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Short Title: Acyclovir-resistant HSV-1 keratitis

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Introduction

Herpes simplex keratitis (HSK) is the most frequent ocular manifestation of herpes simplex virus 1 (HSV-1) infection, with an incidence ranging from 18.2 to 25.8/100,000 inhabitants per year,[1, 2] and a prevalence estimated at 150/100,000 inhabitants,[2] i.e. approximately 100,000 patients in countries such as UK, France or Germany, and 500,000 in the USA. After the first episode, recurrences of HSK may occur in up to 63% of patients,[2] and 51% of them experience more than 2 relapses after a follow-up duration of 15 years.[3] Multiple recurrences can eventually lead to visual dysfunction, due to optical aberrations,[4] corneal opacification and/or neovascularization, with 10% of affected eyes below 20/100 vision after 10 years of disease progression.[5] Moreover, recurrent HSK leads to chronic changes in the ocular surface and trigeminal nerve,[6, 7] ultimately causing dry eye and neurotrophic keratopathy.[8] Taken together, HSK is a leading cause of infection-related blindness in industrialized countries,[9] and severely impairs quality of life in most patients.[10]

To defeat the burden of recurrent HSK, the only currently approved preventive strategy is the long-term use of antiviral prophylaxis (AVP).[11] The results of the Herpetic Eye Disease Study (HEDS), performed in the USA, indicated that acyclovir (ACV) 400 mg b.i.d. causes a 2-fold decrease in recurrences.[11] Given its bioequivalence, 500 mg q.d. of valacyclovir (VACV), (the oral prodrug of ACV), can replace ACV in this setting.[12] However, at the recommended regimen, neither ACV nor VACV block 100% of clinical recurrences, or prevent viral shedding in tears between recurrences.[13] More concerning, as observed in several other chronic viral diseases,[14] long term AVP is associated with the risk of developing antiviral drug resistance, specifically, ACV-resistant (ACV^R) HSV-1 isolates.[15] As VACV is transformed in ACV, both drugs have similar modes of action. These antivirals target the HSV-1 viral DNA polymerase (DNA pol) after initial phosphorylation that is

mandatory for these drugs to be effective and can only be performed by viral thymidine kinase (TK). Thus, ACV resistance may be due to a selection of strains with mutations in TK or in DNA pol, which negatively impacts the antiviral efficacy of ACV (and VACV).[16]

Recent studies have alerted the community about the increasing proportion of ACV^R isolates in patients with recurrent HSK,[15, 17] and the decreased efficacy of AVP.[18, 19] A recent study of immunocompetent patients presenting with HSK recurrences despite AVP, reported that HSV-1 resistance to ACV was a significant cause of AVP failure in these patients, and was associated with longer disease duration.[20] Notably, HSV-1 polymerase chain reaction (PCR) of tear samples in these patients was positive despite long-term AVP, with up to 83% of the positive samples containing genotypically ACV^R virus.[20] This finding provided clinical relevant information to ophthalmologists with patients with recurrent HSK despite AVP, and highlighted that identifying ACV^R may require changing the antiviral regimen.

In the current study, we report a series of patients with HSK caused by ACV^R HSV-1 strains in which we analyzed the relationships between virologic and clinical data. We report outcomes that may encourage clinicians to actively investigate for HSV-1 resistance to ACV in appropriate cases. Additionally, we discuss current and future therapeutic strategies that could help to manage the challenging issue of HSV-1 resistance to ACV in HSK.

Methods

Study design

This multicentre retrospective study evaluated consecutive patients from 9 university hospitals with recurrent HSK with an ocular sample that was positive for HSV-1 resistance to ACV, from January 2010 to March 2018. The French reference center for HSK (Department of Ophthalmology, Bicêtre Hospital, Kremlin-Bicêtre, France)

coordinated this study. This study adhered to the tenets of the Declaration of Helsinki. Institutional Review Board (IRB)/Ethics Committee approval was obtained by the French Society of Ophthalmology (IRB 00008855 Société Française d'Ophtalmologie IRB#1). All participants provided informed consent.

Patients and clinical samples

The date of inclusion corresponded to the diagnosis of HSV-1 ACV resistance. Clinical samples were gathered from corneal swabs or tears collected using Schirmer strips, as previously described,[20] from patients with suspected antiviral-refractory HSK, defined by highly frequent recurrences despite appropriate AVP and/or clinical resistance to suppressive antiviral treatment of an ongoing relapse. A chart review was performed to collect data on: i) reason(s) for genotypic resistance testing (frequent recurrences despite appropriate AVP and/or clinical resistance to suppressive antiviral treatment), ii) number of HSK episodes before and after inclusion, iii) duration of HSK history (i.e., time between first HSK episode and inclusion), iv) medical history of patients, including any disease or treatment that could induce complete or partial immunodeficiency, v) ophthalmological features of HSK and related ocular complications, vi) best corrected visual acuity (BCVA) at inclusion and at the end of follow-up, recorded in logarithm of the minimum angle of resolution (LogMAR) notation for statistical analysis. Patients were grouped based on the number of recurrences before inclusion, from 2-5, 6-10, 11-20, > 20. The value allocated for each patient corresponded to the mean of the interval, except for the highest group (>20), for which conservative hypothesis dictates using the lowest number of recurrence (20).

Virological analysis

HSV-1 resistance to ACV was confirmed with a genotyping method based on sequencing the UL23 (encoding TK) and UL30 (encoding DNA pol) viral genes. All samples were processed as follows: after isolating the nucleic acid from ocular fluids

as previously described,[20] real-time PCR (Artus® HSV-1/2 RG kit; Qiagen, Hilden, Germany) was used to detect the HSV-1 DNA. For positive samples, HSV-1 genotypic resistance profile was investigated by sequencing full-length UL23 and UL30 genes, using the Sanger method, as previously described.[21] This sequencing approach detects resistance mutations in the majority strain of a clinical sample, which has the highest probability of being responsible for the clinical phenotype.[22] Mutations detected by the genotyping method were considered resistance mutations based on previous data reported in the literature or from phenotypic testing results performed in the laboratory.[21]

Statistical analysis

Patient anonymity was maintained for the duration of the study. Descriptive analysis was performed with Excel (Mac Version 14.4.1; Microsoft Corp., Redmond, WA, USA) and statistical analysis was performed with R version 3.2.0 software.[23] Continuous variables are presented as mean ± standard error, discrete variables as median (interquartile range [IQR]). The Kruskall-Wallis test was used for quantitative outcomes. Statistical significance was indicated by $P \leqslant 0.05$ (2-tailed).

Results

Patients

Eighteen HSK patients (13 males, 5 females, aged 66.8±4.7 years) with an HSV-1 positive ocular sample with genotypic resistance to ACV, were retrospectively included (**Table 1**). HSV-1 genotypic resistance testing was performed because of frequent recurrences despite appropriate AVP (13 patients, 72%), or poor efficacy of suppressive antiviral treatment (5 patients, 28%). At inclusion, the mean duration of disease was 29.8±9.4 years and 15 patients (83%) had experienced more than 10 recurrences. Six patients (33%) presented with definite causes of immunodeficiency at inclusion: 3 were receiving systemic corticosteroid and/or immunosuppressive

drugs, 1 patient had an idiopathic combined immune deficiency with hypogammaglobulinemia and CD4+ T cell lymphocytopenia, 1 patient had undergone allogeneic hematopoietic stem cell transplantation (HSCT) for a refractory diffuse large B-cell lymphoma 3 months prior to inclusion, and 1 patient had non-Hodgkin's B cell lymphoma and was receiving chemotherapy and anti-CD20 monoclonal antibody (rituximab) (**Table 2**).

Ophthalmological findings

All patients presented with unilateral disease. The recurrences leading to the diagnosis of HSV-1 resistance to ACV were diagnosed as epithelial keratitis in 11 cases, stromal keratitis with epithelial ulceration in 6 cases and neurotrophic ulcer in one case. All patients had epithelial involvement at inclusion. Genotypic resistance testing was performed for suspected ACV resistance, due to frequent recurrences despite appropriate AVP in 13 patients (72%), or poor efficacy of suppressive antiviral treatment in 2 patients, (11%), and both conditions occurring in 3 patients (17%). Two patients were lost to follow-up after diagnosis of resistance. Remaining patients were followed for 31.5±14.5 months after inclusion. The mean BCVA at inclusion was 0.95±0.42 LogMAR (20/200) and the mean final BCVA was 1.00±0.50 LogMAR (20/200) (**Table1 & 2**). There was a mean of 2.8±1.3 recurrences between inclusion and end of follow-up. Neurotrophic ulcers were noted in 1 patient at inclusion and in 5 patients during follow-up.

Virological data

PCR was performed from corneal swabs for 12 patients and on tear samples for 6 patients. Mutations conferring resistance to ACV were observed in HSV-1 TK for 15 patients (83%) and in 3 patients (17%) for DNA Pol. Mutations in TK consisted of one amino acid change for 13 of 15 patients, two different amino acid changes for 1 patient, and one frameshift for 1 patient. Mutations in TK (including stop codons)

known to confer resistance to ACV were found in 13 patients (**Table 3**). For 2 patients, resistance to ACV was confirmed using phenotypic testing (**Table 3**). Noteworthy, HSV-1 strains with stop codons or frameshift mutations leading to absence of TK activity are viable. Indeed, TK-negative HSV-1 strains can replicate and establish latent infection. [24, 25] In trigeminal ganglia, TK-negative viruses can be rescued by the TK from wild-type viruses coinfections, a phenomenon known as complementation.[26] In peripheral tissue, where host cells actively divide and grow, and where nucleotides are available in quantity, TK is dispensable for HSV-1 replication.[24]

For the 3 remaining patients, one amino acid change conferring resistance to ACV was detected in the DNA pol. Interestingly, no HSV-1 strains exhibited resistance mutations within both TK and DNA pol genes (**Table 3**). Of note, the DNA pol gene could not be sequenced for 3 patients because the HSV-1 load was too low in the ocular sample.

Treatments

At inclusion, all patients were treated with oral VACV, ranging from 500 to 3000 mg/day [median 1500 mg/day, interquartile range (IQR): 1000-1500 mg/day] (**Table 2**). When HSV-1 antiviral resistance was diagnosed, topical antiviral treatment was added to oral therapy in 9 patients, either as topical 0.15% ganciclovir (GCV) (5 patients), or topical 1% trifluridine (TFT) (4 patients). Oral treatment was switched to famciclovir (FCV) (500 mg t.i.d.) in 5 patients and to valganciclovir (VGCV) (900 mg b.i.d.) in 1 patient. The epithelial ulcer healed in all cases with follow-up. Once the acute phase of the relapse was controlled, VACV was used for AVP in 11 patients, ranging from 500 to 3000 mg/day (median 1500 mg/day, IQR: 750-1500mg/day), FCV was used for AVP in 5 patients, ranging from 500 to 1000mg/day (median, 1000 mg/day) and VGCV in 1 patient (900 mg/day). Topical GCV (0.15%, t.i.d.), was used alone for 1 patient, or in combination with VACV for 1 patient as prophylaxis.

Immunocompetent versus immunocompromised patients

The number of recurrences between the first episode and the diagnosis of resistance was statistically significantly higher in immunocompetent patients (12 patients, 67%) than in immunocompromised patients (6 patients, 33%) (16.4±1.9 versus 11.5±4.9, *P*=0.05) (**Table 1**). The duration of disease before the diagnosis of resistance was 19.5±15.3 months in immunocompromised patients and 35.8±11.2 months in immunocompetent patients (*P*=0.11). Mean initial BCVA was statistically significantly better in immunocompromised patients than immunocompetent patients (0.41±0.24 LogMAR versus 1.26±0.59 LogMAR respectively, *P*=0.05). BCVA at final visit was statistically significantly better in immunocompromised patients than immunocompetent patients (0.26±0.23 LogMAR versus 1.34±0.63 LogMAR, *P*=0.04).

Discussion

The outcomes of this study highlight the importance of investigating HSV-1 resistance to ACV, in patients presenting with HSK who are recalcitrant to typical antiviral regimens, which allows for planning of future management. In this case series, HSV-1 resistance to ACV was evaluated in suspected antiviral-refractory HSK, defined by frequent recurrences occurring despite AVP and/or acute keratitis that was clinically refractory to high dose antiviral treatment.

In the current study, HSV-1 resistance to ACV was associated to mutations within TK in 15 patients and within DNA pol in 3 patients. Additionally, ACV resistance was diagnosed in patients with long-standing HSK. Interestingly, resistance to ACV was diagnosed mostly when viral replication was clinically obvious (i.e., at a pure epithelial keratitis episode, or during stromal keratitis with epithelial ulceration), and the confirmation of resistance to ACV seemed to occur earlier in the course of herpetic ocular disease in immunocompromised patients.

Long term AVP has been the standard of care for over two decades for HSK.[11] While clinical HSV-1 isolates may contain some ACV^R strains, their proportion within a clinical sample - which determines the global susceptibility of the whole isolate to ACV, and thus the clinical efficacy of ACV (and VACV) for a given patient -[18, 38] may increase under the selective pressure of AVP. Based on a virological method specifically isolating as many different strains as possible from a given clinical sample, van Velzen et al identified long term AVP (> 12 months) as an independent risk factor (odds ratio = 3.42) for the presence of ACV^R strains in corneal swabs from HSK patients.[15] However, this retrospective study relied on samples taken for diagnosis, potentially influencing the findings (and the odds ratio) by ascertainment bias.

In our series, all positive ACV^R HSV-1 samples originated from keratitis cases with corneal epithelium involvement, such as epithelial keratitis or stromal keratitis with epithelial ulceration. These replicative forms are associated with the highest viral loads on the ocular surface, which allow PCR to be more sensitive.[19, 39-41,42] Alternately, stromal keratitis without ulceration mostly involves an exacerbated immune response to HSV-1 antigens,[43] hence, virological investigations are much less likely to provide positive PCR results.[44]

Eleven of 18 of the cases had epithelial keratitis at the time diagnosis of resistance was made. While this clinical form has a good prognosis *per se*, and these patients were efficiently managed with currently available antiviral treatments, sufferers are at high risk of presenting other more severe forms of the disease on further recurrences,[2] having greatly impacted quality of life[10] and developing dry eye and neurotrophic keratopathy. [6]

We observed a better visual outcome in immunocompromised patients, both at inclusion and at the end of follow-up. Immunocompromised patients may have a decreased inflammatory response during HSK recurrences, that is less likely to

induce stromal immune infiltration and corneal opacification. Immune compromise may also explain the faster selection of resistant strains as it facilitates the emergence of ACV^R HSV-1 strains that are usually controlled by normal immune effectors in immunocompetent individuals. In immunocompromised individuals, wild-type HSV-1 is inhibited by ACV whereas ACV^R HSV-1 strains are not, allowing them to become the predominant strain quicker.[16, 45]

Available options for managing ACV^R HSK

In patients infected with TK-mutant HSV-1, TK-dependent anti-HSV-1 drugs (i.e., ACV/VACV, FCV/penciclovir [PCV], GCV/VGCV) are theoretically ineffective. They all depend on TK for their mode action, explaining observations of cross-resistance for these three drugs.[17, 46-48] In this context, TK-independent antiviral drugs, such as topical TFT or intravenous foscarnet (for the most severe cases) seem to be most logical options for suppression therapy.[41, 49] However, in the current series, some patients were successfully managed with the addition of topical GCV. Topical GCV has very high intracellular penetration and may occasionally overcome resistance due to the high intracellular concentrations that will eventually allow phosphorylation of the drug by altered TK, or even the host cellular TK.[50] Koseoglu et al[51] recently reported 4 cases of HSK unresponsive to conventional treatment that were successfully treated with oral VGCV.[51] Although virus resistance was not demonstrated by Koseoglu et al,[51] the authors speculated that VGCV could be an option in cases with virus resistance caused by DNA pol mutations, as long it showed *in vitro* efficacy against specific DNA pol-mutant HSV-1 strains.[52] In the current study, FCV (the prodrug of PCV) was empirically used as an alternative to VACV as preventive or suppressive treatment for patients with ACV^R-HSK. Although PCV shares the same mode of action as ACV, it has a 10-fold longer half-life in HSV-1 infected cells and a 100-fold greater affinity for TK than ACV.[53] Therefore, PCV is a potential option in strains with TK mutations associated with a decreased ACV-affinity

for TK.[46] Unfortunately, current genotypic assays do not always discriminate between HSV-1 strains with altered TK activity or decreased ACV-affinity.

In the rare setting of antiviral resistance caused by the DNA pol mutation, there is currently no treatment approved for HSK. In this rare situation, interferon eye drops can be considered for enhancing the local immune response and overcome viral replication.[54]

Similarly, in cases of both TK or DNA pol-mutant strains, there is currently no approved available options for long-term AVP. Some authors have suggested that long term topical GCV, in the context of cytomegalovirus uveitis / endotheliitis,[55] could be an option, but the tolerance and efficacy for HSK remain unknown.

Future strategies to manage ACV^R HSK

New anti-HSV-1 molecules, such as helicase primase inhibitors (namely amenamevir and pritelivir), which do not depend on TK to be efficient, could be beneficial in the context of ACV^R HSK. Amenamevir has been approved in Japan for varicella-zoster virus (VZV) infections but requires investigation in the context of herpetic ocular disease.[56] Treatments combining antivirals with different modes of action may also provide additive effects to control viral replication.[57] In parallel, other strategies involving heparan sulfate mimetics could be considered for managing these patients, as they compete for adsorption of viruses into susceptible cells and have shown some in vitro efficacy.[58]

Limitations

Our study has some limitations including the small sample size and the retrospective nature (including missing data for follow-up of 2 patients). We acknowledge that it would have been interesting to provide the total number of HSK cases managed during the study period. While this data would likely implicate ACV^R HSK as rare events, we believe this condition is underdiagnosed, and rarely considered by clinicians.

The number of recurrences and the duration of disease between first episode and diagnosis of resistance are likely covariant, and this may impact the statistical significance of our results.

Additionally, only trained corneal specialists managed these patients and they were likely aware of the risk of acquired resistance to antiviral drugs and had access to genotyping, which is yet not widely available. However, in this study we provide comprehensive clinical data on a series 18 cases of both clinically and virologically resistant HSK, where previously published comparable studies presented 1 to 3 cases.[19, 39-41] Although the outcomes may preclude drawing firm conclusions on therapeutic management of these cases, they do permit us to propose some options in addressing this challenging clinical entity.

Another limitation is the absence of data on cumulative duration of antiviral intake prior to the diagnosis of resistance, which may be factor in developing HSV-1 resistance. Nevertheless, we assume that this variable is at least partly correlated with the duration of the disease. Finally, a notable limitation of genotypic resistance assay is the absence of concomitant phenotypic assays, which would allow testing of antiviral treatments. However, unlike phenotypic assays based on plaque reduction or plating efficiency,[15] the genotyping approach does not require strain isolation from the sample, which may be hazardous with ocular swabs or tears, and is less time consuming.[21] As genotyping is performed on viral DNA, specimens can be readily transported to a specialized virology laboratory for analysis, as in this study. Sequencing is relatively simple and is now widely accepted as a reliable method for detecting clinically relevant HSV-1 drug resistance.

To summarize, ACV^R HSK must be suspected in cases with highly frequent recurrences despite appropriate AVP and/or resistance to antiviral treatment. ACV^R is more likely in patients with long-standing disease and multiple recurrences and develops more rapidly in immunocompromised patients. These findings should

neither deter from the use of AVP as a first line strategy for patients with recurrent HSK, as this saves vision over the long-term for HSK patients,[5] nor indicate that AVP is harmful on a large population scale. However, they highlight the fact that some patients may develop ACV^R HSK. Ophthalmologists should consider this rare, but challenging condition, as it requires specific diagnostic and therapeutic strategies. As there is currently no approved effective AVP for ACV^R HSK, new therapeutic strategies are warranted to manage this concerning and emerging clinical challenge.

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References

1. Labetoulle M, Auquier P, Conrad H, et al. Incidence of herpes simplex virus keratitis in France. Ophthalmology 2005; 112 (5):888-95.

2. Liesegang TJ. Epidemiology of ocular herpes simplex. Natural history in Rochester, Minn, 1950 through 1982. Arch Ophthalmol 1989; 107 (8):1160-5.
3. Wishart MS, Darougar S, Viswalingam ND. Recurrent herpes simplex virus ocular infection: epidemiological and clinical features. Br J Ophthalmol 1987; 71 (9):669-72.
4. Kaswin G, Rousseau A, M'Garrech M, et al. Optical aberrations in patients with recurrent herpes simplex keratitis and apparently normal vision. Br J Ophthalmol 2013; 97 (9):1113-7.
5. Young RC, Hodge DO, Liesegang TJ, Baratz KH. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976-2007: the effect of oral antiviral prophylaxis. Arch Ophthalmol 2010; 128 (9):1178-83.
6. M'Garrech M, Rousseau A, Kaswin G, et al. Impairment of lacrimal secretion in the unaffected fellow eye of patients with recurrent unilateral herpetic keratitis. Ophthalmology 2013; 120 (10):1959-67.
7. Rousseau A, Nasser G, Chiquet C, et al. Diffusion tensor magnetic resonance imaging of trigeminal nerves in relapsing herpetic keratouveitis. PloS one 2015; 10 (4):e0122186.
8. Saad S, Abdelmassih Y, Saad R, et al. Neurotrophic keratitis: Frequency, etiologies, clinical management and outcomes. Ocul Surf 2020; 18 (2):231-236.
9. Farooq AV, Shukla D. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol 2012; 57 (5):448-62.
10. Reynaud C, Rousseau A, Kaswin G, M'Garrech M, Barreau E, Labetoulle M. Persistent Impairment of Quality of Life in Patients with Herpes Simplex Keratitis. Ophthalmology 2017; 124 (2):160-169.
11. Acyclovir for the prevention of recurrent herpes simplex virus eye disease. Herpetic Eye Disease Study Group. N Engl J Med 1998; 339 (5):300-6.
12. Miserocchi E, Modorati G, Galli L, Rama P. Efficacy of valacyclovir vs acyclovir for the prevention of recurrent herpes simplex virus eye disease: a pilot study. American J Ophthalmol 2007; 144 (4):547-51.
13. Kumar M, Hill JM, Clement C, Varnell ED, Thompson HW, Kaufman HE. A double-blind placebo-controlled study to evaluate valacyclovir alone and with aspirin for asymptomatic HSV-1 DNA shedding in human tears and saliva. Invest Ophthalmol Vis Sci 2009; 50 (12):5601-8.
14. Wyles DL. Antiviral resistance and the future landscape of hepatitis C virus infection therapy. J Infect Dis 2013; 207 Suppl 1:S33-9.
15. van Velzen M, van de Vijver DA, van Loenen FB, Osterhaus AD, Remeijer L, Verjans GM. Acyclovir prophylaxis predisposes to antiviral-resistant recurrent herpetic keratitis. J Infect Dis 2013; 208 (9):1359-65.
16. Piret J, Boivin G. Antiviral resistance in herpes simplex virus and varicella-zoster virus infections: diagnosis and management. Current Opin Infect Dis 2016; 29 (6):654-662.
17. Duan R, de Vries RD, Osterhaus AD, Remeijer L, Verjans GM. Acyclovir-resistant corneal HSV-1 isolates from patients with herpetic keratitis. J Infect Dis 2008; 198 (5):659-63.
18. Duan R, de Vries RD, van Dun JM, et al. Acyclovir susceptibility and genetic characteristics of sequential herpes simplex virus type 1 corneal isolates from patients with recurrent herpetic keratitis. J Infect Dis 2009; 200 (9):1402-14.
19. Burrel S, Boutolleau D, Azar G, et al. Phenotypic and genotypic characterization of acyclovir-resistant corneal HSV-1 isolates from immunocompetent patients with recurrent herpetic keratitis. J Clin Virol 2013; 58 (1):321-4.
20. Rousseau A, Boutolleau D, Titier K, et al. Recurrent herpetic keratitis despite antiviral prophylaxis: A virological and pharmacological study. Antiviral Res 2017; 146:205-212.

21. Burrel S, Deback C, Agut H, Boutolleau D. Genotypic characterization of UL23 thymidine kinase and UL30 DNA polymerase of clinical isolates of herpes simplex virus: natural polymorphism and mutations associated with resistance to antivirals. Antimicrob Agents Chemother 2010; 54 (11):4833-42.
22. Mercier-Darty M, Boutolleau D, Rodriguez C, Burrel S. Added value of ultra-deep sequencing (UDS) approach for detection of genotypic antiviral resistance of herpes simplex virus (HSV). Antiviral Res 2019; 168:128-133.
23. Team RDC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2011.
24. Coen DM, Kosz-Vnenchak M, Jacobson JG, et al. Thymidine kinase-negative herpes simplex virus mutants establish latency in mouse trigeminal ganglia but do not reactivate. Proc Natl Acad Sci USA 1989; 86 (12):4736-40.
25. Huang CY, Yao HW, Wang LC, Shen FH, Hsu SM, Chen SH. Thymidine Kinase-Negative Herpes Simplex Virus 1 Can Efficiently Establish Persistent Infection in Neural Tissues of Nude Mice. J Virol 2017; 91 (4).
26. Chen SH, Lin YW, Griffiths A, Huang WY, Chen SH. Competition and complementation between thymidine kinase-negative and wild-type herpes simplex virus during co-infection of mouse trigeminal ganglia. J G Virol 2006; 87 (Pt 12):3495-502.
27. Hussin A, Md Nor NS, Ibrahim N. Phenotypic and genotypic characterization of induced acyclovir-resistant clinical isolates of herpes simplex virus type 1. Antiviral Res 2013; 100 (2):306-13.
28. Piret J, Boivin G. Antiviral drug resistance in herpesviruses other than cytomegalovirus. Rev Med Virol 2014; 24 (3):186-218.
29. Sauerbrei A, Bohn-Wippert K, Kaspar M, Krumbholz A, Karrasch M, Zell R. Database on natural polymorphisms and resistance-related non-synonymous mutations in thymidine kinase and DNA polymerase genes of herpes simplex virus types 1 and 2. J Antimicrob Chemother 2016; 71 (1):6-16.
30. Chibo D, Druce J, Sasadeusz J, Birch C. Molecular analysis of clinical isolates of acyclovir resistant herpes simplex virus. Antiviral Res 2004; 61 (2):83-91.
31. Andrei G, Georgala A, Topalis D, et al. Heterogeneity and evolution of thymidine kinase and DNA polymerase mutants of herpes simplex virus type 1: implications for antiviral therapy. J Infect Dis 2013; 207 (8):1295-305.
32. Schubert A, Gentner E, Bohn K, Schwarz M, Mertens T, Sauerbrei A. Single nucleotide polymorphisms of thymidine kinase and DNA polymerase genes in clinical herpes simplex virus type 1 isolates associated with different resistance phenotypes. Antiviral Res 2014; 107:16-22.
33. Sauerbrei A, Vödisch S, Bohn K, Schacke M, Gronowitz S. Screening of herpes simplex virus type 1 isolates for acyclovir resistance using DiviTum® assay. J Virol Methods 2013; 188 (1-2):70-2.
34. Malartre N, Boulieu R, Falah N, et al. Effects of mutations on herpes simplex virus 1 thymidine kinase functionality: an in vitro assay based on detection of monophosphate forms of acyclovir and thymidine using HPLC/DAD. Antiviral Res 2012; 95 (3):224-8.
35. Saijo M, Suzutani T, De Clercq E, et al. Genotypic and phenotypic characterization of the thymidine kinase of ACV-resistant HSV-1 derived from an acyclovir-sensitive herpes simplex virus type 1 strain. Antiviral Res 2002; 56 (3):253-62.
36. Burrel S, Bonnafous P, Hubacek P, Agut H, Boutolleau D. Impact of novel mutations of herpes simplex virus 1 and 2 thymidine kinases on acyclovir phosphorylation activity. Antiviral Res 2012; 96 (3):386-90.
37. Frobert E, Ooka T, Cortay JC, Lina B, Thouvenot D, Morfin F. Herpes simplex virus thymidine kinase mutations associated with resistance to acyclovir: a site-directed mutagenesis study. Antimicrob Agents Chemother 2005; 49 (3):1055-9.

38. Shin YK, Cai GY, Weinberg A, Leary JJ, Levin MJ. Frequency of acyclovir-resistant herpes simplex virus in clinical specimens and laboratory isolates. J Clin Microbiol 2001; 39 (3):913-7.
39. Carter SB, Cohen EJ. Development of Herpes Simplex Virus Infectious Epithelial Keratitis During Oral Acyclovir Therapy and Response to Topical Antivirals. Cornea 2016; 35 (5):692-5.
40. Choong K, Walker NJ, Apel AJ, Whitby M. Aciclovir-resistant herpes keratitis. Clin Exp Ophthalmol 2010; 38 (3):309-13.
41. Zhang W, Suzuki T, Shiraishi A, Shimamura I, Inoue Y, Ohashi Y. Dendritic keratitis caused by an acyclovir-resistant herpes simplex virus with frameshift mutation. Cornea 2007; 26 (1):105-6.
42. Kakimaru-Hasegawa A, Kuo CH, Komatsu N, Komatsu K, Miyazaki D, Inoue Y. Clinical application of real-time polymerase chain reaction for diagnosis of herpetic diseases of the anterior segment of the eye. Jpn J Ophthalmol 2008; 52 (1):24-31.
43. Rowe AM, St Leger AJ, Jeon S, Dhaliwal DK, Knickelbein JE, Hendricks RL. Herpes keratitis. Prog Retin Eye Res 2013; 32:88-101.
44. Fukuda M, Deai T, Hibino T, Higaki S, Hayashi K, Shimomura Y. Quantitative analysis of herpes simplex virus genome in tears from patients with herpetic keratitis. Cornea 2003; 22 (7 Suppl):S55-60.
45. Tenser RB, Nath A. Re: Atypical manifestations and poor outcome of herpes simplex encephalitis in the immunocompromised. Neurology 2013; 81 (5):514.
46. Bacon TH, Levin MJ, Leary JJ, Sarisky RT, Sutton D. Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. Clin Microbiol Rev 2003; 16 (1):114-28.
47. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. Antimicrob Agents Chemother 2011; 55 (2):459-72.
48. van Velzen M, Missotten T, van Loenen FB, et al. Acyclovir-resistant herpes simplex virus type 1 in intra-ocular fluid samples of herpetic uveitis patients. J Clin Viroly 2013; 57 (3):215-21.
49. Toriyama K, Inoue T, Suzuki T, Kobayashi T, Ohashi Y. Necrotizing keratitis caused by acyclovir-resistant herpes simplex virus. Case Rep Ophthalmol 2014; 5 (3):325-8.
50. Colin J, Hoh HB, Easty DL, et al. Ganciclovir ophthalmic gel (Virgan; 0.15%) in the treatment of herpes simplex keratitis. Cornea 1997; 16 (4):393-9.
51. Koseoglu ND, Strauss BR, Hamrah P. Successful Management of Herpes Simplex Keratitis With Oral Valganciclovir in Patients Unresponsive or Allergic to Conventional Antiviral Therapy. Cornea 2019; 38 (6):663-667.
52. Wang LX, Takayama-Ito M, Kinoshita-Yamaguchi H, et al. Characterization of DNA polymerase-associated acyclovir-resistant herpes simplex virus type 1: mutations, sensitivity to antiviral compounds, neurovirulence, and in-vivo sensitivity to treatment. Jpn J Infect Dis 2013; 66 (5):404-10.
53. Vere Hodge RA, Field HJ. Antiviral agents for herpes simplex virus. Adv Pharmacol 2013; 67:1-38.
54. Wilhelmus KR. Antiviral treatment and other therapeutic interventions for herpes simplex virus epithelial keratitis. Cochrane Database Syst Rev 2015; 1:Cd002898.
55. Koizumi N, Miyazaki D, Inoue T, et al. The effect of topical application of 0.15% ganciclovir gel on cytomegalovirus corneal endotheliitis. Br J Ophthalmol 2017; 101 (2):114-119.
56. Shiraki K. Antiviral Drugs Against Alphaherpesvirus. Adv Exp Med Biol 2018; 1045:103-122.

57. Greeley ZW, Giannasca NJ, Porter MJ, Margulies BJ. Acyclovir, cidofovir, and amenamevir have additive antiviral effects on herpes simplex virus TYPE 1. Antiviral Res 2020; 176:104754.
58. Deback C, Rousseau A, Breckler M, et al. Antiviral effects of Cacicol((R)), a heparan sulfate biomimetic for corneal regeneration therapy, for herpes simplex virus type-1 and varicella zoster virus infection. Antiviral Ther 2018; 23 (8):665-675.

Table 1: Main clinical findings at inclusion and during follow-up.

	All patients	**Immunocompetent patients**	**Immunocompromised patients**	***P*** **(Immunocompetent vs immunocompromised patients using the Kruskall-Wallis test)**
N	18	12	6	-
At inclusion				
Age (years) mean±SE	66.8±4.7	68.0±6.2	64.7±5.8	0.45
Number of previous recurrences mean±SE	14.7±2.3	16.4±1.9	11.5±4.9	**0.05**
Disease duration (years) mean±SE	29.8±9.4	35.8±11.2	19.5±15.3	0.11
BCVA (LogMAR) mean±SE	0.95±0.42	1.26±0.59	0.41±0.24	**0.05**
Type of keratitis: Epithelial Stromal with ulceration Neurotrophic	 11 6 1	 6 5 1	 5 1 0	
Follow-up				
Follow-up (months) mean±SE	31.5±14.5	32.3±18.4	29.7±28.9	1.00
Number of recurrences mean±SE	2.8±1.3	2.5±1.4	2.4±1.6	0.80
Final BCVA (LogMAR) mean±SE	1.00±0.50	1.34±0.63	0.26±0.23	**0.04**

N = number of cases, SE = standard error, BCVA = best-corrected visual acuity, LogMAR = logarithm of the minimum angle of resolution, $P \leqslant 0.05$ is statistically significant, indicated in bold numbers.

Table 2: detailed clinical data:

Case #	Gender / Age	Immune status	Cause of Immuno-deficiency	Duration of disease before the diagnosis of resistance (year)	Number of recurrences before the diagnosis of resistance	Antiviral treatment at inclusion (mg/day)	Reason eliciting genotypic resistance testing (1= frequent recurrences despite appropriate AVP, 2= poor efficacy of suppressive antiviral treatment)	Type of HSK	Initial BCVA (LogMAR)	Follow-up after the diagnosis of resistance (months)	Number of recurrences after the diagnosis of resistance	Final BCVA (LogMAR)
1	F/53	IC	N/Ap	44.1	>20	VACV 1000	1	stromal with ulceration	0.4	91	8	0.7
2	M/67	IC	N/Ap	25.3	11-20	VACV 1000	1	neurotrophic	2.8	89	1	2.8
3	M/61	ID	CID	20.8	11-20	VACV 500	1	epithelial	0	86	9	0.3
4	F/61	IC	N/Ap	6.8	11-20	VACV 1500	1	epithelial	2.8	49	1	2.8
5	M/88	IC	N/Ap	68.4	>20	VACV 1500	1	stromal with ulceration	N/A	N/A*	N/A*	N/A*
6	F/74	IC	N/Ap	18.5	11-20	VACV 1500	1+2	stromal with ulceration	0.7	10	3	0.8
7	M/65	IC	N/Ap	31.6	11-20	VACV 1500	1+2	epithelial	0	16	3	0
8	F/53	IC	N/Ap	45.6	>20	VACV 1500	1	epithelial	2.3	23	1	2.3
9	M/77	ID	diffuse B cell lymphoma	0.1	2-5	VACV 1000	1	epithelial	0.8	N/A*	N/A*	N/A*
10	M/63	IC	N/Ap	6.1	>20	VACV 1500	1	epithelial	0.8	18	1	1.7
11	M/59	ID	allogenic BMT	50.3	11-20	VACV 3000	2	epithelial	0.4	6	0	0
12	M/65	ID	oral prednisone + rituximab	15.5	11-20	VACV 1000	1	epithelial	0.7	7	1	0.7
13	M/76	IC	N/Ap	41.5	6-10	VACV 1000	1	epithelial	0.4	34	6	0.1
14	M/57	ID	high dose oral prednisone	0.3	2-5	VACV 1500	1	stromal with ulceration	0.2	24	4	0.1
15	M/67	ID	high dose oral prednisone	30.0	11-20	VACV 1000	1	epithelial	0.3	26	3	0.1
16	M/64	IC	N/Ap	50.4	11-20	VACV 500	1	stromal with ulceration	1	11	0	0.8
17	M/84	IC	N/Ap	50.8	>20	VACV 3000	1+2	stromal with ulceration	0.8	11	2	0.3
18	F/68	IC	N/Ap	29.7	11-20	VACV 3000	2	neurotrophic	1.8	4	2	2.3

F = female, M = male, IC = immunocompetent, ID = immunodeficient, VACV = valacyclovir, CID = combined immune deficiency, BMT = bone marrow transplantation, N/Ap = not applicable, N/A = not available. N/A* = not available, patient lost to follow-up.

Table 3: HSV-1 genotypic resistance profiles

Case #	Type of sample	Mutations associated with ACV resistance in TK (UL23)	Mutations associated with ACV resistance in DNA pol (UL30)	Genbank accession number UL23	Genbank Accession number UL30	Reference
1	SS	**ACVR = R222H**; NP = C6G N23S K36E A192V G251C A265T V267L P268T D286E N376H	NP = S33G A330R V905M P1124H T1208A	OL514323	OL542697	Hussin *et al*., 2013[27]
2	CS	**ACVR = R222H** ; NP = C6G N23S K36E A192V G251C A265T V267L P268T D286E N376H	NP = S33G A330R V905M A1203T T1208A	OL514324	OL542698	Hussin *et al*., 2013[27]
3	CS	**ACVR = E225Stop;** NP= C6G N23S K36E R41H	N/A	OL514325	N/Ap	Piret and Boivin, 2014;[28] Sauerbrei *et al*., 2016[29]
4	CS	**ACVR = DelC553 Frameshift**; NP = N23S K36E R89Q	NP = S33G A330R V905M P1124H T1208A	OL514326	OL542699	Piret and Boivin, 2014;[28] Sauerbrei *et al*., 2016[29]
5	SS	NP = C6G N23S K36E A192V A265T	**ACVR = S775N** NP = S33G A330R V905M A1203T T1208A	OL514327	OL542700	Chibo *et al*., 2004[30]
6	SS	**ACVR = D228Stop;** NP = N23S K36E R89Q	N/A	OL514328	N/Ap	Piret and Boivin, 2014;[28] Sauerbrei *et al*., 2016[29]
7	SS	**ACVR = M128L**; NP = N23S K36E R89Q G240E A265T N376T	NP = S33G A330R V905M A1203T T1208A	OL514329	OL542701	Duan *et al*., 2009[18]
8	CS	NP = C6G N23S K36E A192V G251C A265T V267L P268T D286E	**ACVR = E798K ;** NP = S33G A330R V905M S1123L P1124H T1208A	OL514330	OL542702	Andrei *et al*., 2013[31]
9	CS	**ACVR = H58Y ;** NP = N23S K36E R89Q G240E A265T	NP = S33G L267M A330R V905M S1123L P1124H T1208A	OL514331	OL542703	Schubert *et al*. 2014[32]
10	CS	**ACVR = M121R + A156V;** NP = N23S K36E R89Q	NP = A25V S33G A330R A562T D672N V905M P920S	OL514332	OL542704	Sauerbrei *et al*., 2013[33]
11	SS	**ACVR = T287M** ; NP = N23S K36E R89Q A265T	NP = S33G A330R V905M A1203T T1208A	OL514333	OL542705	Sauerbrei *et al*., 2013[33]
12	SS	**ACVR = L291P**; NP = C6G N23S K36E L42P A265T I326V; MUS = E43A I235N	NP = S33G A330R A1099T S1113C P1124H T1208A	OL514334	OL542706	Malartre *et al*., 2012[34]
13	CS	**ACVR = S123G**; NP = C6G N23S K36E R41H R89Q A192V G251C A265T V267L P268T D286E N376H	NP = S33G A330R V905M T1208A	OL514335	OL542707	*
14	CS	**ACVR = T65P ;** NP = N23S K36E R89Q G240E A265T	N/A	OL514336	N/Ap	Saijo *et al*.,2002[35]
15	CS	**ACVR = F308L ;** NP = N23S K36E R89Q G240E A265T	NP = S33G A330R V905M T1208A	OL514337	OL542708	*
16	CS	NP = G240E A265T R281Q	**ACVR = E798E/K ;** NP = SA330R T530P	OL514338	OL542709	Andrei *et al*. 2013[31]
17	CS	**ACVR = Y53D ;** NP = K36E R89Q A265T	NP = S33G A330R D672N A905M E1005K	OL514339	OL542710	Burrel *et al*., 2012[36]
18	CS	**ACVR = R51W;** NP = N23S K36E R89Q A265T	N/A	OL514340	N/Ap	Frobert et al., 2005[37]

SS= Schirmer strip, CS = corneal swab, ACV = acyclovir, N/A = not available (DNA pol gene could not be sequenced because of a too low HSV-1 load), NP = natural polymorphism, N/Ap = not applicable.

* The role of those unpreviously described mutations in resistance to ACV was assessed by phenotypic testing in the laboratory, as previously described[21]. HSV isolates were considered to be resistant to ACV if the effective concentration 50% (EC_{50}) was ≥7 µM. EC_{50} obtained for ACV were as follows: case#13: 31.8 µM; case#15: 18.0 µM; ACV-sensitive HSV-1 isolate (negative control): 1.6 µM; ACV-resistant HSV-1 isolate (positive control): 19 µM.