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HSV-1 acyclovir drug resistance

The most common treatment for Herpes Simplex virus (HSV) infections is acyclovir. Acyclovir is an antiviral drug that is a structural analogue of deoxyguanosine. The drug is activated when phosphorylated by both viral and host cell kinases. It is effective as it only targets cells that have been infected with the virus; this is because the first phosphorylation step requires viral thymidine kinase, to convert acyclovir into acyclovir monophosphate. The next phosphorylation steps, by enzymes found in the host cells, convert the acyclovir monophosphate into acyclovir triphosphate, which competes with naturally occurring deoxyguanosine triphosphate. This results in the selective inhibition of viral DNA polymerase, preventing the continued elongation of the DNA during viral replication (Bacon *et al.*, 2003).

Mutations in the thymidine kinase gene (UL23) and the DNA polymerase gene (UL30) of HSV can lead to acyclovir resistance. In 95 % of acyclovir resistant isolates, the mutation responsible is found within the thymidine kinase gene (Bacon *et al.*, 2003). There are three types of phenotypic mutations in thymidine kinase (TK) that confer resistance to acyclovir. The most commonly occurring mutations are TK-null mutants, which have no thymidine kinase activity at all, and TK-low-producer mutants, which have lower levels of enzyme activity. Less common mutations, which confer acyclovir resistance, are TK-altered isolates. TK-altered isolates affect the specificity of the thymidine kinase active site, and the enzyme is able to phosphorylate thymidine but it is unable to phosphorylate acyclovir, preventing activation of the antiviral (Piret and Boivin, 2011).

Mutations in DNA polymerase are less common; this is because of its role in elongating the viral DNA, making it essential for virus replication. Mutations in DNA polymerase usually occur in the most conserved sites of the enzyme (Morfin and Thouvenot, 2003).

Resistant strains of HSV are more commonly found in immunocompromised patients. The prevalence of resistant HSV isolates in immunocompetent patients has been reported to be less than 1% whereas in immunocompromised individuals it ranges from between 2.5% to 10.9% (Schmidt *et al.*, 2015). A higher prevalence of around 30% has been reported in patients who have received hematopoietic stem cells and bone marrow transplants (Piret and Biovin, 2011). Resistance may also be a problem in patients treated with acyclovir as a prophylaxis for recurrent herpetic keratitis. Long-term acyclovir prophylaxis has been found to result in the emergence of acyclovir resistant HSV-1. Susceptibility testing may thus be warranted in patients with recurrent HSV keratitis (van Velzen *et al.*, 2013).

At Micropathology Ltd., we have designed a molecular test for the identification of resistance mutations in the thymidine kinase (UL23) and DNA polymerase (UL30) genes of HSV-1. The UL23 and UL30 amplicons are sequenced and analysed using next generation sequencing (NGS) methods (awaiting UKAS-accreditation). This allows us to report low-level resistant mutations down to a nucleotide frequency of 5%. Resistance mutations are confirmed using a curated mutation database. Resistance to other antiviral drugs is reported where the information is available.

References

Bacon TH., Levin MJ., Leary JJ., Sarisky RT., and Sutton D. (2003). Herpes simplex virus resistance to acyclovir and penciclovir after two decades of antiviral therapy. *Clin Microbiol rev* **16** 114-128.

Burrel S., Deback C., Agut H., and Boutolleau D. (2010). Genotypic characterisation 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* **54** 4833-4842.

Morfin F., Thouvenot D (2003). Herpes simplex virus resistance to antiviral drugs. *J Clin Virol* **26** 29-37.

Piret J., and Boivin G. (2011). Resistance of herpes simplex viruses to nucleoside analogues: Mechanisms, prevalence, and management. *Antimicrob agents and chemotherapy* **55** 459-472.

Schmidt S., Bohn-Wippert K., Schlattmann P., Zell R., and Sauerbrei A. (2015). Sequence Analysis of Herpes Simplex Virus 1 Thymidine Kinase and DNA Polymerase Genes from over 300 Clinical Isolates from 1973 to 2014 Finds Novel Mutations That May Be Relevant for Development of Antiviral Resistance. *Antimicrob agents and chemotherapy* **59** 4938-4945.

van Velzen M., van der Vijver DA., van Loenen FB., Osterhaus AD, Remeijer L., and Verjans GM (2013). Acyclovir prophylaxis predisposes to antiviral-resistant recurrent herpetic keratitis. *J Infect Dis.* **208** (1359-1365).