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RAPPORT

2018

Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2017

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Innhold

Introduction

It is a pleasure to present the fifth report from the surveillance system Resistance against Antivirals in Norway (RAVN).

This report presents new data for 2017 on resistance against agents for treatment of influenza, HIV-1 infection, hepatitis B virus infection and human herpes virus infections. The surveys have been conducted by the Norwegian Institute of Public Health and at the Oslo University Hospital.

In addition to surveillance data, we have focused on two relevant topics: Cytomegalovirus (CMV) and hepatitis C virus (HCV). In 2018, the Norwegian Pediatric Association published a preliminary protocol recommending follow-up of congenital CMV infection in newborns failing to pass the newborn hearing screening. This may have consequences for future use of prophylactic and pre-emptive treatment of CMV infections, and Grete Birkeland Kro discusses the topic in this report.

Furthermore, we have chosen to focus on hepatitis C. New effective drugs have improved the treatment outcome dramatically over the last few years, but the implementation is complicated by high costs of the drugs, debated reimbursement rules, and limited access to resistance testing. The impact of resistance testing on the outcome of hepatitis C treatment is under discussion, and we have invited two clinicians presenting slightly different perspectives: Olav Dalgard, Department of Infectious diseases at Akershus University Hospital, is an invited author on hepatitis C, and gives a review on available therapy and recommendations of resistance testing. Bente Magny Bergersen describes a practical approach to treatment without baseline drug resistance testing, presenting data from the local quality registry at the Department of Infectious diseases, Oslo University Hospital Ullevål.

It is our hope that the report contains valuable data for clinicians, microbiologists and those developing treatment guidelines and strategies to prevent transmission of viral infection.

RAVN would like to thank those who contributed with data and writing this report, for excellent work.

Oslo, 2018

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Sammendrag

Bruk av antivirale midler

Salget av antivirale medikamenter fortsetter å øke målt i definerte døgndoser (DDD). Kostnadene for antiinfeksiøse agens for systemisk bruk (ATC gruppe J) har også økt de seneste år, men i mindre grad etter 2016. Introduksjon av nye antiviralia for behandling av HCV infeksjon har influert sterkt på de økte kostnadene, men prisreduksjon på noen av disse medikamentene har gitt mindre økning i kostnader sammenlignet med økning i salg. For hiv-medikamenter har salget målt i antall DDD vært relativt stabilt de siste årene, men det har vært en dreining fra medikamenter med ett virkestoff til kombinasjonspreparater.

Influensavirus

Som i forrige sesong, ble det i 2017/18-sesongen ikke påvist influensavirus med resistens mot oseltamivir eller zanamivir. De få H1N1-virus som sirkulerte denne sesongen, videreførte dermed ikke den resistensen som ble påvist på slutten av 2015/16-sesongen. Alle sirkulerende influensavirusgrupper er for tiden resistente overfor adamantaner, og FHI har derfor sluttet å teste rutinemessig for adamantanresistens. Kun oseltamivir er nå tilgjengelig på det norske markedet.

Hiv-1

Surveillance drug-resistance mutations (SDRMs) ble påvist i 5.6% av prøvene fra pasienter med ny diagnostisert hiv-1 infeksjon i Norge i 2017. Prevalensen av overført resistens har vært stabil de siste årene med bare små variasjoner. Innføring av profylaktisk behandling (PrEP og PEP) kan endre denne situasjonen.

En studie pågår i regi av RAVN der resistensdata kobles mot ulike epidemiologiske variabler fra MSIS. Foreløpige resultater fra denne studien viser at bare 30% av de tilfellene der primær resistensundersøkelse ble utført i 2016, var smittet i Norge. I denne gruppen ble det funnet SDRM i 7.5% av prøvene.

Hepatitt B virus

I 2017 ble 23 pasientprøver sendt til referanselaboratoriet for hepatitt B virus (HBV) resistenstesting. Det ble påvist antiviral resistens i 2 av disse prøvene. Blant pasienter der genotyping primært var rekvirert (N=223), ble ingen resistensmutasjoner påvist.

Resistens mot antivirale midler brukt i behandling av HBV ser ut til å være et lite problem i Norge. Antall prøver som undersøkes er imidlertid lite, og resultatene må derfor tolkes med forsiktighet.

Humane herpesvirus

I 2017 mottok referanselaboratoriet prøver fra 27 pasienter for cytomegalovirus (CMV) resistenstesting, og hos 7 (26%) kunne det påvises mutasjoner i CMV-UL97 genet som gir resistens mot ganciclovir. Hos en pasient ble det funnet ytterlige mutasjoner i CMV-DNA-polymerasegenet (UL54) som medfører resistens mot cidofovir og foscarnet. De fleste tilfeller av terapisvikt skyldes ikke utvikling av resistens mot CMV-midler.

Aciclovir og dets prodrug valaciclovir er de mest benyttede medikamenter for behandling og profylakse mot herpes simplex virus (HSV) -1 og -2 infeksjoner. Til tross for et stort forbruk er forekomsten av behandlingssvikt, som skyldes resistens svært lav. I 2017 ble det sendt ti prøver fra pasienter med slik behandlingssvikt til Folkhälsomyndigheten i Sverige. I to av prøvene ble det funnet mutasjoner som medfører resistens mot aciclovir og valaciclovir.

Hepatitt C virus

Med enkelte unntak vil hepatitt C infeksjon kureres med en 12-ukers behandling med kombinasjon av 2 direktevirkende antivirale medikamenter. Behandlingen har få bivirkninger, og alle infiserte bør tilbys behandling. Behandlingssvikt hos behandlingsnaive vil kun skje hos ca. 3% av alle som behandles. Prediktorer for behandlingssvikt er bl.a. genotype 3 infeksjon, tilstedeværelse av resistensassosierte varianter (RAV) og avansert leverfibrose. Resistenstesting før behandling med enkelte unntak er ikke nødvendig.

Summary

The usage of antivirals

The sales of antiviral drugs continued to increase measured in defined daily doses (DDDs), according to The Norwegian Drug Wholesales statistics database. The cost of antiinfectives for systemic use (ATC group J) has also increased during recent years although the yearly increase has been smaller since 2016. Introduction of new antivirals for treatment of HCV infections has highly influenced the increased costs in this group, but price reduction for some of these drugs has resulted in a relatively lower increase in cost compared to the increase in sales. For HIV drugs the sales measured in number of DDDs have been relatively stable in recent years, but there has been a significant change in the pattern of use with a transition from single ingredient products to fixed combinations.

Influenza virus

No mutations conferring resistance against oseltamivir and zanamivir were detected in the 2017/18 season. Resistance mutations found at the end of the 2015/16 season were apparently not transferred to the few H1N1-viruses circulating this season. All circulating influenza strains are currently resistant to adamantanes. The reference laboratory therefore do not routinely analyse for adamantan resistance. In Norway, only oseltamivir is now available for antiviral treatment of influenza.

HIV-1

Surveillance drug-resistance mutations (SDRMs) were detected in 5.6% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2017. The prevalence of transmitted drug resistance has been stable for the past few years with only minor variation. The introduction of prophylactic treatment (PrEP and PEP) could challenge this situation.

RAVN is currently conducting a study which aims to connect epidemiological data from MSIS with resistance data. Preliminary results from this study show that only 30% of the reported cases in 2016 were infected in Norway. In this group, 7.5% of the samples had SDRMs.

Hepatitis B virus

In 2017, 23 patient samples were submitted for HBV antiviral resistance testing. Among these, only two cases of resistance were detected. Among samples submitted primarily for HBV-genotyping (N=223), no drug resistance mutations were detected.

The burden of resistance against HBV antivirals seems to be low in Norway given the large number of patients on treatment. However, the data is very limited due to the low frequency of testing and should therefore be interpreted carefully.

Human herpes viruses

In 2017 the reference laboratory received specimens from 27 patients for genotypic analysis of CMV resistance and mutations in the CMV-UL97 gene, conferring resistance to ganciclovir, were recorded in 7 cases (26%). In one patient additional mutations in the

CMV-UL54 gene conferring foscarnet- and cidofovir resistance were seen. Most cases of treatment failure are thus not due to the development of resistance to anti-CMV-therapy.

Aciclovir and its prodrug valacicklovir are extensively used for both treatment and prophylaxis of herpes simplex virus-1 and -2 (HSV) infections. Despite the high consumption of these antiviral drugs treatment failure due to drug resistance is quite uncommon. In 2017 ten specimens were sent from Norway to Folkhälsomyndigheten, Sweden for genotypic resistance testing. Mutations conferring resistance to aciclovir/valaciclovir were seen in two of these specimens.

Hepatitis C virus

In most cases, Hepatitis C can be cured by a 12-week oral regimen with a combination of two direct-acting antiviral drugs (DAAs). Hepatitis C treatment has few side effects and everyone who is infected should be identified and offered treatment. Treatment failure after combination therapy with DAAs is rare and will only occur in approximately 3% of those treated. Predictors of treatment failure include genotype 3 infection, the presence of resistance associated variants (RAVs) and advanced liver fibrosis. With such good response rates to the first-line treatments, there is seldom need for resistance testing before hepatitis C treatment is administered.

1 The usage of antivirals in Norway

In the last twenty-five years, the development of new specific antivirals has accelerated, especially due to development of new HIV drugs (1) and hepatitis C virus (HCV) drugs. The sales of antiviral drugs, measured in defined daily doses (DDDs), have been increasing every year. According to The Drug Consumption in Norway 2013-2017, the cost of antiinfectives for systemic use (Anatomical Therapeutic Chemical Classification (ATC) group J) has continued to increase during the past few years, although the yearly increase has been smaller since 2016 (2). Introduction of new antivirals for treatment of HCV infections has highly influenced the increased costs in this group in recent years. However, price reduction for some of these drugs has resulted in a relatively lower increase in cost compared to the increase in sales measured in DDDs and number of users (fig. 1.1). For the HIV drugs, sales measured in number of DDDs have been relatively stable the last years, but there has been a significant change in the pattern of use with a transition from single ingredient products to fixed combinations. The result is use of fewer pharmaceutical products per person per day. Figure 1.1 shows the sales of all direct acting antiviral drugs (DAA) (ATC group JO5A), measured in DDDs during the past five years.

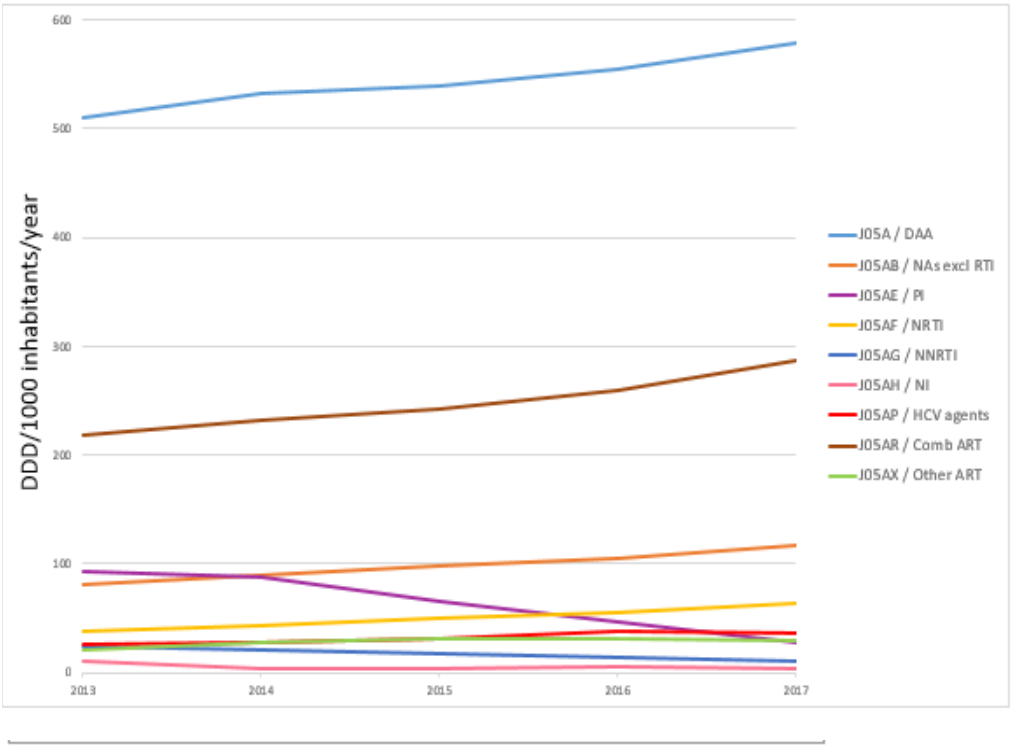

Figure 1.1. Sales of direct acting antiviral drugs (DAA), ATC group J05A for 2013-2017 given in DDD/1000 inhabitants/year (2).

Influenza virus

The usage of antivirals for the treatment of influenza (ATC group J05AH), is shown in Table 1.1. The variation since 2013 is probably linked to the size of the yearly influenza epidemic. The use of zanamivir has declined steadily during this period and in 2017 oseltamivir was the only neuraminidase inhibitor (NI) used in Norway.

Table 1.1. Number of individuals with at least one prescription of a neuraminidase inhibitor (NI) per year, ATC group J05AH, in the period 2013-2017.

NI drug	Number of individuals with one or more prescription per annum				
	2013	2014	2015	2016	2017
Zanamivir	85	18	52	25	0
Oseltamivir	3911	1080	1477	2129	1923

HIV

There are currently 31 antivirals, defined as different ATC codes, used in treatment of HIV in Norway. The number of patients retrieving at least one prescription of these drugs has increased by more than 50 % from 2013 to 2017. Figure 1.2 shows the number of patients retrieving at least one prescription of the 10 most prescribed HIV agents per year. During the whole period, more than 95 % of treated persons received a combination product containing more than one active substance. In 2017, more than 4800 persons retrieved a prescription of one of these fixed combinations in Norway. Single substance products could be given in addition to the fixed combination for some patients.

The single substance products, tenofovir disoproxil, adefovir dipivoxil and emtricitabine are approved for treatment of HIV and HBV infections, but are rarely used for HIV therapy. The users of these products are neither included in the total number of users of HIV treatment nor in the different subgroups in figure 1.2. The sum of the patients using the different products is higher than the total number of patients treated with HIV agents in figure 1.2. This is because some patients receive more than one product during a year.

Several new fixed combination products have been introduced over the last years and it is expected that this trend will continue. Changes in usage may be due to these new combinations.

The fixed combination of emtricitabine and tenofovir disoproxil (TDF) has been the most used combination product the last years. A small decrease was seen in 2015 and 2016 before the use again increased in 2017. In 2016, TDF was approved for PrEP (Pre-Expositional Prophylaxis) to reduce the risk of sexually acquired HIV-1 infection in adults at high risk and given reimbursement for this indication. This may explain the increased number of patients retrieving at least one prescription of this drug in 2017. A new prodrug of tenofovir, tenofovir alafenamide (TAF), was introduced in three different fixed combinations in 2016; one 2-component combination (emtricitabine + TAF), one 3-component combination (emtricitabine + TAF + rilpivirine) and one combination with 4 substances (emtricitabine + TAF + elvitegravir + cobicistat). TAF is given in lower doses and has a greater bioavailability in relevant body tissues than TDF. The use of the new TAF

containing combinations has increased in 2017 while the use of most of the TDF combinations is reduced. The fixed combination of emtricitabine and TDF is the only TDF containing combination that is still increasing in 2017.

Since 2012 three new 3-component combinations and two 4-component combinations (emtricitabine + TDF + elvitegravir + cobicistat) and (emtricitabine + TAF + elvitegravir + cobicistat) have been introduced. Of the combinations introduced after 2013, the most commonly prescribed in 2017 was the combination of lamivudine, abacavir and dolutegravir (integrase inhibitor), introduced in 2014.

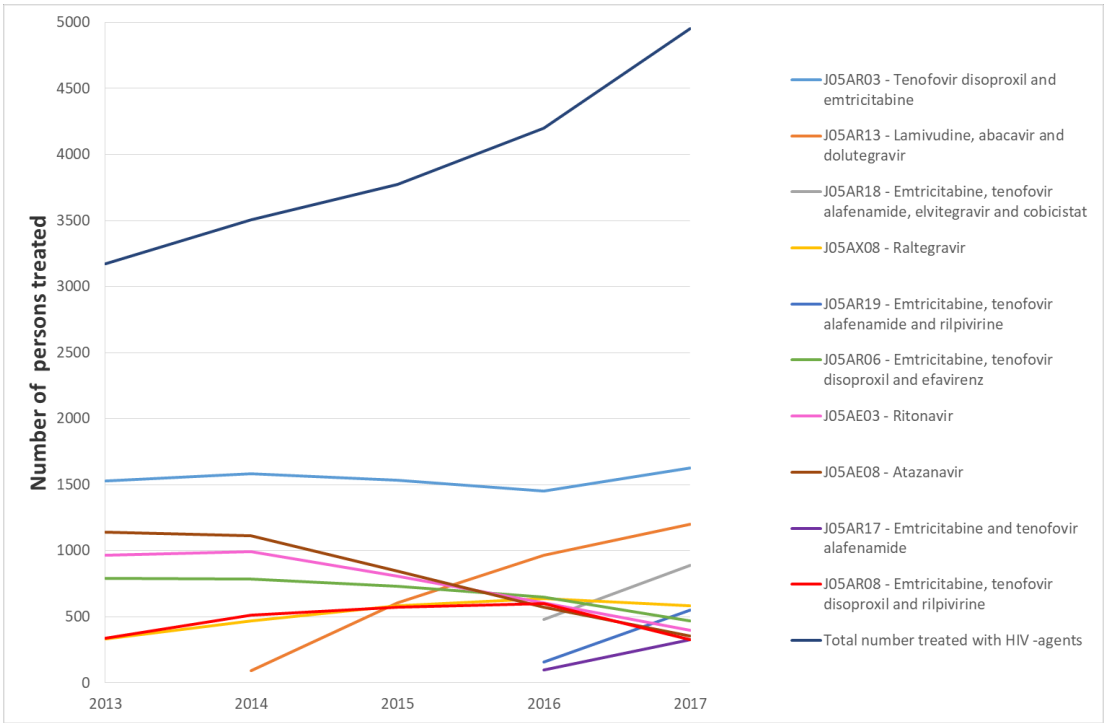

Figure 1.2. Trends in the use of antivirals for treatment of HIV for the period 2013-2017. The 10 most used agents based on the number of patients given at least one prescription per year (2). Six of these agents contain tenofovir (TDF or TAF).

The changes in therapy during the 5 years period is illustrated in figure 1.3. Only 6 of the 10 most used drugs in 2017 have been on the market the whole period. These drugs are indicated in the figure. The group “other HIV drugs” includes drugs introduced during the last 5 years. In this group the fixed combinations of lamivudine, abacavir and dolutegravir (J05AR13), emtricitabine, TAF, elvitegravir and cobicistat (J05AR18), emtricitabine, TAF and rilpivirine (J05AR19) and emtricitabine and TAF (J05AR17) are the products with the highest number of users in 2017. Only the combination of emtricitabine and TDF (J05AR03) has more users than these combinations in 2017.

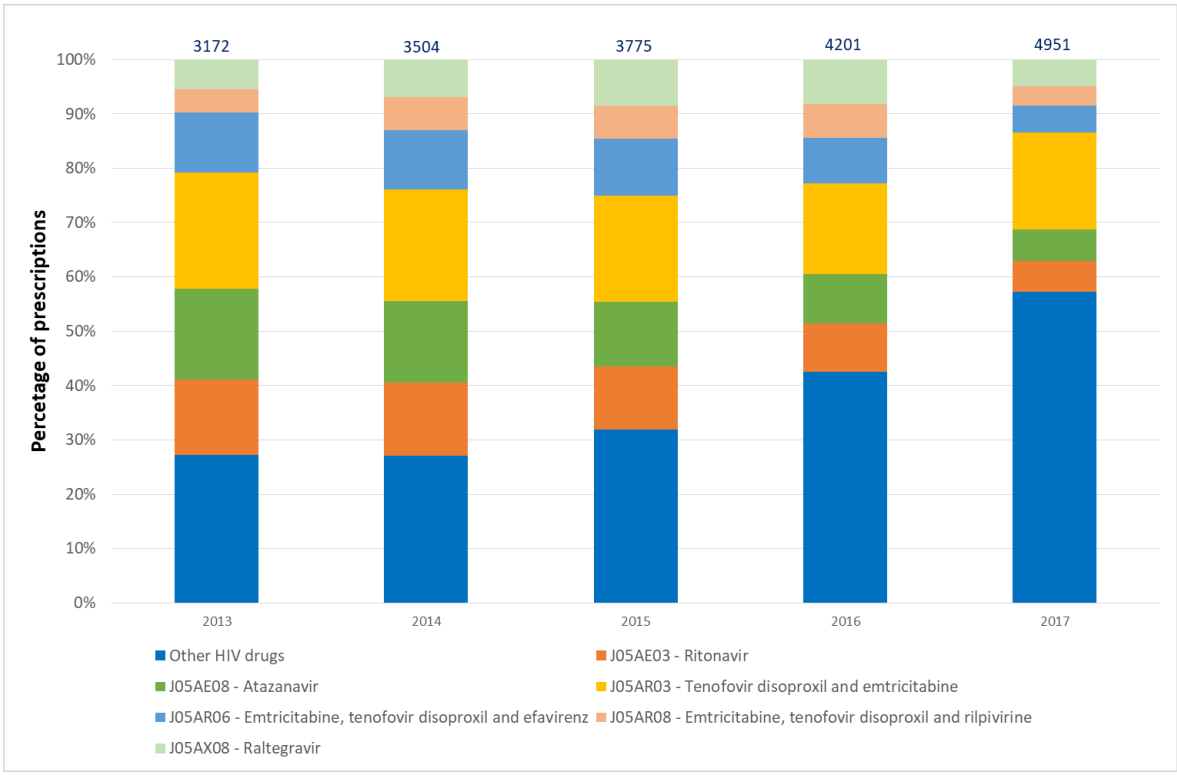

Figure 1.3. Patterns of prescriptions for HIV-treatment from 2013–2017 based on the number of prescriptions for the different products in the period. The 6 single ingredient/fixed combination products that are among the 10 most prescribed in 2017 and for the whole five years period are specified. Total number of patients with HIV prescriptions is given on top of the columns (3).

Hepatitis B virus

There are currently ten approved therapies for HBV infection including three interferon based and seven nucleoside/nucleotide analogues (NA) lamivudine, adefovir dipivoxil, emtricitabine, entecavir, telbivudine, TDF and the new prodrug of tenofovir, TAF. Treatment of HBV with antivirals is generally given as monotherapy. The use of the NA-drugs is shown in figure 1.4.

The data is based on the annual number of patients retrieving at least one prescription per year for the period 2013-2017. Lamivudine, adefovir dipivoxil, TDF and emtricitabine are drugs that are approved for both HBV and HIV, while entecavir, telbuvidine and TAF (as a single ingredient product) are approved for HBV only. An estimate of the number of patients treated for HBV with antivirals in Norway will therefore be in the range of 540-1023 in 2017. The lowest number is based on the number of patients prescribed drugs approved for HBV only. The highest number is the total number of patients prescribed one of the seven NA-drugs (excluding lamivudine containing products approved for HIV only). The number of persons treated has been increasing during the last 5 years. TAF, which was approved for monotherapy of HBV in January 2017, in addition to entecavir and TDF are considered first line therapies for HBV. Of the patients receiving HBV treatments with the seven NA drugs, 98% received one of these three drugs in 2017. The introduction of TAF may further influence the pattern of use of anti-HBV drugs the next years.

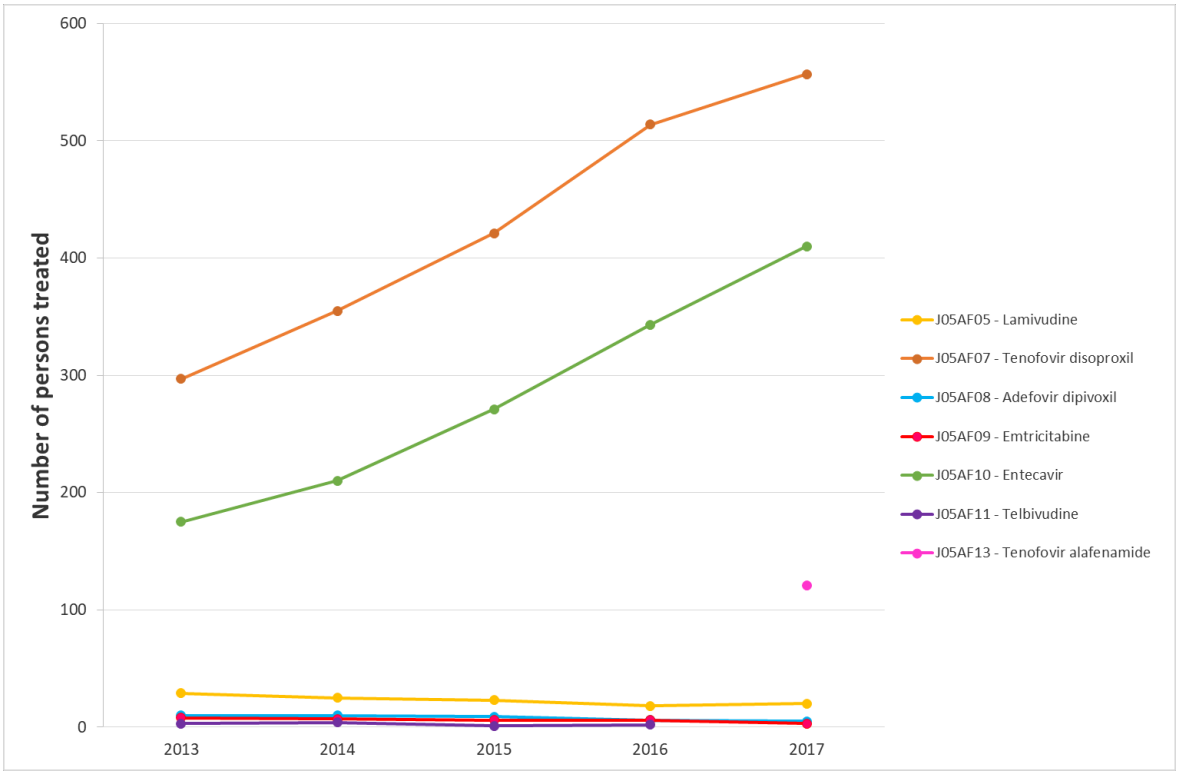

Figure 1.4. Patterns of prescriptions for HBV-treatment from 2013-2017 based on the number of patients given at least one prescription per year (3).

Hepatitis C virus

Until 2011, hepatitis C virus (HCV)-therapy was based on a combination of pegylated interferon and ribavirin for an up to a year, depending on HCV-genotype. In 2011, two new protease inhibitors (PI), telaprevir and boceprevir, were licensed for combination therapy with ribavirin and interferon in HCV genotype 1 infections. In 2014, three new antiviral drugs targeting HCV entered the market: sofosbuvir; a pangenotypic polymerase inhibitor, simeprevir; a second-wave protease inhibitor and daclatasvir; a pangenotypic NS5A (non-structural protein 5A) inhibitor. The NS5B polymerase inhibitor dasabuvir entered the market in 2015. This substance is used in combination with a new fixed combination product including ombitasvir (NS5A inhibitor), paritaprevir (NS3 protease inhibitor) and ritonavir (pharmacokinetic (PK) enhancer). A fixed combination of ledipasvir (NS5A inhibitor) and sofosbuvir was also marketed. In addition, a new fixed combination of sofosbuvir and velpatasvir (NS5A inhibitor) was introduced in 2016. In 2017, the most used fixed combination was the new combination of elbasvir (NS5A inhibitor) and the pangenotypic NS3/4A protease inhibitor grazoprevir. In addition, a combination of sofosbuvir, velpatasvir and voxilaprevir (NS3/4A protease inhibitor) and one with glecaprevir (NS3/4A protease inhibitor) and pibrentasvir (NS5A inhibitor) were introduced but are still in limited use. With the new DAAs the therapy for chronic HCV-infection has totally changed. One of the advantages with many of the new HCV products is the possibility to avoid the use of interferon. The overall number of patients on treatment has increased during the last five years with the new drugs on the market (figure 1.5). Number of persons given at least one prescription for a HCV drug (except interferons) has increased almost 70% from 2016 to 2017. The usage of DAAs is expected to increase further in the coming years in connection with the introduction of these new drugs. The

pattern of use (figure 1.5) is also predicted to change further because of changes in treatment guidelines and reimbursement rules.

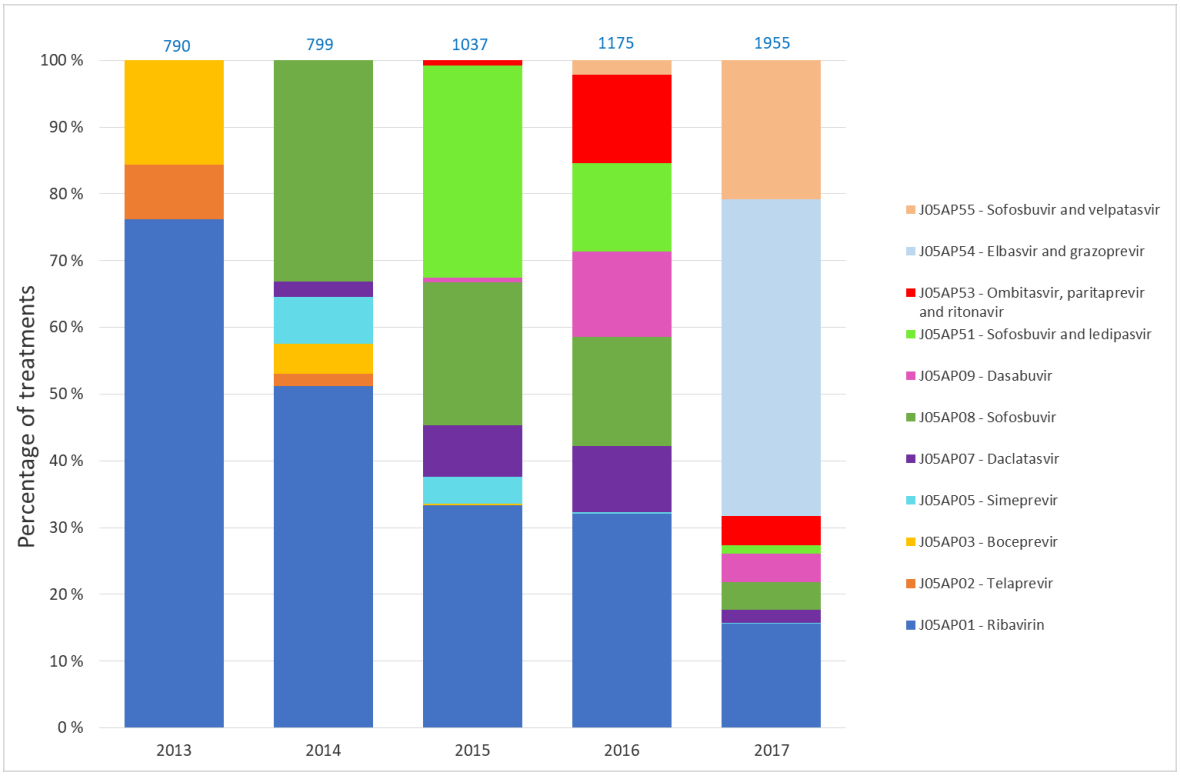

Figure 1.5. Patterns of prescriptions for HCV-treatment from 2013-2017 based on the number of patients given at least one prescription per year. Total number of patients retrieving HCV prescriptions is given on top of the columns (3).

Human herpesviruses

Figure 1.6 shows the two most prescribed drugs for human herpes virus infections over the last five years. The use of the other drugs approved for treatment of human herpes virus is limited (table 1.2). While ganciclovir and famciclovir have been prescribed very rarely in this period, aciclovir is prescribed more often and valaciclovir is the substance most commonly prescribed. The use of this drug is steadily increasing.

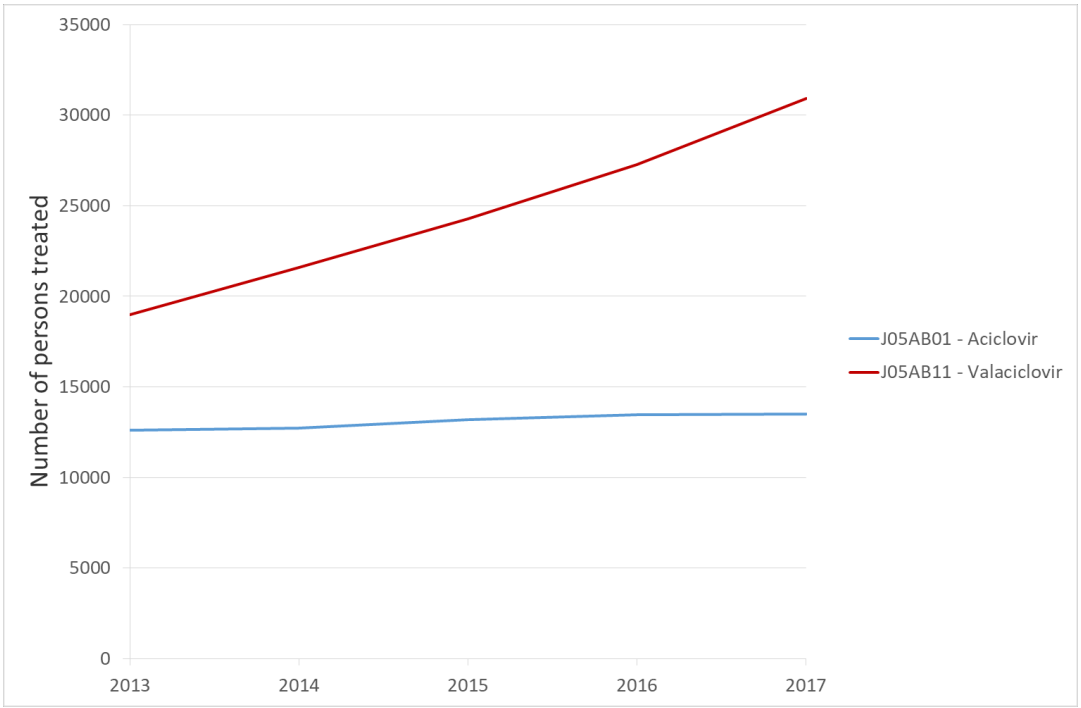

Figure 1.6. Number of individuals with at least one prescription of aciclovir and valaciclovir per year for the period 2013-2017 (3).

Table 1.2. Number of patients given prescription for human herpes virus infections per year for the period 2013-2017 (3).

	2013	2014	2015	2016	2017
J05AB01 - Aciclovir	12 598	12 720	13 191	13 449	13 519
J05AB06 - Ganciclovir	1	2	2	1	2
J05AB09 - Famciclovir	2	4	3	3	4
J05AB11 - Valaciclovir	18 985	21 583	24 257	27 263	30 907
J05AB14 - Valganciclovir	365	379	371	377	439

References

1. Arts, E and Hazuda D. HIV-1 Antiretroviral Drug Therapy. Cold Spring Harb Perspect Med 2012; 2:a007161.

2. Sakshaug, S (red), Drug Consumption in Norway 2013-2017 (Legemiddelforbruket i Norge 2013-2017), Norwegian Institute of Public Health, Oslo, Rapport 2018. ISBN (electronic): 978-82-8082-916-0, ISSN (electronic): 1890-9647

3. Norwegian Prescription database (NorPD) www.norpd.no.

2 Influenza virus

Fact box: Influenza virus drug resistance	
Treatment	Oseltamivir (Tamiflu®) As of 2016, zanamivir is no longer available on the Norwegian market
Resistance testing method	Genotypic by pyrosequencing or Sanger sequencing Phenotypic by neuraminidase susceptibility assay (MUNANA) The Norwegian Institute of Public Health performs influenza drug resistance testing in Norway
Target gene	Neuraminidase (Currently all viruses are resistant towards adamantanes, which inhibits the function of the matrix protein. The matrix gene is therefore now not regularly screened for resistance.)
Indication for resistance testing	- Patients treated with influenza antiviral drugs; with a particular focus on immunocompromised patients and young children as they often shed virus long-term, patients with severe or progressive illness who do not clinically improve, and patients with evidence of ongoing influenza virus replication through virus load monitoring. - Patients developing illness after or during antiviral chemoprophylaxis. - Patients infected after exposure to individuals receiving antiviral drugs. - Antiviral resistance surveillance: Patients in the community, particularly those without known exposure to antiviral drugs, to facilitate detection of emergence and spread of viruses with resistance. Performed by the Norwegian influenza surveillance program.
Surveillance	Screening for resistance as part of the national influenza surveillance program which involves samples from both untreated and treated patients. Currently no active surveillance program for treatment induced resistance.
Further information	ECDC expert opinion on neuraminidase inhibitors Guidance for clinical and public health laboratories testing for influenza virus antiviral drug susceptibility in Europe ISIRV antiviral group

Surveillance of influenza virus resistance

Monitoring of antiviral susceptibility has not revealed any neuraminidase inhibitor resistance in Norwegian viruses during the season 2017-18. The National Influenza Centre Norway, NIPH, has analysed 8% of viruses received for phenotypic resistance. In total, 6 % of the H3 viruses, 25 % of the H1 viruses, 3 % of the B-Yamagata viruses and 29 % of the B-Victoria viruses have been analysed for antiviral drug resistance (oseltamivir and zanamivir) either phenotypically or genetically.

The quite few H1N1-viruses circulating this season apparently did not carry the H275Y resistance mutation found in H1 viruses at the end of the 2015/16 season. H1N1 did not circulate during 2016/17.

All circulating influenza strains are currently resistant against adamantanes. These drugs are no longer used for treatment of influenza in Norway or most other countries. The National Influenza Centre Norway therefore does not routinely analyse for amantadine resistance. In December 2016, zanamivir was withdrawn from the Norwegian marked, because of low consumption. In Norway, only oseltamivir is now available for antiviral treatment of influenza.

Table 2.1. Norwegian influenza viruses resistant to the NIs oseltamivir and zanamivir and M2 blockers (adamantanes), during the influenza seasons 2005/6 through 2017/18.

Season	Oseltamivir resistance			Zanamivir resistance			Adamantane resistance	
	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)
2005/06	0% (n=6)	0% (n=13)	0% (n=21)	0% (n=6)	0% (n=13)	0% (n=21)	Nd	75% (n=4)
2006/07	0% (n=5)	0% (n=10)	nd	0% (n=5)	0% (n=10)	Nd	0% (n=6)	90% (n=10)
2007/08	67,8% (n=272)	0% (n=2)	0% (n=59)	0% (n=114)	0% (n=2)	0% (n=59)	0% (n=112)	100% (n=2)
2008/09	100% (n=33)	0% (n=13)	0% (n=1)	0% (n=5)	0% (n=12)	0% (n=1)	0% (n=5)	100% (n=65)
2009-pdmH1	0% (n=884)	nd	0% (n=11)	0% (n=36)	nd	0% (n=9)	100% (n=258)	100% (n=2)
2010/11	0,82% (n=244)	0% (n=1)	0% (n=30)	0% (n=2)	0% (n=1)	0% (n=24)	100% (n=54)	100% (n=10)
2011/12	0% (n=27)	0% (n=72)	0% (n=5)	nd	0% (n=60)	0% (n=4)	100% (n=21)	100% (n=56)
2012/13	0% (n=256)	0% (n=22)	0% (n=24)	0% (n=20)	0% (n=22)	0% (n=19)	100% (n=11)	100% (n=5)
2013/14	0% (n=183)	0% (n=43)	0% (n=27)	0% (n=32)	0% (n=43)	0% (n=27)	100% (n=77)	100% (n=67)
2014/15	0,74% (n=136)	0% (n=169)	0% (n=92)	0% (n=136)	0% (n=166)	0% (n=92)	nd	100% (n=30)
2015/16	3,0 (n=339)	0% (n=32)	0% (n=50)	0% (n=106)	0% (n=31)	0% (n=48)	nd	nd
2016/17	0% (n=10)	0% (n=174)	0% (n=54)	0% (n=8)	0% (n=161)	0% (n=54)	nd	nd
2017/18	0% (n=121)	0% (n=66)	0% (n=41)	0% (n=28)	0% (n=54)	0% (n=30)	100% (n=1)	100% (n=1)

3 Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV) drug resistance	
Treatment	*Five classes:* Reverse transcriptase inhibitors (RTI's), which are subdivided in NRTI's (nucleoside RTI's) and NNRTI's (non-nucleoside RTI's) Integrase inhibitors Protease inhibitors CCR5 antagonists Fusion inhibitors
Resistance testing method	Genotypic assays based on amplification by RT-PCR and Sanger sequencing of the product. The sequences are analysed for amino acid mutations associated with drug resistance. A plasma viral load > 500 copies/ml is required for the analysis. In Norway, all HIV-1 drug resistance tests are performed at the National Reference laboratory for HIV-1 at the Department of Microbiology at Oslo University Hospital, Ullevål.
Target genes	Reverse transcriptase Protease Integrase gp120 (envelope), V3 region (for CCR5 antagonist resistance testing)
Indication for resistance testing	Virological failure during antiviral treatment
Surveillance	It is recommended that samples from all patients with newly diagnosed HIV-1 infections are tested for resistance mutations in the protease and reverse transcriptase genes.

Surveillance of transmitted HIV-1 drug resistance

Surveillance methods

Transmitted (primary) drug resistance refers to resistance detected in previously untreated persons. Acquired (secondary) drug resistance results from selection of drug-resistant variants from a genetically heterogeneous virus population during antiretroviral therapy. Drug resistance rarely occurs without drug exposure. Transmitted drug resistance therefore implies that resistant virus was transmitted either directly, or through intermediates, from a person with acquired drug resistance.

The World Health Organization (WHO) recommends the use of a consensus genotypic definition of transmitted HIV-1 drug resistance to compare transmitted HIV-1 drug resistance rates across geographic regions and time (1). The list of mutations for drug-resistance surveillance (SDRMs) is based on the following criteria (2): 1) Mutations should be commonly recognized as causing or contributing to resistance. 2) Mutations should be nonpolymorphic and should not occur at highly polymorphic positions. That is, they should not commonly occur in the absence of therapy or at sites with a naturally high level

of variation. 3) The mutation list should be applicable to the eight most common HIV-1 subtypes. 4) The list should be short and unambiguous. The SDRM list is not designed for individual patient management as it excludes certain clinically relevant drug resistance mutations and includes certain mutations, which lack clinical relevance, but are robust markers of transmitted drug resistance.

In Norway the national surveillance system for HIV-1 monitors the prevalence of transmitted drug resistance against protease inhibitors (PI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTI). The monitoring is conducted according to WHOs SDRM-list of 2009 and analysed by using the Calibrated Population Resistance (CPR) tool at Stanford HIV Drug Resistance Database (1).

The surveillance is based on resistance testing of samples taken from newly diagnosed patients. Results are included in the surveillance system when the MSIS report number is specified on the referral form. However, this report number is lacking in a number of cases. The reference laboratory for HIV is taking action to increase the percentage of newly diagnosed cases of HIV-1 infection represented in the surveillance. The annual number of sequences analysed for primary HIV-1 drug resistance from newly diagnosed cases of HIV-1 in Norway since 2010 is shown in figure 3.1.

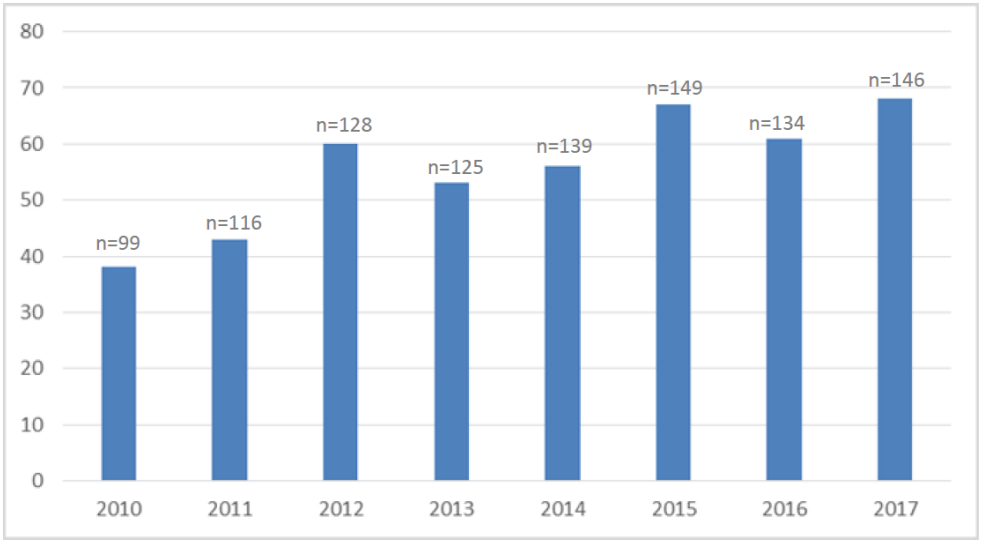

Figure 3.1. Number of newly diagnosed cases of HIV-1 infection where sequences were sent for resistance testing (2010-2017).

Surveillance findings in Norway in 2017

SDRMs detected by monitoring for primary HIV-1 resistance are presented in figure 3.2. The bars show the percentage of the sequences with detected SDRMs during each year of surveillance. There may be several SDRMs per sequence. The lines show the percentage of sequences with SDRMs affecting NNRTI, NRTI and PI, respectively.

In 2017, the total number of analysed samples was 146. SDRMs from the WHO list were detected in 5.6% of the analysed sequences. The different mutations found are specified in table 3.1. Of the analysed sequences, 0.7% had SDRMs associated with PI, 3.5% with NNRTI and 3.5% with NRTI.

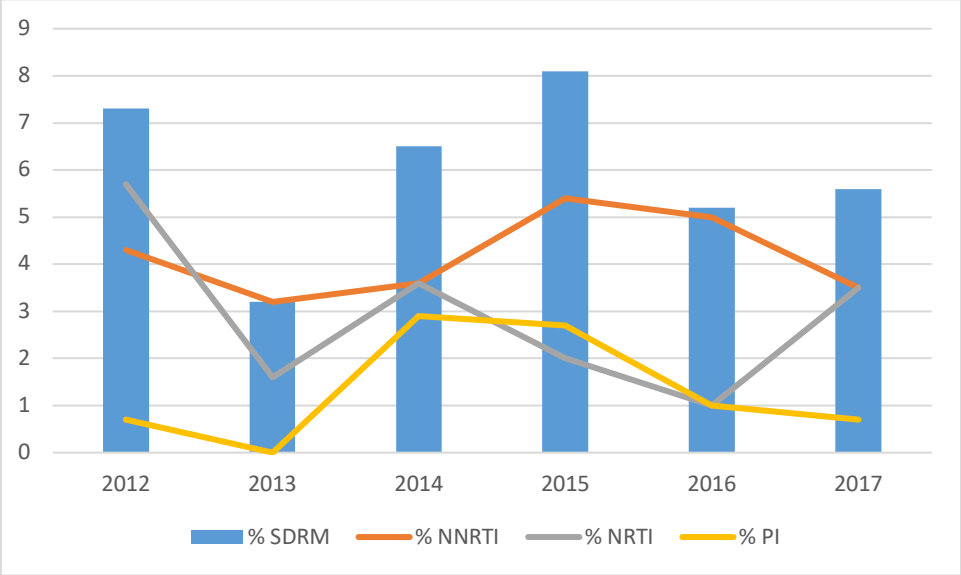

Figure 3.2. Percentage of analysed sequences containing one or more SDRMs (Surveillance Drug Resistance Mutations) and the distribution on NNRTI, NRTI and PI, respectively (2012-2017).

Table 3.1. The different mutations found in the sequences with SDRMs in 2017.

SequenceID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	M41L	None	None
2	T215S	Y181C	None
3	M184V	None	None
4	None	None	M46I
5	D67G, K70R, V75M, M184V, T215I, K219E	V106M, Y181C	None
6	None	K103N	None
7	None	G190A	None
8	D67N, K70R, M184V, T215Y, K219EQ	K101E, G190A	None

Discussion

The surveillance data are based on data from patients who had their HIV-1 infection confirmed in Norway and where the diagnoses were anonymously reported to MSIS during the respective year (3). A number of these patients are immigrants who were infected before arrival in Norway. Some of these patients may have received treatment in their home countries. Thus, the data do not reflect the risk of being infected in Norway with a drug resistant strain of HIV-1.

In an ongoing study, connecting epidemiological data from MSIS with resistance data, we aim to describe the prevalence of SDRM in different subgroups, such as risk group or country of infection etc. Preliminary results from this study show that only 30% (40 out of 133) of the reported cases in 2016 were infected in Norway.

Conclusions

Surveillance drug-resistance mutations were detected in 5.6% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2017, mainly mutations associated with resistance against NRTI and NNRTI. The prevalence of transmitted drug resistance has been stable for the last years with only minor variation. The introduction of

prophylactic treatment (PrEP and PEP) could challenge this situation, and the recent implementation of PrEP calls for intensified surveillance. Surveillance of HIV-1 resistance over time is important in order to make decisions on implementing preventive measures to control dissemination of resistant HIV-1 strains.

References

1. https://hivdb.stanford.edu/page/who-sdrm-list/
2. Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, et al. (2009) Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update. PLOS ONE 4(3): e4724. https://doi.org/10.1371/journal.pone.0004724
3. www.msis.no

4 Hepatitis B virus

Fact box: Hepatitis B virus (HBV) drug resistance	
Treatment	Nucleoside/nucleotide analogues (lamivudine, adefovir dipivoxil, emtricitabine, entecavir, telbivudine, tenofovir disoproxil and tenofovir alafenamide) Interferon Treatment of HBV infection with antivirals is generally given as monotherapy.
Resistance testing method	Genotypic assays based on amplification by RT-PCR and Sanger sequencing of the product. The sequences are analyzed for amino acid mutations associated with drug resistance using geno2pheno (version 2.0) resistance database (1) from Max Planck Institut Informatik. A plasma viral load > 800 IU/ml is required for the analysis. In Norway, all HBV drug resistance tests are performed at the Norwegian Institute of Public Health.
Target gene	Polymerase gene (a segment overlapping with the small S gene)
Indication for resistance testing	Virological failure/breakthrough on antiviral treatment
Surveillance	Population-level surveillance of a treatment naive population selected from samples submitted for genotyping

Surveillance of HBV drug resistance

Drug resistance surveillance data in Norway in 2017

The surveillance of HBV resistance in Norway aims to monitor two populations; 1) patients that have been tested for drug resistance primarily in relation to treatment and 2) patients that are genotyped for HBV as part of the elucidation of infectious status, generally before treatment. The latter population can therefore be regarded as surveillance of primary resistance.

Mutations altering specific amino acids positions within the polymerase gene, alone or in combination, give rise to resistance to all the approved antivirals for the treatment of HBV (Table 4.1).

Table 4.1. Nucleos(t)ide analogue cross-resistance data for resistant HBV variants (The table is adapted from the revised EASL 2017 Hepatitis B Guideline (2)).

Cross-resistance data for resistant HBV variants					
HBV-variants (mutations)	LAM	LDT	ETV	ADV	TDF/TAF*
Wild type	S	S	S	S	S
M204V	R	S	I	I	S
M204I	R	R	I	I	S
L180M + M204V	R	R	I	I	S
A181T/V	I	I	S	R	I
N236T	S	S	S	R	I
L180M + M204V/I ± I169T ± V173L ±M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

The amino acid substitution profiles are shown in the left column and the level of susceptibility is given for each drug: S (sensitive), I (intermediate/reduced susceptibility), R (resistance). ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; LAM, lamivudine; ADV, adefovir * No clinical data for TAF available.

In 2017, 23 patient samples were submitted for HBV drug resistance testing. Resistance mutations were detected in only 2 of the 23 samples (table 4.2), both from patients on entecavir treatment. One of the samples had mutations L180M and M204V indicating reduced susceptibility to entecavir. The other sample had mutation S202G in addition to L180M and M204V indicating resistance to entecavir. Among cases, where no resistance was detected, information on antiviral treatment was indicated as follows; four on tenofovir treatment, seven on entecavir, one on tenofovir/emtricitabine (Truvada) and one on an unknown type of antiviral treatment. No information was given regarding treatment in 4/23 cases. Four samples were tested prior to treatment (baseline resistance), all with no resistance. Among patients only submitted for HBV-genotyping (N=223) no drug resistance mutations were detected.

Table 4.2. HBV antiviral resistance in samples submitted for drug resistance testing in 2011–17.

HBV–variants resistant to antivirals	Drug resistant mutations						
Year	2011	2012	2013	2014	**2015**	**2016**	**2017**
Total analysed	14	3	9	17	**10**	**23**	**23**
Wild type	11	2	8	15	**8**	**17**	**21**
M204I	1a	0	1a	1c	**1e**	**1[d]**	
L180M + M204V	1b	1a		1c		**1[d]**	**1[a]**
A181T/V eller N236T	1a	0	0	0	**0**	**0**	
L180M + M204V/I ± I169T ± M250V	0	0	0	0	**0**	**1[d]**	
L180M + M204V/I ± T184G ± S202I/G	0	0	0	0	**0**	**2[c,d]**	**1[a]**
Sequencing inconclusive					**1**	**1**	

a=entecavir, b=tenofovir, c=lamivudine, d=treatment unknown, e= telbuvidin

Conclusion

Only two cases of antiviral resistance against HBV were detected in 2017. Therefore, resistance against antivirals seems to be a small problem in Norway given the large number of patients on treatment. However, the data is very limited due to the low frequency of testing for antiviral resistance and should therefore be interpreted carefully. No resistance was detected among patients tested for genotyping only, as we have seen over the previous years.

References

1. Geno2pheno resistance database at Max Planck Institute of Informatik https://hbv.geno2pheno.org/
2. European Association for the Study of the Liver (EASL) 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection (2017). J Hepatol. 18. april 2017.
3. (http://www.easl.eu/research/our-contributions/clinical-practice-guidelines/detail/easl-2017-clinical-practice-guidelines-on-the-management-of-hepatitis-b-virus-infection)

5 Surveillance of human herpes viruses drug resistance

Cytomegalovirus

Fact box: Human cytomegalovirus (CMV) drug resistance	
Treatment	Ganciclovir/valganciclovir (nucleoside analogue) Cidofovir (nucleotide analogue) Foscarnet (polymerase inhibitor)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analysed for amino acid mutations associated with drug resistance. In Norway, all CMV drug resistance tests are performed at the National Reference laboratory for CMV at the Department of Microbiology at Oslo University Hospital, Rikshospitalet.
Target genes	CMV-genes UL97 and UL54
Indication for resistance testing	Persistent high viral load in blood or other compartments during antiviral treatment
Surveillance	Population-level surveillance is currently not necessary

Serious CMV-infections are mainly encountered in immunosuppressed patients and congenitally infected newborns. Antiviral drugs for the therapy of CMV-infections are ganciclovir (GCV) and its prodrug valganciclovir (VGCV), cidofovir (CDV) and foscarnet (FOS). All these drugs target the viral DNA-polymerase encoded by CMV UL54. GCV and VGCV are the drugs of choice since they are quite effective in inhibiting virus replication and have few side effects. To become active GCV is monophosphorylated by the CMV UL97 kinase and then di- and tri-phosphorylated by cellular kinases. GCV-triphosphate is specifically accepted by the CMV-DNA polymerase and becomes integrated in the growing CMV-DNA chain where it acts as a chain terminator.

Some new anti-CMV-drugs are in clinical trials. Maribavir, a UL97-kinase inhibitor, has been used in clinical trials with favorable outcomes but is not yet approved by the FDA. Brincidofovir is a lipidconjugate of CDV with an increased antiviral potency and reduced renal toxicity relative to CDV. Letermovir binds and inhibits the CMV-DNA terminase complex, which is involved in cleaving of concatemeric CMV-DNA and packaging the genome into the capsid. The drug is approved by the FDA for prophylactic use after stem cell transplantation (1).

During GCV anti-CMV therapy, resistance mutations usually develop after a cumulative exposure of six weeks or more. Resistance mutations are first seen in the UL97 kinase gene. The UL54 (DNA- polymerase) mutations are more likely to emerge later and add to the level of resistance conferred by preexisting UL97 mutations. UL54 resistance mutations in the absence of UL97 mutations are rare (2). UL97 mutations that confer GCV resistance are mainly clustered at UL97 codon 460, 520 or 590-607. UL54 GCV/CDV resistance mutations are mainly located in the exonuclease domain whereas the FOS resistance mutations range widely. Resistance mutations may confer insignificant resistance, low-grade resistance and moderate resistance depending on whether the effective drug concentration (EC50) is increased by <2x, 2-5x or 5-15x respectively. Patients infected with a GCV –low grade resistant strain may be treated by increasing the

dose of GCV or VGCV whereas a switch to FOS or CDV is recommended when infected with a moderately resistant strain.

In 2017, 39 specimens were received for genotypic analysis of CMV resistance mutations. The specimens were from patients with treatment failures when on GCV-therapy. Seven specimens contained insufficient amounts of CMV-DNA for PCR-amplification and sequencing. UL97 and UL54 genes were successfully amplified and sequenced in 32 specimens from 27 patients. GCV-resistant CMV was detected in 7 patients. All patients had CMV-UL97 GCV resistance mutations and one patient had additionally two CMV-UL54 resistance mutations that confer resistance to GCV, CDV and FOS (Table 5.1).

Table 5.1. CMV-resistance mutations recorded in patients tested in 2017

Patient	UL97 mutations1	UL54 mutations
1	C603W	--
2	L595S	-
3	L595W	-
4	H520Q	-
5	A594V	-
6	M460V	-
7	L595W	K513N[2] D588N[3]

[1]GCV moderate resistance
[2]CDV moderate and GCV low grade resistance
[3]FOS moderate and GCV/CDV low grade resistance

As can be seen from Table 5.2, the reference laboratory has during the last ten years received specimens from 215 patients. In 65 (30%) patients anti-CMV drug resistance mutations were detected. Accordingly, the majority of the patients (70%) had treatment failures while on anti-CMV drugs caused by other factors than drug resistance. Clinical suspicion of drug resistance is in most cases not genotypically confirmed. This may be caused by insufficient dosage of the drug, inadequate anti-CMV T-cell response in immunosuppressed patients or the specimens were collected to early after initiation of therapy. Increases in viral load in the first two weeks of treatment are not predictors of drug resistance.

Table 5.2. Number of patients analyzed for anti-CMV drug resistance mutations and number of patients in whom CMV- drug resistance mutations were detected.

Year	Number of patients tested	Number of patients with CMV resistance mutations
2008	14	5
2009	12	8
2010	22	5
2011	18	4
2012	23	5
2013	23	8
2014	21	7
2015	27	5
2016	28	8
2017	27	7
Sum	215	65 (30%)

Herpes simplex virus

Fact box: Herpes simplex virus (HSV) drug resistance	
Treatment	Aciclovir/Valaciclovir (nucleoside analogue)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analyzed for amino acid mutations associated with drug resistance. All HSV drug resistance tests for Norway are performed at The Public Health Agency of Sweden, Stockholm.
Target gene	HSV thymidine kinase gene
Indication for resistance testing	Persistent HSV-infection despite ongoing therapy
Surveillance	Population-level surveillance is currently not necessary

Herpes virus-infections are caused by either herpes simplex virus 1 (HSV-1) or herpes simplex virus 2 (HSV-2). The anti-HSV agents currently in use in Norway target the viral DNA-polymerase (UL30). The first line drugs are aciclovir (ACV) or its prodrug valaciclovir (VACV). In 2016 almost 40.000 persons were given ACV or VACV. Penciclovir (PCV) is available as ointment for treatment of herpes labialis. Second line drugs include foscarnet (FOS) and cidofovir (CDV). Several new drugs are in clinical trials or in a preclinical phase.

ACV has to be phosphorylated first by the HSV thymidine kinase (TK) and then by the cellular kinases to the active ACV-triphosphate. About 95% of the resistance mutations are localized in the thymidine-kinase gene (UL23) whereas 5% are localized in the DNA-polymerase gene (UL30) (3).

Although the prevalence of HSV resistance mutations are reported to be 0.1% -0.7% in immunocompetent patients and 3.5% to 10% in immunocompromised patients, treatment failures are relatively rare (4). In 2017, ten HSV samples (three HSV-1 and seven HSV-2) from Norwegian patients with persistent HSV infections while on ACV treatment were sent to Folkhälsomyndigheten, Sweden for genotypic resistance testing. Based on TK resistance mutations specimens from two patients contained HSV resistant to ACV, one HSV-1 and one HSV-2 (table 5.3). Six specimens contained ACV sensitive HSV and two specimens had insufficient amounts of HSV-DNA for analysis. Additionally, HSV-DNA-polymerase (UL30) mutations were looked for in specimens from four patients and no resistance mutations for ACV, FOS and CDV were found.

Table 5.3. HSV-1 and HSV-2 acyclovir resistance mutations

Patient	HSV-type	TK-mutations	DNA-pol mutations
1	HSV-1	436 del G	-
2	HSV-2	G201D	-

Conclusion

In 2017, we received specimens from 27 patients with treatment failure while on anti-CMV therapy. Seven patients had GCV resistance mutations in the CMV-UL97-kinase gene and one patient had additional UL54-polymerase resistance mutations that conferred resistance to FOS and CDV. In the years 2008-2017 specimens from 215 patients were analyzed, however, only 65 (30%) had GCV-resistance mutations. Most of the anti-CMV treatment failures are thus not due to drug resistant CMV.

Despite the fact that ACV, its prodrug VACV, and prescription-free PCV are widely used for treatment and prophylaxis of HSV-infections, treatment failures due to resistance mutations are rare. Ten specimens were received and two of them harbored ACV-resistant HSV. The situation is as in previous years.

References

1. Frenge P, Leruez-Ville M. Efficacy and safety of new antiviral drugs for treating cytomegalovirus infections. Med Mal Infect, 2018 e-pub ahead of print.
2. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev 23:689-712,2010
3. Sauerbrei A, Bohn-Wippert, Kaspar M, Krumbholz A, Karrash 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,
4. Piret J, Boivin G. Antiviral resistance in herpes simplex virus varicella zoster virus infections: diagnosis and management. Curr Opin Infect Dis 2016;29:654-662.

Cytomegalovirus: prophylaxis and resistance

Grete Birkeland Kro

Prophylactic treatment, such as vaccination or passive immunization that prevents primary CMV infection is not currently available. There are vaccines under development, but none are thus far available on the market. Basic hygiene measures remain the only means to avoid contracting CMV. Hence, the term CMV prophylaxis currently refers to prevention of CMV disease, not prevention of primary CMV infection.

The main group that receives prophylactic treatment against CMV disease is transplanted patients. Prior to transplantation the CMV serostatus of the donor and the recipient are determined. The guidelines for solid organ transplantation recommend prophylactic treatment only for CMV seronegative recipients who receive organs from CMV seropositive donors, except for lung transplantation, where CMV seropositive recipients also receive prophylaxis. The treatment regimes differ depending on the transplanted organ; Valganciclovir per os, 900mg x 1 or 450 mg x 2, duration 3 to 12 months. Those who do not receive prophylactic treatment are treated preemptively; which entails monitoring with CMV DNA quantitation (CMV PCR) and initiating treatment if CMV DNA levels rise above a certain threshold. Valganciclovir per os is the drug of choice also in this group, unless the patient is a bone marrow transplanted with insufficient bone marrow function. In such cases, foscarnet is preferred to avoid further deterioration of bone

marrow function. The treatment normally continues until no CMV DNA has been detected in two sequential CMV DNA quantitations, or for at least two weeks.

Prophylactic treatment is also considered in newborns diagnosed with congenital CMV infection. The goal when treating newborns with congenital CMV infection is to improve hearing and neurodevelopmental outcomes. There is an ongoing discussion on whether to treat only newborns with moderate to severe CMV disease or also newborns with isolated hearing loss. In 2018, the Norwegian Pediatric Association published a preliminary protocol for diagnosis and follow-up of congenital CMV infection in newborns that do not pass the newborn hearing screening. In cases where treatment is indicated, the Norwegian protocol recommends Valganciclovir mixt p.o. (16mg/kg x 2) for 6 months or in case of severe infection Ganciclovir iv (6mg/kg x 2) for 2-4 weeks followed by Valganciclovir mixt p.o.

Does the use of prophylactic treatment increase resistance?

CMV drug resistance results from single or multiple mutations that confer various levels of resistance. Over time the mutations accumulate, and thus the likelihood of CMV resistance increases with the number of viruses replicating and the duration of the drug exposure. The number of replicating viruses is influenced by insufficient antiviral drug activity, category, and degree of host immunosuppression.

Prophylactic and preemptive treatment that suppresses viral replication effectively prevents the high viral loads seen in patients with CMV disease. Patients with high viral loads require treatment for a longer period of time than those with a low viral load. Thus prophylactic and preemptive treatment that effectively suppresses viral replication may actually reduce the risk of CMV resistance.

Referanser:

1. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation. [1] et al, Transplantation. 2018 Jun;102(6):900-931.
2. Forslag til protokoll for utredning og oppfølging av medfødt CMV infeksjon hos barn som ikke passerer eller lar seg teste med hørselsscreening i nyfødtperioden. Støen R, Rønnestad A, Salvesen B, Barlinn R, Stokkereit Mattsson T, Klingenberg C. http://legeforeningen.no/Fagmed/Norsk-barnelegeforening. Mars 2018
3. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Rawlinson WD, et al. Lancet Infect Dis. 2017;17: e177-88
4. Congenital Cytomegalovirus – A European Expert Consensus Statement on Diagnosis and Management. Luck SE, et al. Ped Inf Dis J 2017; 36: 1205-12
5. Antiviral drug resistance of human cytomegalovirus. Lurain NS[1], Chou S. Clin Microbiol Rev. 2010 Oct;23(4):689-712.

6 Hepatitis C virus

Fact box: Hepatitis C virus (HCV) drug resistance	
Treatment	Pegylated interferon and ribavirin Telaprevir (NS3/4A-inhibitor) Boceprevir (NS3/4A-inhibitor) Sofosbuvir (NS5B-inhibitor) Simeprevir (NS3/4A-inhibitor) Daclatasvir (NS5A-inhibitor) Dasabuvir (NS5B-inhibitor) Fixed combinations: Sofosbuvir + ledipasvir (NS5A-inhibitor) Paritaprevir/ritonavir (NS3/4A-inhibitor) + ombitasvir (NS5A-inhibitor)
Resistance testing method	Sequencing of relevant genes and/or the complete HCV genome Routine resistance testing is currently not available in Norway, but a NGS-based method is under development at the Norwegian Institute of Public Health.
Target genes	NS3–NS4A (protease) NS5A (replication and assembly factor) NS5B (polymerase)
Indication for resistance testing	Virological failure during treatment
Surveillance	Primary resistance testing on selected genotypes and genomic regions from clinical routine samples

Testing for hepatitis C virus drug resistance is currently not available in Norway, but a method based on next generation sequencing is under development at The National Institute of Public Health. Until this method is established, samples may be sent to Uppsala University Hospital or Sahlgrenska University Hospital in Gothenburg. They offer Sanger sequencing of target regions in the NS3 and NS5A genes. The occurrence of baseline resistance against most DAA's is very low, but there are a few exceptions. According to current international guidelines, baseline testing is recommended in the following two cases:

- When using grazoprevir/elbasvir for treatment-naive patients with genotype 1a infections
- When using sofosbuvir/velpatasvir for genotype 3 infections in patients with cirrhosis.

Depending on the local availability of resistance testing, different strategies may be used to take these two cases into account. Below, Bente Magny Bergersen describes an empirical strategy without the use of resistance testing, and finally Olav Dalgard gives the background for the international consensus.

In the Norwegian guidelines for treatment of hepatitis C the two strategies are regarded as equivalent. However, only testing of patients with genotype 1a and high VL (>800 000 IU/ml) is emphasised in the guidelines.

Hepatitis C, treatment and resistance testing- a clinicians view

Bente Magny Bergersen

The large majority of hepatitis C patients who received direct acting antiviral drugs (DAAs) at the Department of Infectious Diseases Oslo University Hospital, has been entered into a local HCV quality registry. The following presentation of data is based on estimates from this registry.

In the period from August 2014 to June 2018, an estimated 530 patients have been treated with one or more of the following DAAs: daclatasvir (Daclinza®), sofosbuvir (Sovaldi®), dasabuvir (Exviera®), ombitasvir/paritaprevir/ritonavir (Viekirax®), sofosbuvir/ledipasvir (Harvoni®), elbasvir/ grazoprevir (Zepatier®) and sofosbuvir/velpatasvir (Epclusa®). Sofosbuvir is a robust, pangenotypic nucleotide analogue that inhibits the NS5B polymerase. Ledipasvir (found only in combination with sofosbuvir) is a NS5A inhibitor (first generation). Elbasvir and velpatasvir are NS5A inhibitors (second generation). Paritaprevir, grazoprevir and voxilaprevir are NS5 3/4 A protease inhibitors. See next Section by Olav Dalgard for pharmacological details.

The effective and pan genotypic combination of sofosbuvir/velpatasvir for 12 weeks is the treatment of choice in HCV genotype 2 and 3. Resistance testing only becomes relevant in discontinuations and/or relapse. At present, none of our 146 patients treated with sofosbuvir/velpatasvir have relapsed.

Due to the high prices of DAAs, the choice of drugs has been highly influenced by economic restrictions. In March 2017, the restrictions on DAA treatment of HCV genotype 1 were repealed, and all patients with genotype 1 and 4 could be treated with elbasvir/grazoprevir.

In HCV genotype 1a with high viral load, a higher number of relapses is expected, and an increased duration of treatment is recommended if resistance testing cannot be performed (1). LIS Guidelines March 2017 recommend that in cases of HCV genotype 1a with HCV RNA> 800,000 IU/ml and/or NS5A-polymorphism which leads to a five-fold decrease in elbasvir activity, ribavirin should be added, and the duration of treatment should be extended to 16 weeks. The local hepatitis C registry at that time had a total of about 250 HCV genotype1 patients, and 60% (150/250) had HCV RNA> 800 000 IU/ml. HCV resistance testing is not available at Oslo University Hospital, and tests are forwarded to Sahlgrenska laboratory in Gothenburg, Sweden.

The clinic at Ullevål Hospital identified three treatment options: 1) treat a considerable number of patients with unnecessary drugs with significant adverse events 2) have most of the genotype 1 patients resistance tested, and wait several weeks for the results, or 3) treat all HCV genotype 1 with elbasvir/ grazoprevir for 12 weeks and re-treat the relapses. In mutual understanding with hospital management, the clinic opted for treatment option 3. The recommended treatment of HCV relapse is sofosbuvir/velpatasvir/voxilaprevir (Vosevi®), where sustained viral response (SVR) in 1a is reported to be achieved in 96% (phase 3, POLARIS-1). Genotype or resistance profile does not appear to affect the treatment success rate. The only patient group that cannot use protease inhibitors is patients with decompensated cirrhosis.

In the period from August 2014 to June 2018 the clinic has treated 184 HCV genotype 1 patients with elbasvir/ grazoprevir; 116 are registered as SVR, 33 as “treatment

concluded, awaiting final HCV RNA", 28 as "under treatment". Three of 184 patients (2%) are registered as relapses – all three are genotype 1a with high viral load. One of them is under re-treatment with sofosbuvir/ velpatasvir/voxilaprevir. Four patients chose to terminate treatment, for a variety of reasons.

The combination of sofosbuvir/ledipasvir for eight weeks has also been used in HCV genotype 1a patients with high viral load because it is of shorter duration, has few side effects and is cheaper than 16-week treatment with elbasvir/grazoprevir/ribavirin. From March 2015 to June 2018 the clinic has treated 133 HCV patients with sofosbuvir/ledipasvir, most of them genotype 3. Ninety-five of 133 are registered as SVR, four of 133 (3%) are registered as relapses.

Conclusion: Based on our clinical experience with DAA's, resistance testing does not appear to significantly impact the outcome of hepatitis C treatment.

References

(1) Kwo P, Gane EJ, Peng CY, Pearlman B, Vierling JM, Serfaty L, Buti M, et al. Effectiveness of Elbasvir and Grazoprevir Combination, With or Without Ribavirin, for Treatment-Experienced Patients With Chronic Hepatitis C Infection

Direct-acting antivirals for the treatment of chronic hepatitis C

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The hepatitis C virus (HCV) is a hepatotrophic RNA virus that, in contrast to hepatitis B, is not incorporated in the host's DNA. This contributes to the fact that hepatitis C treatment, and not hepatitis B treatment, usually causes a lasting viral cure. It has been shown that undetectable HCV RNA at 12 weeks after the end of treatment is a reliable marker for sustained virological response (SVR). Until 2011, SVR could only be achieved by immunomodulatory treatment in the form of subcutaneous administration of interferon alpha combined with oral ribavirin. With this combination, approximately 60 % achieved SVR, but since interferon had an effect on a variety of organ systems, frequent and sometimes serious adverse reactions were seen. Because of that, there was a great need for more efficient and tolerable treatment. Already in the 1990s, attempts were made to inhibit the HCV protease, but because the active site of the protease was shallow, this proved difficult to achieve. When a protease inhibitor with good antiviral effect was finally identified, the drug appeared toxic in animal models and development was interrupted. Lack of a cell culture model system made the way from candidate molecule to testing in patients long. This changed in 2005 when a model system, fully permissive to cell culture replication of HCV, was made available. The system not only allowed rapid identification of new and better protease inhibitors, but also molecules that blocked other virus-specific proteins. Thus, inhibitors of the HCV polymerase and inhibitors of gene products encoded in the NS5A region of HCV were identified. The group of new oral HCV drugs was termed direct acting antiviral drugs (DAAs). In 2012, it was for the first time shown that it is possible to achieve SVR with a combination of DAAs without the use of interferon (1).

Direct acting antiviral drugs

There are four main groups of direct acting antiviral drugs: nucleoside analogue polymerase inhibitors, non-nucleoside analogue polymerase inhibitors, protease inhibitors and NS5A inhibitors (2).

Nucleoside analogue polymerase inhibitors

In this group, sofosbuvir is the only drug. Sofosbuvir competes with nucleosides for the active site of the HCV polymerase. The drug has a moderate antiviral effect against all genotypes and has a very high genetic barrier to resistance preventing resistant variants to develop entirely. Side effects are insignificant, and interactions are rarely a major problem.

Non-nucleoside analogue polymerase inhibitors

Drugs in this group alter the form of the polymerase and thus inhibit replication of HCV. They have a weak antiviral effect and a low genetic barrier to resistance. They are only effective against HCV genotype 1 and dasabuvir is the only drug within this group that is in clinical use.

Protease inhibitors

The following protease inhibitors are available: paritaprevir grazoprevir, glecaprevir and voxilaprevir. Protease inhibitors have good antiviral effects. First-generation protease inhibitors (paritaprevir and grazoprevir) have a low genetic barrier against resistance and are only effective against genotypes 1 and 4, whereas second-generation protease inhibitors (glecaprevir and voxilaprevir) have a high genetic barrier and are pangenotypic. Resistance associated variants (RAVs) that are selected during treatment are not fit and will usually not be detectable a few months after a treatment failure. With the exception of voxilaprevir that may cause diarrhea and nausea, protease inhibitors have few side effects, but one should be aware of interactions with other medications. In addition, protease inhibitors undergo hepatic metabolism. In patients with liver failure a many fold increase in serum concentration is seen during treatment with protease inhibitors followed by a risk of hepatic toxicity. Therefore, these drugs should not be given to patients with hepatic failure.

NS5A inhibitors

The functions of the proteins encoded by the NS5A region of the virus genome are not well known, but they affect the assembly and release of the virus. Inhibitors of NS5A-encoded proteins have a good antiviral effect against all genotypes. However, the drugs have a low genetic barrier to resistance and RAVs are fit and will often remain dominant following treatment failure. NS5A inhibitors have insignificant side effects and few drug-drug interactions. Five medications in this group are available: ombitasvir, ledipasvir, velpatasvir elbasvir and pibrentasvir.

How can DAAs be combined?

Pangenotypic combinations

There are three pangenotypic combination tablets (sofosbuvir/velpatasvir, sofosbuvir/ velpatasvir/voxilaprevir, and glecaprevir/pibrentasvir). The combinations all have high genetic barriers to resistance, and strong antiviral effects with very good treatment responses. In those with genotype 1, 2 and 4, SVR is obtained in 97-100% while in those with genotype 3 infection approximately 95% achieve SVR (3). Since it is desirable to treat with as few drugs as possible and there are several excellent duo treatments, the triple regimen of sofosbuvir/velpatasvir/ voxilaprevir is basically a second line treatment (4).

Genotype specific combinations

N*ucleoside analogue polymerase inhibitor + NS5A inhibitor*

The combination of sofosbuvir/ledipasvir for 12 weeks gives SVR in 97% of those with genotype 1 (5). In those without cirrhosis, treatment can be shortened to eight weeks. The regime is also effective against genotype 4.

Protease inhibitor + NS5A inhibitor + non-nucleoside analogue polymerase inhibitor

Among the first generation DAAs, a quadruple regimen was required to achieve good response without sofosbuvir in patients with genotype 1 infection. The combination of ombitasvir / paritaprevir/ritonavir + dasabuvir yielded 96% SVR. In this regimen, the protease inhibitor paritaprevir is pharmacokinetically boosted by ritonavir as is also done in HIV treatment.

Protease inhibitor + NS5A inhibitor

The protease inhibitor grazoprevir combined with elbasvir was the first sofosbuvir-free duoregimen that gave adequate responses. In those infected with HCV genotype 3, a double dose was needed to achieve high rates of SVR. Unfortunately, the high dose regimen gave increased liver transaminase activity and the combination therefore should only be used at low dosage against genotype 1 and 4. The NS5A inhibitor elbasvir has good antiviral effect, but a single mutation in the NS5A region that is often present before treatment produces a reduced antiviral effect against subtype 1a and SVR rates were 92%.

Resistance testing

Treatment failure after combination treatment with DAAs is rare and will only occur in approximately 3% of those treated. Predictors of treatment failure include genotype 3 infection, the presence of RAVs and advanced liver fibrosis. Retreatment with the triple regimen sofosbuvir/velpatasvir/voxilaprevir is successful in 95% of those who did not achieve SVR. This holds true also in those who failed to respond to a NS5a inhibitor containing regime whom we know often will harbour RAVs.

With such good response rates to the first-line treatments, there is often no need for resistance testing before hepatitis C treatment is administered. There are however two exceptions; the first one with use of grazoprevir/elbasvir in treatment-naive patients with genotype 1a, and the second one with the use of sofosbuvir/velpatasvir in genotype 3 infection and cirrhosis (6, 7). In 15% of the wild-type viruses of genotype 1a, one or more

RAVs will be present in the NS5A region. In those with high viral load (> 800,000 IU / ml) these may have clinical significance and only 50% of patients carrying these RAVs will achieve SVR. These patients should therefore be offered a different regime than grazoprevir/elbasvir. Patients with the Y93H RAV, genotype 3a and cirrhosis will have reduced effect of sofosbuvir/velpatasvir. Therefore, these patients should receive ribavirin in addition.

Conclusion

In most cases, Hepatitis C can be cured by a 12-week tablet cure with a combination of two direct-acting antiviral drugs. Hepatitis C treatment has few side effects and everyone who is infected should be identified and offered treatment.

References

1. Lok AS, Gardiner DF, Lawitz E, Martorell C, Everson GT, Ghalib R, Reindollar R, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. N Engl J Med2012;366:216-224.

2. Pawlotsky JM. New hepatitis C therapies: the toolbox, strategies, and challenges. Gastroenterology 2014;146:1176-1192.

3. Zeuzem S, Foster GR, Wang S, Asatryan A, Gane E, Feld JJ, Asselah T, et al. Glecaprevir-Pibrentasvir for 8 or 12 Weeks in HCV Genotype 1 or 3 Infection. N Engl J Med 2018;378:354-369.

4. Bourliere M, Gordon SC, Flamm SL, Cooper CL, Ramji A, Tong M, Ravendhran N, et al. Sofosbuvir, Velpatasvir, and Voxilaprevir for Previously Treated HCV Infection. N Engl J Med 2017;376:2134-2146.

5. Kowdley KV, Gordon SC, Reddy KR, Rossaro L, Bernstein DE, Lawitz E, Shiffman ML, et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med 2014;370:1879-1888.

6. Zeuzem S, Ghalib R, Reddy KR, Pockros PJ, Ben Ari Z, Zhao Y, Brown DD, et al. Grazoprevir-Elbasvir Combination Therapy for Treatment-Naive Cirrhotic and Noncirrhotic Patients With Chronic Hepatitis C Virus Genotype 1, 4, or 6 Infection: A Randomized Trial. Ann Intern Med 2015;163:1-13.

7. Foster GR, Afdhal N, Roberts SK, Brau N, Gane EJ, Pianko S, Lawitz E, et al. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. N Engl J Med 2015;373:2608-2617.

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