mutations in chronic hepatitis B: evaluation and clinical usefulness of three new commercial assays

Quantizzazione del DNA del virus dell'epatite B ed evidenziazione di mutazioni nelle regioni core promoter, precore e polimerasi nell'epatite cronica B: valutazione ed utilità clinica di tre nuovi tests commerciali

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INTRODUCTION

New diagnostic procedures and therapeutic approaches for chronic hepatitis B are currently under investigation. They are justified by the high incidence of HBV infection in the world and by the possible progression of the disease to cirrhosis and hepatocellular carcinoma [1].

Several aspects have to be taken into account in the evaluation of chronic hepatitis B and its treatment. During chronic HBV infection, mutations in the core promoter and precore regions have been described in association with the HBeAg status and with the severity of the liver disease [2-8]. The double core-promoter mutation A1762T/G1764A has been associated with HBeAg/anti-HBe seroconversion and seems to influence HBV replication and hepatitis B core antigen/HBeAg synthesis [6, 9-14].

However, the absence of HBeAg has been associated to the precore stop codon mutation at nucleotide position G1896A [15, 16]. Anti-HBe chronic hepatitis is the most common clinical presentation in the Mediterranean area and is frequently characterized by a severe or fulminant course and by a poor response to IFN treatment [17-20]. It has been observed that the precore mutation occurs during the natural course of chronic hepatitis B and follows the appearance of core promoter mutations [21].

The management of chronic hepatitis B depends on the HBeAg status, ALT values and HBV-DNA levels. The major favorable predictive factors of response to treatment with α-interferon (IFN) or lamivudine are high ALT and low HBV-DNA levels at baseline [22-25].

Lamivudine, a well-tolerated nucleoside analogue, has been shown to be efficient in reducing HBV-DNA levels and inducing anti-HBe seroconversion, particularly in patients with very active liver disease [26-28].

A major problem is the emergence of drug resistant strains of HBV during lamivudine treatment, which is enhanced the longer the duration of treatment [29, 30]. These mutations concern the reverse transcriptase (rt) domain of the polymerase gene in the B and C subdomains; particular drug resistance is conferred by the

mutation in the YMDD motif (M552V/I), which is often associated with the L528M mutation [26, 31-33]. According to a new numbering system recently proposed, the above mutations are identified as rtM204V/I and rtL180M, respectively [34]. The baseline HBV DNA serum levels and ALT values of patients with chronic hepatitis B and the appearance of mutations before and/or during treatment with IFN or lamivudine are therefore crucial points for the management of the disease.

A number of methods for the detection of HBV-DNA and HBV mutations are now available, but they need to be performed separately with different equipment and different molecular biology techniques. An easier determination of HBV-DNA and HBV mutations from the same DNA extraction and using the same method would further help clinicians in the evaluation and treatment of the disease.

Accordingly, we performed a technical evaluation of three new commercial assays, labeled according to the European In Vitro Diagnostic Directive 98/79, for the quantitation of HBV DNA (affigene® HBV VL, Sangtec), detection of core promoter G1764A and precore G1896A mutations (affigene® HBV mutant VL, Sangtec) and of polymerase region L528M and M552V mutations (affigene® HBV DE/3TC, Sangtec). We studied the general performance of the assays and their correlation with methods currently used. In particular, we compared the viral loads generated with the affigene® HBV VL assay versus the Amplicor HBV Monitor™ assay (Manual Test, Roche), the detection of mutation with affigene® HBV mutant VL assay versus direct sequencing and with affigene® HBV DE/3TC assay versus direct sequencing and the Inno-LIPA HBV DR (Innogenetics). Furthermore, we evaluated the clinical application of these tests in the management of a small group of chronic HBeAg positive patients.

PATIENTS AND METHODS

Frozen sera from 11 patients (6 male/5 female; median age 19 years; range 12-26) with chronic hepatitis B were studied. All patients were HBeAg/HBV DNA positive with median basal ALT 1.7 UNL and histologically ascertained mild chronic hepatitis. They had been treated for 18 months with a sequential combination of lamivudine and α-interferon (IFN); particularly they had received lamivudine 100 mg/die for 3 months, followed by lamivudine plus IFN (5MU/m^2 t.i.w.) for 6 months and lamivudine alone for other 9 months. During treatment specimens of patients had been tested for quantitative HBV-DNA by Amplicor HBV Monitor™ assay (Manual Test, Roche Diagnostics SpA, Milan, Italy); at the end of therapy core promoter and precore region changes were determined by direct sequencing and polymerase mutations by direct sequencing and Inno-LIPA HBV DR (Innogenetics, Ghent, Belgium) (35).

In the present study we compared the molecular results obtained with the above-mentioned techniques with the affigene tests. Similarly, sera from each patient were evaluated at T0, before starting treatment, at Te, at the end of treatment, and, when available, at T9, at month 9 during treatment.

HBV-DNA detection

Quantitative HBV-DNA was detected by Amplicor HBV Monitor™ assay (Manual Test, Roche Diagnostics SpA, Milan, Italy), following the manufacturer's instructions.

Core promoter, pre core and polymerase region analysis by direct sequencing.

HBV-DNA extraction

DNA was extracted from 100 µl of serum mixed with 100 µl of a digestion mix containing a final concentration of 25 mmol/L sodium acetate, 2.5 mmol/L ethylenediaminotetracetic acid, 1% sodium dodecyl sulfate, 2 mg/ml proteinase K and 10 µg/ml of yeast transfer RNA as carrier. Digestion was performed at 37° C overnight and the digests were extracted twice with phenol/chloroform, once with chloroform, followed by ethanol precipitation before resuspension in water.

Amplification of the core promoter, precore and polymerase regions

HBV-DNA was amplified by PCR twice using primers BP1: 5'TCTGTGCCTTCTCATCTG, sense, nt 1554, and BP2: 5'AATGCTCAGGAGACTCTAAG, antisense, nt 2044 for core promoter-precore regions and primers S: 5'GGTTATCGCTGGATGTGT, sense, nt 367, AS: 5'ACCCAGAGACAAAAGAAAA, antisense nt 826 for polymerase region.

The reaction was performed in a volume of 100 µl containing 10 mM Tris-HCl pH 8.3, 50 mM potassium chloride, 200 µmol of each dNTP,

2.5 mM magnesium chloride, 1 unit of Taq polymerase (Applied Biosystem PE Italia, Branch of PE Europe B.V.). The PCR products were electrophoresed in 1.5% agarose gels and visualised by UV transillumination, following staining with ethidium bromide.

Sequencing
Amplicons were purified by Qiaquick spin columns (Qiagen, Hilden; Germany) according to the manufacturer's instructions, and then sequenced by the chain-termination method, using an automated capillary sequence reader (ABI Prism 310, Perkin-Elmer). Sequence analysis was performed using the DNASIS package for Windows 95 and amino-acid alignments with the PROSIS package (Hitachi, Japan).

Detection of polymerase mutations by Inno-LIPA HBV DR
Detection of polymerase mutations by Inno-LIPA HBV DR (Innogenetics, Ghent, Belgium) was performed according to manufacturer's instructions.

Affigene® HBV VL assay
The HBV DNA levels were measured using the affigene® HBV VL assay (Sangtec Molecular Diagnostics) according to the manufacturer's instructions (IFU 3-31-79990).

Affigene® HBV mutant VL assay
The fractions of precore/core mutations compared to wildtype were measured using the affigene® HBV mutant VL assay (Sangtec Molecular Diagnostics) according to the manufacturer's instructions (IFU 3-29-79990). Details about this method are shown in Figure 1. The fractions of precore/core mutations compared

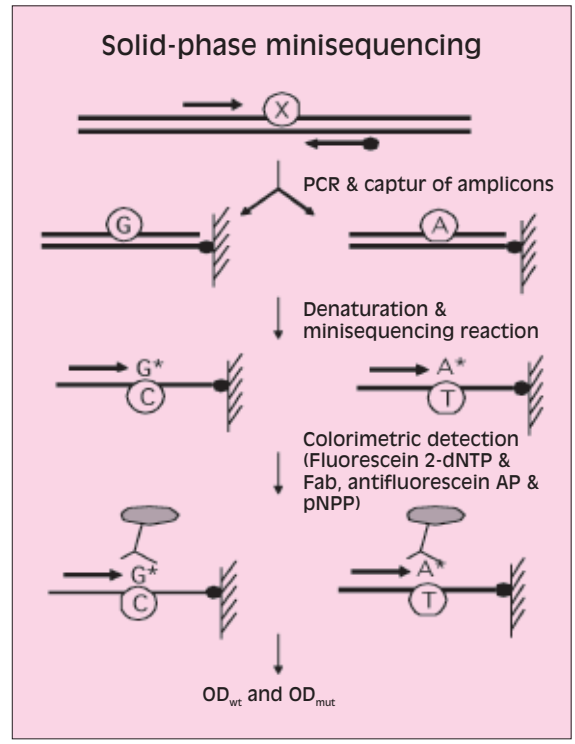

Figure 1 - Principle of solid-phase minisequencing.

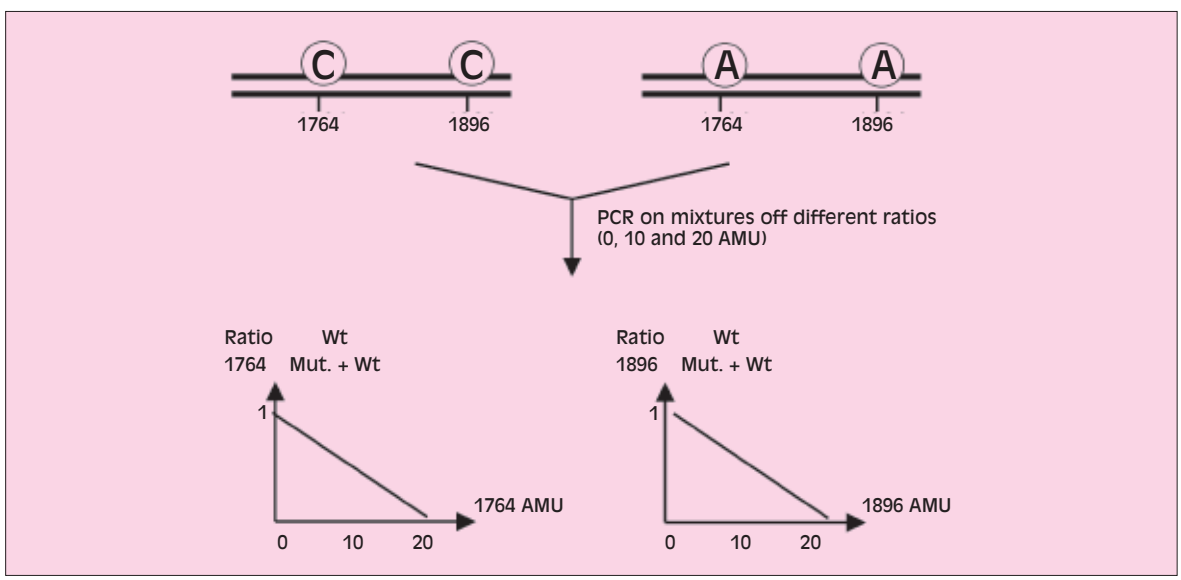

Figure 2 - Estimation of the fractions of the precore/core mutant compared to wild type from two standard curves, one for each investigated position.

to wildtype were estimated from the two standard curves, one for each investigated position, which were prepared for each amplification run. For a detailed illustration see Figure 2. The detection limit (defined as 100% positivity rate) of the assay according to the manufacturer's instructions (IFU 3-29-79990) is 2 AMU (corresponding to ~ 10% mutant), provided that the viral load is ≥5 x 10^3 IU or 2.5 x 10^4 genome copies per mL.

Affigene® HBV DE/3TC assay. The evaluation of mutations in the rt-M204V/I motif was performed using the affigene® HBV DE/3TC assay (Sangtec Molecular Diagnostics) according to the manufacturer's instructions (IFU 3-28-

Table 1 - Comparison of HBV DNA quantitation by affigene HBV VL and Amplicor HBV Monitor.

Patient and time point	*Affigene HBV VL Copies/mL*	*Amplicor HBV Monitor Copies/mL*	*Affigene HBV VL Log copies/mL*	*Amplicor HBV Monitor log copies/mL*
1 - T0	>4 x 10^7	>4 x 10^7		
1 - Tm	1,6 x 10^5	1,07 x 10^5	5,20	5,03
1 - Te	>4 x 10^7	1,19 x 10^7		
2 - T0	>4 x 10^7	>4 x 10^7		
2 - Tm	5,93 x 10^4	2,64 x 10^4	4,77	4,42
2 - Te	1,06 x 10^5	7,45 x 10^4	5,03	4,87
3 - T0	No serum	>4 x 10^7		
3 - Tm	3,29 x 10^3	Neg		
3 - Te	>4 x 10^7	1,60 x 10^7		
4 - T0	No serum	3,80 x 10^6		
4 - Tm	4,39 x 10^5	1,13 x 10^6	5,64	6,05
4 - Te	2,07 x 10^6	3,40 x 10^5	6,32	5,53
5 - T0	>4 x 10^7	>4 x 10^7		
5 - Tm	4,46 x 10^6	2,10 x 10^6	6,65	6,32
5 - Te	9,14 x 10^5	2,92 x 10^4	5,96	4,47
6 - T0	>4 x 10^7	1,60 x 10^7		
6 - Tm	3,82 x 10^5	2,74 x 10^5	5,58	5,44
6 - Te	>4 x 10^7	2,52 x 10^7		
7 - T0	No serum	>4 x 10^7		
7 - Tm	4,37 x 10^5	8,28 x 10^4	5,64	4,92
7 - Te	2,53 x 10^7	2,01 x 10^7	7,40	7,30
8 - T0	> 4 x 10^7	>4 x 10^7		
8 - Tm	2,84 x 10^5	2,25 x 10^5	5,45	5,35
8 - Te	>4 x 10^7	3,67 x 10^4		
9 - T0	1,67 x 10^7	7,55 x 10^6	7,22	6,88
9 - Tm	1,41 x 10^5	1,21 x 10^5	5,15	5,08
9 - Te	1,26 x 10^5	5,40 x 10^3	5,10	3,73
10 - T0	>4 x 10^7	>4 x 10^7		
10 - Tm	1,95 x 10^4	1,40 x 10^4	4,29	4,15
10 - Te	3,74 x 10^6	8,90 x 10^6	6,57	6,95
11 - T0	2,37 x 10^6	5,46 x 10^6	6,37	6,74
11 - Tm	3,67 x 10^5	2,05 x 10^4	5,56	4,31
11 - Te	> 4 x 10^7	1,99 x 10^7		

T0: before treatment; Tm: in the middle of treatment; Te: after treatment

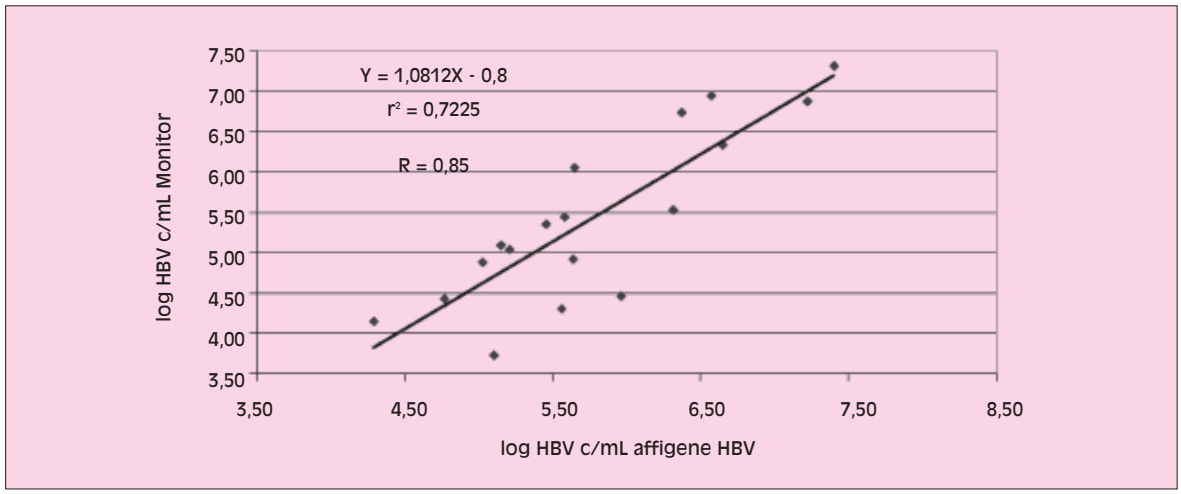

Figure 3 - Correlation of quantitative HBV DNA obtained by Affigene HBV VL and Monitor HBV Amplicor.

79990). The presence of wild type or mutant genotype in each specimen was determined from the signals from each minisequencing reaction. The detection limit (defined as 100% positivity rate) of the assay according to the manufacturer's instructions (IFU 3-28-79990) is 15% mutants, provided that the viral load is $\geq 10^4$ IU or 5×10^4 genome copies per mL.

Statistical analysis
The linear regression analysis was used for the correlation study between the Amplicor HBV Monitor™ test and the affigene® HBV VL test.

RESULTS

Clinical findings
At the end of treatment all patients remained HBeAg positive with elevated HBV-DNA and ALT levels. The rt-204 mutation was observed in 5 patients while G1764A and G1896A were found in only 1 patient.

Affigene® HBV VL
Thirty specimens were tested in single replicates with the affigene® HBV VL. The same specimens had been tested with the Amplicor HBV Monitor™ assay (manual test). Twenty-nine of the thirty (97%) specimens were concordant positive with both assays. Eighteen of the thirty (60%) specimens had HBV genome copy numbers below the upper detection limits for the assays ($4x10^7$ c/mL for both assays). Specimens above the upper detection limits for the assays were not diluted and re-tested. The eighteen specimens below the upper detection limits were included in the correlation analysis, which resulted in a correlation coefficient of r=0.85. One of the thirty specimens was positive with the affigene® HBV VL assay and negative with the Amplicor HBV Monitor™ assay (manual test). These data are reported in Table 1 and Figure 3.

Affigene® HBV mutant VL
Thirty specimens were tested in single replicates with the affigene® HBV mutant VL assay. One specimen showed up as invalid in position G1764A and there was no more serum available from this patient for that time point to repeat the test. Ten of the specimens were tested by direct sequencing. Eight of the ten (80%) specimens were concordant mutant or wild type with both assays at nt position 1764. Seven of the ten (70%) specimens were concordant mutant or wild type with both assays at nt position 1896.
The affigene® HBV mutant VL assay revealed 1-3 AMU mutant DNA at 1764 nucleotide position in two specimens and at 1896 nucleotide position in three, whereas direct sequencing showed no mutations in these specimens. The data are shown in Table 2.
Parallel tests of specimens containing small fractions of mutant virus DNA (plasmids) quantified by limited dilution (2-7 AMU) confirmed the sensitivity observed for the affigene® HBV mutant VL assay (data not shown but provided by the manufacturer).

Affigene® HBV DE 3/TC
Thirty specimens were tested in single replicates with the affigene® HBV DE/3TC assay.

Table 2 - Detection of G1764A and G1896A mutations by affigene HBV Mutant VL in comparison with Direct Sequencing.

Patient and time point	*Affigene HBV VL Copies/mL*	*Affigene HBV Mut G1764A**	*Direct Sequencing G1764A*	*Affigene HBV Mut G1896A**	*Direct Sequencing G1896A*
1 - T0	>4 x 10^7	9	-	7	-
1 - Tm	1,6 x 10^5	9	-	8	-
1 - Te	>4 x 10^7	14	mut	10	Mut
2 - T0	>4 x 10^7	0	-	0	-
2 - Tm	5,93 x 10^4	0	-	1	-
2 - Te	1,06 x 10^5	0	wild	2	Wild
3 - T0	No serum	-	-	-	-
3 - Tm	3,29 x 10^3	0	-	0	-
3 - Te	>4 x 10^7	1	wild	0	Wild
4 - T0	No serum	-	-	-	-
4 - Tm	4,39 x 10^5	0	-	0	-
4 - Te	2,07 x 10^6	-	wild	-	Wild
5 - T0	>4 x 10^7	0	-	0	-
5 - Tm	4,46 x 10^6	0	-	0	-
5 - Te	9,14 x 10^5	0	wild	0	Wild
6 - T0	>4 x 10^7	Invalid	-	0	-
6 - Tm	3,82 x 10^5	0	-	0	-
6 - Te	>4 x 10^7	1	wild	0	Wild
7 - T0	No serum	-	-	-	-
7 - Tm	4,37 x 10^5	0	-	5	-
7 - Te	2,53 x 10^7	0	wild	0	Wild
8 - T0	>4 x 10^7	0	-	0	-
8 - Tm	2,84 x 10^5	0	-	5	-
8 - Te	> 4 x 10^7	0	wild	3	Wild
9 - T0	1,67 x 10^7	0	-	0	-
9 - Tm	1,41 x 10^5	0	-	5	-
9 - Te	1,26 x 10^5	0	wild	0	Wild
10 - T0	>4 x 10^7	0	-	2	-
10 - Tm	1,95 x 10^4	1	-	4	-
10 - Te	3,74 x 10^6	0	wild	2	Wild
11 - T0	2,37 x 10^6	0	-	0	-
11 - Tm	3,67 x10^5	0	-	0	-
11 - Te	>4 x 10^7	0	wild	0	Wild

* Number of mutants are espressed in AMU (Affigene Mutant Unit) from 0 to 20. mut = mutant

Ten specimens had previously been tested by direct sequencing. Four of these ten (40%) specimens were concordant mutant or wild type in both assays.
In six of the ten (60%) specimens affigene® HBV DE/3TC detected mixes of wild type and mutant while direct sequencing showed either pure mutant or pure wild type. Nine specimens were tested both with the affigene® HBV DE/3TC assay and the Inno-LIPA HBV DR assay. Three of these nine (33%) specimens were concordant mutant or wild type with both assays.
In six cases affigene® HBV DE/3TC detected

mixes of wild type and mutant while Inno-LIPA HBV DR detected either pure mutant or pure wild type.
The data are shown in Table 3. Parallel tests of specimens containing small fractions of mutant virus DNA (plasmids) quantified by limited dilution (10-25% mutant) confirmed the sensitivity observed for the affigene® HBV DE/3TC assay (data not shown but provided by the manufacturer).

Table 3 - Detection of 528 and 552 HBV polymerase mutations by affigene HBV DE/3TC in comparison with Direct Sequencing and Inno LIPA HBV DR.

Patient and time point	*Affigene HBV VL Copies/mL*	*HBV DE/3TC Nt position 669*	*HBV DE/3TC Nt position 741*	*HBV DE/3TC Nt position 743*	*HBV DE/3TC Aminoacid 528*	*HBV DE/3TC Aminoacid 552*	*Inno LIPA HBV DR Aminoacid 552*	*Direct Seq. Aminoacid 552*
1 - T0	>4 x 10^7	wild	Mix	Wild	wild	mix M/V		
1 - Tm	1,6 x 10^5	wild	Wild	Wild	wild	Wild		
1 - Te	>4 x 10^7	wild	Mix	Wild	wild	mix M/V	Wild	wild
2 - T0	>4 x 10^7	wild	Wild	Wild	wild	Wild		
2 - Tm	5,93 x 10^4	wild	Wild	Mix	wild	Mix M/I		
2 - Te	1,06E+0^5	wild	Wild	Wild	wild	Wild	Wild	wild
3 - T0	No serum	-	-	-				
3 - Tm	3,29 x 10^3	wild	Wild	Mix	wild	Mix M/I		
3 - Te	>4 x 10^7	wild	Wild	Mut	wild	Mut I	mut I	mut
4 - T0	No serum	-	-	-				
4 - Tm	4,39 x 10^5	wild	Wild	Wild	wild	Wild		
4 - Te	2,07 x 10^6	-	-	-			mut I	mut
5 - T0	>4 x 10^7	wild	Wild	Mix	wild	Mix M/I		
5 - Tm	4,46 x 10^6	wild	Mix	Wild	wild	mix M/V		
5 - Te	9,14 x 10^5	wild	Mix	Mix	wild	Mix M/V/I	Wild	wild
6 - T0	>4 x 10^7	wild	Wild	Wild	wild	Wild		
6 - Tm	3,82 x 10^5	wild	Wild	Wild	wild	Wild		
6 - Te	>4 x 10^7	wild	Wild	Mut	wild	Mut I	mut I	mut
7 - T0	No serum	-	-	-				
7 - Tm	4,37 x 10^5	wild	Wild	Mix	wild	Mix M/I		
7 - Te	2,53 x 10^7	mix	Mix	Mix	mix M/L	Mix M/V/I	mut V	mut
8 - T0	>4 x 10^7	wild	Wild	Wild	Wild	Wild		
8 - Tm	2,84 x 10^5	wild	Wild	Mix	Wild	Mix M/I		
8 - Te	>4 x 10^7	wild	Mix	Mut	Wild	Mut V/I	mut I	mut
9 - T0	1,67 x 10^7	wild	Wild	Wild	Wild	Wild		
9 - Tm	1,41 x 10^5	mix	Wild	Wild	mix M/L	Wild		
9 - Te	1,26 x 10^5	mix	Wild	Mix	mix M/L	Mix M/I	-	wild
10 - T0	>4 x 10^7	wild	Wild	Wild	Wild	Wild		
10 - Tm	1,95 x 10^4	wild	Wild	Wild	Wild	Wild		
10 - Te	3,74 x 10^6	mix	Mix	Wild	mix M/L	mix M/V	Wild	wild
11 - T0	2,37 x 10^6	wild	Wild	Wild	Wild	Wild		
11 - Tm	3,67 x10^5	wild	Wild	Mix	Wild	Mix M/I		
11 - Te	>4 x 10^7	mix	Mix	Mix	mix M/L	Mix M/V/I	mut V	mut

nt = nucleotide; wild = wild type sequence; mut = mutant; mix = wild type + mutant sequence; M, methionine; V, valine; I. isoleucine; L, leucine.

DISCUSSION

The results of this study showed that the general performance of the three affigene® HBV assays was good; they are sensitive, easy to perform and are executed using only one DNA extraction. Their results correlated well with the other tests compared.

The clinical application of these assays is useful for a complete evaluation of the patient. The quantization of HBV DNA before starting and during antiviral treatment is an important parameter for monitoring therapy of chronic hepatitis B [22, 24].

In our patients very high viral loads dropped during treatment, but they rose again at T18 in many patients. As shown in Table 3, at the end of treatment, HBV-DNA levels increased in patients with rt-M204V/I mutation and not in patients with wild-type sequence. The appearance of the rt-polymerase mutations during lamivudine treatment should be evaluated. The identification of these mutations before clinical reactivation can favor the early prediction of a possible breakthrough. In our study the rt-M204V/I was detected as a mixed population with wild-type by the affigene assay, but only the dominant viral population was revealed with the other methods. The identification of a scanty presence of mutant strains might induce the clinician to a stringent follow-up to determine the possible increase of mutants during lamivudine therapy. However, as patients who have presented a reactivation during lamivudine treatment can now be successfully treated with new nucleotide analogues, such as adefovir [36-38], the early detection of rt-M204V/I might suggest shifting to a different drug regimen.

The detection of mixed, wild type or mutant populations in the core promoter and pre core regions is an important feature for determining the state of the disease, thus providing a tool for the most suitable therapy to personalize the dose and duration. The distribution of G1764A and G1896A mutations in the general population has epidemiological importance as it indicates the predominance of more severe or easy-to-treat hepatitis in a specific geographical area. In fact, in HBeAg positive patients the detection of G1764A mutation can be predictive of anti-HBe seroconversion and warrant the starting of treatment [4, 11]. In contrast, the presence of G1896A in anti-HBe patients indicates a generally more severe liver disease with a poor response to therapy; in these patients, as suggested by the recent guidelines for treating chronic HBV infection, therapy should be started only if baseline HBV-DNA levels are $>10^5$ genome copies/ml [18, 19, 39, 40].

In our patients the G1764A mutation was present only in 1 patient (patient 1), who also showed G1896A, and no patient obtained anti-HBe seroconversion or ALT normalization. It has been described that the occurrence of rt M204V/I mutation during lamivudine treatment in anti-HBe positive patients has coincided with severe reacutization of hepatitis, and in-vitro studies show that the G1896A mutation increases the replication efficiency of the lamivudine resistant HBV [41, 42]. In contrast, in our HBeAg positive patients, after the emergence of the rt M204V/I, HBV-DNA rose again to high levels, but the liver disease remained stable.

It is important to establish which of the parameters considered are present so as to optimize the management and treatment of chronic hepatitis B for each single patient. These three assays offer advantages in simplicity, cost and the detection of a small fraction of mutant strains; this information on the status of the disease will be helpful for clinicians in the management of chronic hepatitis B patients.

Key words: HBV-DNA, mutants, lamivudine resistance

SUMMARY

Background: HBV-DNA quantitation, the HBe antigen status and the appearance of mutations in the core promoter, precore and polymerase regions are important elements in the management of chronic HBV infection.

Methods: We performed a technical evaluation of 3 new kits, affigene® HBV VL, affigene® HBV mutant VL and affigene® HBV DE/3TC assays (Sangtec Molecular Diagnostics) in comparison with the Amplicor HBV Monitor™ assay (Manual Test, Roche), direct sequencing and direct sequencing/Inno-LIPA HBV DR (Innogenetics), respec-

tively. We evaluated the clinical application of these tests in the management of patients with chronic (HBeAg positive) hepatitis B. Serial sera of 11 chronic HBeAg positive patients were studied before, during and after lamivudine/interferon treatment.
Results: HBV-DNA quantitation detected with affigene® HBV VL showed a high correlation with the Amplicor HBV Monitor™ test (r=0.85). affigene® HBV mutant VL (positions G1764A, G1896A) and affigene® HBV DE/3TC (positions rtL180M, rtM204V/I) were able to detect a low presence of mutants in a mixed population (wild type and mutant) compared to direct sequencing and Inno-LIPA HBV DR, which identified only the dominant population.
Conclusions: These three sensitive assays, performed with the same DNA extraction, give clinicians useful information for the management of chronic hepatitis B and for timing treatment.

RIASSUNTO

Obiettivi: *La quatizzazione dell'HBV-DNA, la presenza o assenza dell'HBeAg e la comparsa di mutazioni nelle regioni core promoter, precore e polimerasi sono elementi importanti nella gestione della infezione cronica da HBV.*
Metodi: *È stata eseguita una valutazione tecnica di 3 nuovi kits, affigene® HBV VL, affigene® HBV mutant VL e affigene® HBV DE/3TC assays (Sangtec Molecular Diagnostics) comparandoli rispettivamente con Amplicor HBV Monitor™ assay (Test Manuale, Roche), sequenziamento diretto e sequenziamento diretto/Inno-LIPA HBV DR (Innogenetics).*
È stata valutata l'applicazione clinica di questi tests nella gestione di pazienti con epatite cronica B (HBeAg positiva).
Sono stati studiati sieri sequenziali di 11 pazienti con epatite cronica B, HBeAg positiva, prima, durante e dopo terapia con lamivudina ed interferone.
Risultati: *La quantizzazione dell'HBV-DNA ottenuta con affigene® HBV VL mostrava una alta correlazione con l'Amplicor HBV Monitor™ test (r=0.85). affigene® HBV mutant VL (posizioni G1764A, G1896A) e affigene® HBV DE/3TC (posizioni rtL180M, rtM204V/I) erano in grado di individuare una bassa presenza di mutanti in una popolazione mista (wild type e mutanti) comparati al sequenziamento diretto e ad Inno-LIPA HBV DR, che identificavano solo la popolazione dominante.*
Conclusioni: *Questi tre tests, praticati utilizzando la stessa estrazione di DNA, danno ai clinici utili informazioni per la gestione dell'epatite cronica B e per la valutazione dei tempi di trattamento.*

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