Presence of HBV S-gene mutants in immunocompromised patients

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Hepatitis B virus (HBV) is responsible for a serious, potentially fatal disease that affects approximately two billion people worldwide. Although an extended HBV immunization program has run since 1991, representing the most effective preventive measure possible, leading to a dramatic reduction of HBV hepatitis incidence, 4.5 million new HBV infections still occur every year.

In 1988 the emergence of HBV S-gene mutants was first observed in Italian vaccinated children's sera with the presence of both HBs antigen and anti-HBs antibodies. S-gene sequence revealed glycine (G) to arginine (A) substitution at position 145, within the *a*-determinant of S-gene, causing conformational changes that allow for the virus to escape the vaccine-induced response (Zanetti, 1988). Other similar mutations were later found in immunocompromised patients, responsible for HBV reactivation by immune escape in previously anti-HBs immune persons (Sheldon, 2008).

As the prevalence of HBV surface antigen (HBsAg) variants is unknown we investigated the presence of HBsAg mutations in 200 consecutive Ligurian patients, monitored for viral load and drug resistance.

Virus sequences were obtained by the Trugene® HBV Genotyping kit (Siemens Healthcare Diagnostics Inc.), according to the manufacturer's recommendations.

In all samples viral load was evaluated by Artus® HBV-RG PCR (Qiagen) and HBsAg status by Monolisa Ultra (Biorad Laboratories S.r.l.) and EIAgen (Adaltis Italia S.p.A.). In the samples with the G145R mutation HBsAg status was also evaluated by Vitros (Johnson & Johnson Medical S.p.A.), Axsym and Architect (Abbott S.r.l.).

G145R mutation was found in 5/200 (2.5%) examined sequences (4 D genotype and 1 A genotype) all derived from immunocompromised patients (2 neoplastic, 2 haemodialysed and 1 transplanted; this last was on lamivudine). G145R was alone in 3 patients and accompanied by T126I, T131A, C139Y, E/D144G and T126I, M133L, respectively, in 2. Differently from the other 197 patients, in which HBsAg was always detected, in the patients with multiple mutations, HBsAg resulted undetectable by 3 of the 5 assays used (Vitros, Monolisa Ultra and EIAgen), while it was detected by the other two (Axsym and Architect) (Table 1).

G145R is the most common mutation observed in the *a*-determinant (from aa 124 to aa 147) of HBsAg representing the marker of the *escape mutant* under immunological pressure (passive or active) and capable of determining false negative results with some assays for the detection of HBsAg (Carman *et al.,* 1990)

Other mutations, such as the I/T126N/A, Q129H, M133L, P142S and D144A, have been associated with immune escape, either in combination or alone (Torresi *et al.,* 2002). With regard to HBsAg testing, however, the presence of two or more mutations, including G145R was required for detection failure.

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TABLE 1 - *HBsAg detection, quantitative HBV-DNA, HBsAg gene mutations and state of treatment in the five patients with 145R mutation.*

Patient	*HBsAg EIAgen*	*HBsAg AxSYM*	*HBsAg Architect*	*HBsAg Vitros*	*HBsAg Ultra Monolisa*	*HBV-DNA UI/ml*	*HBsAg mutations*	*State of treatment*
1	Negative	Positive	Positive	Negative	Negative	$1.7x10^7$	G145R, T126I, T131A, C139Y, E/D144G	None
2	Negative	Positive	Positive	Negative	Negative	$2.4x10^4$	G145R, T126I, M133L	Lamivudine
3	Positive	Positive	Positive	Positive	Positive	1.6x106	G145R	None
4	Positive	Positive	Positive	Positive	Positive	4.4x103	G145R	None
5	Positive	Positive	Positive	Positive	Positive	8.2x104	G145R	None

In our experience, only when the G145R was present with other mutations did the detection of HBsAg fail (Table 1) (Hsu *et al.*, 2007), likely due to structural alterations of HBs protein leading to reduced affinity to neutralizing anti-HBsAb used in the screening tests (Carman *et al.,* 1990; Torresi, 2002).

Hence, for immunocompromised patients with liver damage and discordant HBV serology, PCR is helpful to detect the potential presence of HBV. Furthermore, larger studies are needed to confirm mutant prevalence, quantify the risk of false negative results in HBsAg-based screening tests and suggest solutions to minimize the problem.

REFERENCES

CARMAN W.F., ZANETTI A.R., KARAYIANNIS P., WATERS J., MANZILLO G., TANZI E., ET AL. (1990). Vaccine-induced escape mutant of hepatitis B virus. *Lancet.* **336**, 325-329.

SHELDON J., SORIANO V. (2008). Hepatitis B virus escape mutants induced by antiviral therapy. *J. Antimicrob. Chemoth.* **61**, 766-768.

TORRESI J. (2002). The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. *J. Clin. Virol.* **25**, 97-106.

ZANETTI A.R., TANZI E., MANZILLO G., MAIO G., SBREGLIA C., CAPORASO N., ET AL. (1988). Hepatitis B variant in Europe. *Lancet.* **2**, 1132-1133.

HSU CW, YEH CT, CHANG ML, LIAW YF. (2007). Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology.* **132**, 543-550.