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Short communication

Problematic molecular diagnosis of HSV-1 infection due to a single nucleotide polymorphism in the US7 gene

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ABSTRACT

Background: HSV-1 infection is very common worldwide and can be associated with severe medical conditions such as encephalitis and neonatal herpes. Current diagnosis relies heavily on molecular assays targeting the viral genome. One of the pitfalls of these assays is genetic variability, as viral polymorphisms located in the target region can impair the detection of HSV-1.

Objectives: The aim of this study was to determine if genetic diversity of HSV-1 could account for equivocal assay results we obtained during routine diagnosis of HSV-1 in a genital specimen with the HSV-1 HSV-2 VZV R-gene® assay.

Study design: The presence of HSV-1 in the genital swab studied was assessed using the HSV-1 HSV-2 VZV R-gene® qPCR assay and viral culture. Genetic variability of the patient's HSV-1 isolate was determined using molecular cloning, sequencing and comparison of the sequence of the isolate with those of previously published strains.

Results: We identified an HSV-1 isolate carrying a novel single-nucleotide polymorphism (SNP) located in the US7 gene from a genital swab. This SNP resulted in a missense substitution in the gI protein. It was responsible for the generation of an altered amplification curve when the genital specimen was checked for HSV-1 presence with the Argene HSV-1 HSV-2 VZV R-gene® assay, preventing unequivocal determination of the HSV-1 status of the sample analyzed.

Conclusions: This study raises awareness of the risk of misdiagnosis of HSV-1 infections when "single-target" molecular assays are employed.

1. Background

Herpes simplex virus type 1 (HSV-1) is a widespread virus, whose prevalence has been estimated to be 67% in 2012 in the 0-49-years-old world population [1]. HSV-1 infection can cause severe conditions, such as encephalitis and neonatal herpes, which are associated with high mortality rates [2,3]. As the outcome of affected patients is drastically improved by treatment with acyclovir, rapid and accurate diagnosis of HSV-1 infections is of utmost importance.

HSV-1 infections are mainly diagnosed using Nucleic Acid Amplification Tests (NAATs) and viral culture [4]. NAATs are the cornerstone of HSV encephalitis diagnosis, due to the poor sensitivity of culture for cerebrospinal fluid (CSF) samples, and molecular assays are progressively replacing viral culture for detection of HSV in genital specimen [5,6]. But detection of HSV-1 by NAATs can be hampered by viral genetic variability. Indeed, Stevenson et al. demonstrated that polymorphisms in the sequence targeted by the assay can strongly reduce sensitivity [7].

In our laboratory, we use the HSV-1 HSV-2 VZV R-gene® assay from Argene/bioMerieux for diagnosis of HSV-1 infections in CSF and genital swabs. It is based on the Taqman real-time PCR chemistry, and targets a unique 142 bp region of the HSV-1 US7 gene.

2. Objectives

During a routine test performed in our laboratory to detect HSV-1 in clinical samples with the R-gene® assay, we observed an altered amplification curve with an atypical non-sigmoidal shape in a genital specimen. This result prevented unequivocal identification of the HSV-1 status of the sample tested. We sought to determine if viral genetic

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variability in the target sequence could be the reason for improper amplification.

3. Study design

3.1. Patient specimen

A genital swab was collected from a pregnant woman presenting with vulvar lesions evocative of HSV infection at Antibes Hospital.

3.2. Assessment of the HSV-1 status of the patient genital swab

DNAs extracted from the patient specimen or the viral culture supernatant using the NucliSENS® easyMAG® platform (bioMérieux, Craponne, France) were amplified with the HSV-1 HSV-2 VZV R-gene® assay (Argene, Verniolle, France) or the US6 targeting in-house qPCR assay designed by Weidmann et al. [8], respectively. Regarding viral culture, MRC-5 and HEPG2 cells were inoculated with the medium containing the patient genital swab, and monitored daily for the presence of a cytopathic effect (CPE). HSV-1 presence in cultures was assessed by staining with the MicroTrak® HSV1/HSV2 Direct Specimen Identification/Typing Test (TrinityBiotech, Bray, Ireland).

3.3. Identification of a novel single-nucleotide polymorphism (SNP) in the HSV-1 US7 gene

US7 amplicon sequences targeted by the R-gene® assay with or without the SNP were cloned into the TA pCR™ 2.1-TOPO® vector (ThermoFisher Scientific, Illkirch-Graffenstaden, France). Three regions covering the whole coding sequence of gI were amplified by PCR with the ThermalAce DNA Polymerase (ThermoFisher Scientific, Illkirch-Graffenstaden, France) and amplicons were sequenced in both directions using PCR primers. For semi-quantitative PCR of HSV-1 US7 gene, 10^5 copies of plasmids were submitted to PCR amplification with the R-gene® assay or T7 and M13 Reverse primers for 25, 30 or 35 cycles. PCR reactions were analyzed by agarose gel electrophoresis.

4. Results

4.1. Equivocal amplification of HSV-1 DNA in an HSV-1 positive genital swab using the HSV-1 HSV-2 VZV R-gene® assay

The DNA extract of the patient specimen was amplified with the R-gene® assay. The QS3 and TSC positive controls of the assay exhibited a sigmoidal curve using a linear fluorescence scale, indicative of proper amplification of HSV-1 DNA. In contrast, the curve obtained from the patient sample showed an altered non-sigmoidal shape with no exponential phase (Fig. 1). Based on these data, the presence of HSV-1 in the patient specimen could not be unequivocally confirmed or ruled out. HSV-1 positivity of the sample was demonstrated by viral culture and positive amplification of the culture supernatant DNA extract with an in-house qPCR assay targeting a different HSV-1 gene (i.e. US6).

4.2. Discovery of a new missense SNP in HSV-1 US7 gene responsible for improper amplification with the R-gene® assay

The US7 amplicon amplified from the culture supernatant by the R-gene® assay was cloned and sequenced. Alignment of the sequence with the previously published US7 sequences of 286 HSV-1 strains allowed us to identify a novel and unique SNP corresponding to a C to T substitution which resulted in an Arginine to Cysteine amino acid substitution at position 139 of the gI glycoprotein (Table S1). We further sequenced the whole coding region of the gI protein, and deposited the sequence in GenBank (Accession number MG386501). We amplified plasmids containing the US7 amplicon sequence with (SNP+) or without (SNP-) the SNP with the R-gene® assay. We observed a non-sigmoidal curve for the SNP + vector, while the curve obtained for the SNP- plasmid was clearly sigmoidal. This demonstrated that the SNP was indeed responsible for the equivocal amplification observed in the patient specimen. As the SNP was located far from the extremities of the US7 amplicon, we hypothesized that the altered shape of the amplification curve was not due to impaired annealing of primers, but rather to a defect in hybridization of the Taqman probe at the SNP site. We performed semi-quantitative PCR with the R-gene® assay on SNP + and SNP- vectors, and measured identical quantities of PCR product after 25, 30 and 35 PCR cycles for both plasmids. These data confirmed that annealing of primers and amplification of the HSV-1 US7 amplicon were not affected by the presence of the SNP.

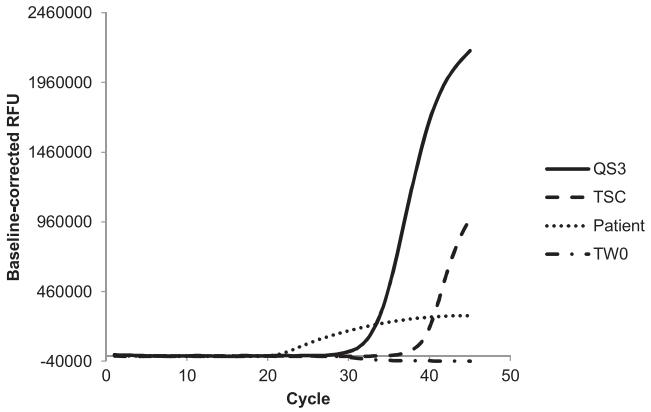

Fig. 1. Real-time PCR curves generated from amplification of QS3, TSC, TW0 and patient sample with the HSV-1 HSV-2 VZV R-gene® assay. QS3 (positive control, 200 HSV-1 copies), TSC (positive control, 5 HSV-1 copies), TW0 (negative control) and patient sample DNA extract were submitted to real-time PCR amplification using the R-gene® assay. This graph shows the baseline-corrected fluorescence (y-axis) measured at each PCR cycle (x-axis) for QS3 (solid line), TSC (dashed line), TW0 (dashed-dotted line) and patient sample (dotted line).

5. Discussion

NAATs are becoming the most prominent tools used by clinical microbiology laboratories for diagnosis of HSV-1 infections, due to their high clinical sensitivity and low turnaround time. However, as our study shows, genetic variability of HSV-1 can impair molecular detection of the virus in clinical specimen and yield false-negative results. This can prove problematic when testing is performed to diagnose encephalitis and neonatal herpes, as a misdiagnosis can seriously compromise patient outcome. This pitfall could be almost completely avoided through the systematic use of two NAATs targeting different regions of the HSV-1 genome, as the probability of occurrence of concurrent polymorphisms in two distinct areas is very low. This is supported by our data showing positive amplification of the patient's HSV-1 strain carrying the US7 SNP with an alternative in-house qPCR assay targeting the US6 gene. In addition, we think that the field would benefit from development of unique multiplex assays. Considering this, our data should fuel changes in clinical practices with the adoption of a "dual-target" strategy to increase the accuracy and reliability of HSV-1 diagnosis, at least for testing of critical specimen such as CSFs, genital swabs from pregnant women, and neonates samples.

Our data strongly suggested that the SNP we identified was located in the Taqman probe hybridization region, leading to the formation of a mismatch during probe annealing. It is well known that mismatches between probes and target sequences result in reduced Tm, which induces dissociation of the probe from the template at a lower temperature [7,9]. Thus, we propose that the alteration of the shape of the amplification curve may be due to instable hybridization of the probe at the annealing/extension temperature. This may have impaired efficient

hydrolysis of the Taqman probe by the DNA polymerase and hindered accumulation of fluorescence.

Author contribution

Sébastien Vitale: Conceptualization, Investigation, Writing – Original Draft. **Céline Loubatier:** Investigation, Writing – Review & Editing. **Isabelle Cannavo:** Resources, Writing – Review & Editing. **Valérie Giordanengo:** Writing – Review & Editing, Supervision.

Ethical approval

Not required.

Competing interests

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2018.12.001.

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