NIPH
Norwegian Institute of Public Health

REPORT

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Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2018

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Introduction

In 2018, the rise in antimicrobial resistance (AMR) was defined by WHO as one of the greatest threats to global health. Antiviral treatment is a young, but rapidly growing field, and the number of antiviral drugs registered is constantly increasing. Better knowledge and increased awareness are essential to be able to control emerging antiviral drug resistance, and surveillance will be a key tool for management.

The Norwegian Surveillance System for Virus Resistance (RAVN) was established in 2014 and is coordinated by the RAVN administration at the Norwegian Institute of Public Health. The RAVN administration works together with the RAVN Advisory council to monitor viral resistance in collaboration with participating regional laboratories.

It is a pleasure to present the sixth report from the surveillance system for Resistance against Antivirals in Norway (RAVN). In this report, we present data for 2018 on resistance against agents for treatment of influenza, HIV-1 infection, hepatitis B virus infection. and human herpes virus infections. The reference laboratories at the Norwegian Institute of Public Health and at the Oslo University Hospital have submitted the data.

In this year's report, we have added a new chapter with a general introduction to antiviral treatment and drug resistance, including a presentation of the different drugs and drug classes. Hopefully, this will provide useful background knowledge when reading other parts of the report, particularly for new colleagues in the field.

In addition to the surveillance data, we have selected two relevant topics that are given special attention in the report, presented by invited authors:

- *National tender on HIV drugs:* This is a process administered by Norwegian health authorities aiming to reduce the cost of antiretroviral treatment of HIV through coordinated procurement of the drugs. The possible consequences of instructing clinicians to prescribe drugs based on an economically prioritized list of HIV treatment regimens are discussed from a drug resistance perspective.
- *New antiviral drugs against influenza*: Currently, oseltamivir is the only drug on the market in Norway, but new drugs are in the pipeline. An overview of new treatment options is presented, and their potential for drug resistance discussed.

Furthermore, we provide an update on the status of the ongoing work with resistance testing for hepatitis C virus (HCV): Drug resistance testing is currently not available in Norway. An assay based on next generation sequencing is being implemented at the NIPH.

It is our hope that the report contains valuable data and interesting perspectives for clinicians, microbiologists, other colleagues with an interest in infectious diseases, and for those developing diagnostic- and treatment guidelines and strategies to prevent transmission of viral infections.

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

Enjoy!

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Sammendrag

Bruk av antivirale midler

Ifølge data fra Reseptregisteret, fortsetter trenden fra tidligere år, nemlig at salget av antivirale medikamenter målt i definerte døgndoser (DDD) fortsetter å øke også i 2018. Til tross for en økning av salget, både målt i antall DDD og i antall behandlede pasienter, er kostnadene i 2018 for første gang redusert. Dette kan forklares av prisreduksjon på medikamenter mot hiv og hepatitt C virus (HCV). For medikamenter mot hiv og HCV har det vært en dreining fra medikamenter med ett virkestoff til kombinasjonspreparater. Denne endringen har vært spesielt tydelig for HCV de siste to år, hvor 95 % av pasientene i 2018 ble behandlet med kombinasjonspreparater mot 27 % i 2016 og ingen i 2014.

Influensavirus

Det ble ikke påvist influensavirus med resistens mot oseltamivir eller zanamivir i 2017/18-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.

Humant immunsviktvirus-1

Resistensmutasjoner som overvåkes ble påvist i 9.2 % av prøvene fra pasienter med nydiagnostisert hiv-1 infeksjon i Norge i 2018. Dette er en noe høyere andel enn foregående år, men det var i 2018 også færre prøver som ble undersøkt for resistens, og totalt færre nye tilfeller av hiv-1 i Norge. Prevalensen av overført resistens har vært stabil de siste årene med bare små variasjoner.

I 2018 var det utelukkende resistensmutasjoner assosiert med revers transkriptase-hemmere som ble påvist i overvåkningen av primærresistens (5.9 % non-nukleosid- og 5.0 % nukleosid revers transkriptasehemmere). Det ble ikke påvist noen mutasjoner assosiert med resistens mot proteasehemmere. For integrasehemmere er det ingen overvåkning av primærresistens.

Hepatitt B virus

I 2018 ble 20 pasientprøver sendt til referanselaboratoriet for hepatitt B virus (HBV) resistenstesting. Det ble påvist antiviral resistens i 4 av disse prøvene, og alle hadde mutasjoner assosiert med resistens mot entecavir. Blant pasienter der det primært var rekvirert genotyping (n=245), 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 2018 mottok referanselaboratoriet 27 prøver fra 22 pasienter for cytomegalovirus-resistenstesting. Resistensmutasjoner ble detektert hos to pasienter. De fleste tilfeller av terapisvikt skyldes ikke resistens mot antivirale midler.

For herpes simplex virus var det i 2018 kun fem prøver som ble analysert med tanke på resistens mot antiviralia, og resistens mot aciklovir ble påvist i en av disse prøvene. På tross av økt bruk av aciklovir, både i behandling og som profylakse, er resistens sjeldent.

Hepatitt C virus

Resistensundersøkelse av HCV er foreløpig ikke tilgjengelig i Norge, og det er heller ingen overvåkning av HCV-resistens i Norge. Prøver videresendes laboratorier i Sverige ved behov for resistensundersøkelser. En sekvenseringsbasert metode for resistens-bestemmelse av HCV er under utvikling ved FHI, og status for dette metodearbeidet er beskrevet i rapporten.

Summary

The usage of antivirals

According to The Norwegian Drug Wholesales statistics database, the sales of antiviral drugs measured in defined daily doses (DDDs) continued to increase in 2018. However, price reduction for some of the drugs used in treatment of HIV and hepatitis C virus (HCV) has resulted in a reduction in cost, despite the continuous increase in sales measured in DDDs and number of users. Both for HIV- and HCV-drugs, there has been a significant change in the pattern of use with a transition from single ingredient drugs to fixed combinations. In 2018, 95% of the patients treated for HCV used fixed combination drugs, a significant increase from 27% in 2016, and none in 2014.

Influenza virus

No mutations conferring resistance against oseltamivir or zanamivir were detected in the 2018/19 season. All circulating influenza strains are currently resistant to adamantanes. The reference laboratory does therefore not routinely analyze for adamantine resistance. In Norway, only oseltamivir is currently available for antiviral treatment of influenza.

Human immunodeficiency virus-1

Surveillance drug-resistance mutations (SDRMs) were detected in 9.2% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2018. This is a small increase compared to previous years, but the number of samples analyzed for primary drug resistance was lower in 2018 compared to 2017. The prevalence of transmitted drug resistance has been stable for the past few years with only minor variation. In 2018 only mutations associated with resistance against non-nucleoside- (5.9%) and nucleoside- (5.0%) reverse transcriptase inhibitors were detected, and no mutations affecting protease inhibitors were found.

Hepatitis B virus

In 2018, 20 patient samples were submitted for hepatitis B virus (HBV) antiviral resistance testing. Among these, only four cases of resistance were detected, all against entecavir. Among samples submitted primarily for HBV-genotyping (n=245), no drug resistance mutations were detected.

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

Human herpes viruses

In 2018, the reference laboratory received 27 samples from 22 patients for cytomegalovirus resistance testing. Resistance mutations were detected in samples from two patients. In most cases, therapy failure is not due to resistance against antiviral drugs.

Five herpes simplex samples were analyzed for antiviral drug resistance. A mutation associated with aciclovir resistance was detected in one of the samples. Despite an increasing consumption of aciclovir for both therapeutic and prophylactic treatment, treatment failure is rare, indicating a low frequency of drug resistance.

Hepatitis C virus

Resistance testing for HCV is currently not available in Norway, and there is no surveillance of drug resistant HCV in Norway. If HCV resistance testing is needed, samples are sent to laboratories in Sweden. A sequencing method for resistance testing of HCV is under development at the NIPH, and the status of this ongoing work is described in the report.

1 Antivirals and development of drug resistance

Antiviral drugs act by inhibiting viral replication, usually targeting a specific step in the virus' replication cycle. Most antiviral drugs are effective only against one particular virus or a group of viruses, and specific antiviral therapy is available only for a few viral infections. In principle, drugs may be designed to inhibit any step in the replication cycle of a virus. Most of the antivirals currently available work by inhibiting viral DNA- or RNA-synthesis, or by direct inhibition of other viral enzymes essential to the virus (1).

Drug resistance against antivirals is caused by genetic changes in the viral genome leading to amino acid alterations in the protein targeted by the drug, thereby affecting the activity of the drug. These genetic changes most commonly arise from random mutations. In addition, recombination or exchange of genetic material may also occur for certain viruses, for example antigenic shifts in influenza. Genetic alteration at a key site of the viral genome is usually a disadvantage for the virus, and most resistance mutations impair viral fitness. However, in the presence of antiviral drugs, resistant variants will have a fitness advantage over wild type virus. Resistant virus variants are therefore selected and may continue replication under these conditions. Compensatory mutations, further increasing viral fitness of the resistant variants, may then be selected by similar mechanisms. This may ultimately lead to the persistence of these variants even in the absence of antiviral drugs.

The risk of developing drug resistance varies significantly between different viruses, depending on factors such as mutation frequency and replication accuracy of the virus, viral load, fitness of mutated virus, and duration of both the infection and the treatment. Immunocompromised patients are at particular risk. Furthermore, different drugs have different genetic barriers, meaning that the number of mutations needed for development of resistance is different for different drugs.

Antivirals against influenza

There are two classes of antiviral drugs for treatment of influenza that are approved in Europe:

1) M2-inhibitors: blocks the M2 ion channel of influenza A virus, thereby inhibiting the early stages of virus replication. No effect on influenza B. (examples: amantadine and rimantadine)

2) Neuraminidase inhibitors: Neuraminidase inhibitors are effective during the last stage of the replication cycle, inhibiting the release of newly formed virus particles. Normally, hemagglutinin on the surface of the virus binds to sialic acid on the cell surface. The virus is released after the viral enzyme neuraminidase cleaves residues on the sialic acid, thus destroying this binding. Neuraminidase inhibitors bind to neuraminidase on the surface of influenzavirus A and B, preventing cleavage of sialic acid and thereby preventing release of the virus from the surface of the host cell (2). (examples: oseltamivir and zanamivir)

Oseltamivir is however the only antiviral drug against influenza currently on the market in Norway. All circulating influenza strains are currently resistant to the two M2-inhibitors, and these drugs are no longer used for treatment of influenza. Zanamivir is still registered but was withdrawn from the marked in 2016 due to limited use.

New drugs are under development, and several are approved for treatment of influenza in the USA and Japan. The first polymerase inhibitor targeting endonuclease (baloxavir

marboxil) was recently approved by FDA in the USA and is expected to be available in Europe in 2020. The drug targets the endonuclease function of influenza RNA polymerase and inhibits viral replication by preventing transcription of viral mRNA.

Drug resistant influenza

Resistance can develop in different ways. Most often, the resistant form develops by de novo mutations and may be selected when an antiviral substance is present at suboptimal concentrations, or when the virus is not fully sensitive. For immunocompromised patients and children, the risk of drug resistance during treatment is increased. However, drug resistant influenza virus may also develop in the absence of antiviral agents as long as the mutation that confers resistance does not cause any significant evolutionary disadvantage for the virus. The largest outbreak of such a virus was in 2007, when a resistant H1N1 virus completely replaced the wildtype virus within one year after its first occurrence. Resistance may 'hitch-hike' on another advantageous feature that promotes one virus strain over others, such as fitness-enhancing mutations at other genomic sites (3). Furthermore, reassortation of the segmented genome may rapidly lead to major genetic changes that could involve domains of importance for drug resistance characteristics.

Antivirals against HIV

There are five different classes of antiretroviral drugs used in the treatment of HIV-infection, targeting different phases of HIV's lifecycle:

1) Entry inhibitors: CCR5 blockers that block the binding between viral gp120 and the chemokine receptor CCR5 (example: maraviroc). Fusion inhibitors preventing fusion between the viral gp41 and the cell membrane (example: enfuvirtide), are no longer registered.

2) Nucleoside reverse transcriptase inhibitors (NRTI): Analogues of naturally occurring deoxynucleotides that are incorporated into the viral DNA chain in competition with the natural substrate. When incorporated, the drug stops further elongation of the viral DNA chain (chain termination), thereby inhibiting transcription of RNA into DNA (examples: abacavir, lamivudine, emtricitabine, tenofovir, and zidovudine).

3) Non-nucleoside reverse transcriptase inhibitors (NNRTI): Bind to the reverse transcriptase, thereby inhibiting transcription of RNA into DNA (examples: rilpivirine, etravirine, nevirapine, and efavirenz).

4) Integrase inhibitors: Prevent integration of pro-viral DNA into the host cell DNA (examples: dolutegravir, raltegravir, and elvitegravir).

5) Protease inhibitors: Bind to the protease, thereby preventing the cleavage of polyproteins in the maturing virus particle (examples: darunavir, atazanavir, and lopinavir). Effect is improved by addition of a pharmacokinetic enhancer (ritonavir or cobicistat).

In antiretroviral therapy (ART) for HIV-1, combinations of at least two drugs from different classes are used in order to reduce the risk of drug resistance. Currently recommended first line regimens consist of an integrase inhibitor in combination with two NRTIs. Alternatively, a boosted protease inhibitor or an NNRTI may replace the integrase inhibitor. Fixed-dose combination drugs are widely available.

Drug resistant HIV

HIV has a very high mutation rate and a considerable risk for development of resistant variants, mainly due to inaccuracy in viral replication and the lack of proofreading of the viral enzyme reverse transcriptase. There is vast genetic variation in the HIV-1 genome, and each patient harbors a mixture of coexisting genetic variants. This genetic variation increases over the course of the infection. Drug resistant viruses may evolve from wild-type viruses if viral replication persists during antiretroviral treatment. Because most drug resistance mutations impair viral fitness, wild type virus often rapidly reemerge when treatment is interrupted, but not always. Drug resistance rarely occurs without previous drug exposure, but individuals carrying virus with resistance mutations may transmit this virus to others. Drug resistance emerging during antiviral treatment is called acquired drug resistance. Drug resistance detected in previously untreated persons is usually transmitted from a person with acquired drug resistance, either directly, or through intermediates. The term transmitted drug resistance is used when previously uninfected individuals are infected with virus that has drug resistance mutations (4).

Antivirals against hepatitis B virus

Only one class of antivirals is used for treating chronic hepatitis B infection:

1) Nucleoside/nucleotide analogues (NAs): Analogues of naturally occurring deoxynucleotides that are incorporated into the viral DNA chain in competition with the natural substrate. When incorporated, the drug stops further elongation of the viral DNA chain (chain termination), thereby inhibiting transcription of RNA into DNA by the HBV polymerase. (examples: entecavir, tenofovir disoproxil, and tenofovir alafenamide)

The activity of the HBV polymerase is similar to that of HIV reverse transcriptase, and several of the NAs have activity against both viruses. Currently, monotherapy with entecavir or tenofovir is recommended as first-line treatment, given their antiviral potency and favorable resistance profile (5). Another treatment option is interferon therapy, which works by enhancing the host immune response. Although interferon-based treatment strategies confer an opportunity for seroconversion its current use in treatment is limited, mainly due to considerable side effects.

Drug resistant HBV

The mutations associated with HBV drug-resistance are located in the reverse transcriptase domain of the HBV polymerase, and lead to reduced inhibitory effect of the drug on the viral polymerase. Aside from reducing the virus's sensitivity to the drug, primary mutations often simultaneously reduce viral fitness. Compensatory resistance mutations restoring replication capacity, and secondary resistance mutations increasing drug resistance, may arise after the emergence of primary resistance mutations. Drug resistant HBV may develop under antiviral treatment but is rarely (if ever) transmitted. Reported resistance in HBV is mainly towards the less potent drugs lamivudine and adefovir, which have a low genetic barrier to resistance compared to tenofovir and entecavir. For entecavir, several mutations are required to confer drug resistance. Resistance to entecavir may still occur, but it is rare. For tenofovir, only a few cases of clinically significant resistance are described worldwide, all of them as part of multidrug resistance (6). Because of the rarity of resistant cases, the relevant mutation sites for tenofovir-resistance are not fully confirmed.

Antivirals against cytomegalovirus (CMV)

Only one class of antivirals is used for treating CMV-infection:

1) Nucleoside analogues (NAs): Analogues of naturally occurring deoxynucleotides that are incorporated into the growing strand of viral DNA by CMV polymerase (UL54), causing termination of the growing viral DNA strand (chain termination). Drugs of choice: Ganciclovir or its prodrug valganciclovir.

Ganciclovir and valganciclovir are the drugs of choice since they are quite effective in inhibiting virus replication and have few side effects. To become active, ganciclovir is monophosphorylated by the CMV UL97 kinase and then di- and tri-phosphorylated by cellular kinases. Cidofovir and foscarnet are also incorporated by the CMV-DNA polymerase but work independently of the CMV kinases. Because they do not require activation by viral enzymes, their action is not limited to infected cells. These drugs have more side-effects and are used only in special situations such as CMV retinitis or retinal necrosis.

Some new anti-CMV-drugs are in clinical trials. Letermovir binds to and inhibits the CMV-DNA terminase complex which is involved in cleaving and packaging of CMV-DNA genome into the capsid. The drug is approved by both the FDA and the European Medicines Agency for prophylactic use after stem cell transplantation and is already available in Norway. Maribavir, a UL97-kinase inhibitor, has been used in clinical trials with favorable outcomes but is not yet approved by the FDA.

Drug resistant CMV

During ganciclovir anti-CMV therapy, resistance mutations usually develop after a cumulative exposure of six weeks or more. Since ganciclovir has two points of interaction with CMV, two main types of resistance mutations arise. Resistance mutations are usually first seen in the UL97 kinase gene. The UL54 (DNA- polymerase) mutations tend 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 uncommon.

Antivirals against herpes simplex virus (HSV)

Only one class of antivirals is used for treating HSV-infection:

1) Nucleoside analogues (NAs): Analogues of naturally occurring guanosine that are incorporated into the growing strand of viral DNA by HSV DNA polymerase (UL30), causing termination of the growing viral DNA strand (chain termination). Drugs of choice: aciclovir or its prodrug valaciclovir.

To be effective, aciclovir has to be triphosphorylated, first by a viral thymidine kinase (TK) and then by the cellular kinases to the active aciclovir -triphosphate. Aciclovir and valaciclovir are effective against both HSV-1 and HSV-2, as well as varicella zoster virus. Penciclovir is available as ointment for topical treatment of herpes labialis. Second line drugs include foscarnet and cidofovir.

Drug resistant HSV

Resistance to aciclovir develops by mutations of either the HSV-TK- or HSV DNA polymerase gene. Mutations in HSV-TK are by far the most common, and about 95% of the resistance mutations are localized in the thymidine-kinase gene (UL23) whereas 5% are localized in the DNA-polymerase gene (UL30) (7).

Cidofovir and foscarnet are independent of HSV-TK and therefore active against most of the strains that are resistant to aciclovir. 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 (8).

Antivirals against hepatitis C virus (HCV)

Until 2011, hepatitis C virus (HCV)-therapy was based on a combination of pegylated interferon and ribavirin for 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 direct acting antiviral drugs (DAA) targeting HCV entered the market: sofosbuvir (SOF); a pangenotypic polymerase inhibitor, simeprevir; a second-wave protease inhibitor, and daclatasvir; a pangenotypic NS5A (nonstructural protein 5A) inhibitor. Most of the first DAAs were used for a limited period of time in Norway, before newer substances took over the market after a few years.

There has been a rapid development of new and better drugs against HCV over the last few years, replacing the early generations of direct acting antivirals. There are now several pangenotypic combination tablets available, with high genetic barriers to resistance and excellent treatment responses. The goal of HCV therapy is to cure the infection. Treatment is usually given over 8-12 weeks, and most patients obtain sustained virological response (defined as absence of viremia) 12 or 24 weeks after completion of treatment (9).

There are currently four main groups of DAA against HCV (10):

1) Nucleoside analogue polymerase inhibitors (NS5B): Compete with nucleosides for the active site of the HCV polymerase (example: sofosbuvir).

2) Non-nucleoside analogue polymerase inhibitors (NS5B): Alter the shape of the polymerase and thus inhibit replication of HCV (example: dasabuvir).

3) Protease inhibitors (NS3/4A): Target the active site of the protease enzyme, inhibiting proteolysis of the HCV polyprotein. Genotype specific. (example: voxilaprevir, grazoprevir).

4) NS5A inhibitors: Target the proteins encoded by the NS5A region of the virus genome, thereby affecting the replication, assembly and release of the virus (examples: velpatasvir, ledipasvir).

Drug resistant HCV

Similar to HIV, HCV exhibits considerable genetic variation. The HCV RNA polymerase is relatively inaccurate and lacks proofreading, leading to a high mutation rate. As a result, a single infected person may harbour a vast population of variants, or quasispecies, dominated by the variants with the best viral fitness. Some of these random mutations may lead to amino acid substitutions associated with reduced susceptibility to antiviral drugs, called resistance-associated substitutions (RASs). The RASs can be present prior to

treatment, or they may develop during treatment. Continued replication under antiviral pressure increase selection of viruses with RASs. The clinical significance of the different RASs is variable, and the presence of a RAS does not necessarily predict treatment failure. After interruption of treatment, most RASs are reversed. However, some RASs may persist also in the absence of antiviral drugs, affecting future treatment options.

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2 The usage of antivirals in Norway

In the last decades, the development of new specific antivirals has accelerated, especially due to development of new drugs against HIV (1) and hepatitis C virus (HCV). The sales of direct acting antiviral drugs (DAA), measured in both defined daily doses (DDDs) and number of patients treated have been increasing every year (Figure 2.1 and Figure 2.2, respectively), and the introduction of new antivirals for treatment of HCV infections has highly contributed to increased costs. Recent price reduction for some of the drugs used in treatment of HIV and HCV has however resulted in reduced costs despite continued increase in sales. According to The Drug Consumption in Norway 2014-2018 (2), the cost of anti-infectives for systemic use (Anatomical Therapeutic Chemical Classification (ATC) group J) was, for the first time in the five-year period, reduced in 2018. This is mainly due to reduced costs for the DAA (J05).

For HIV drugs, sales measured in number of DDDs have been relatively stable in recent years, apart from a slight increase in 2018. There has however, been a significant change in the pattern of use with a transition from single ingredient drugs to fixed combinations. This has resulted in the use of fewer pharmaceutical products per person per day, as illustrated in Figure 2.1.

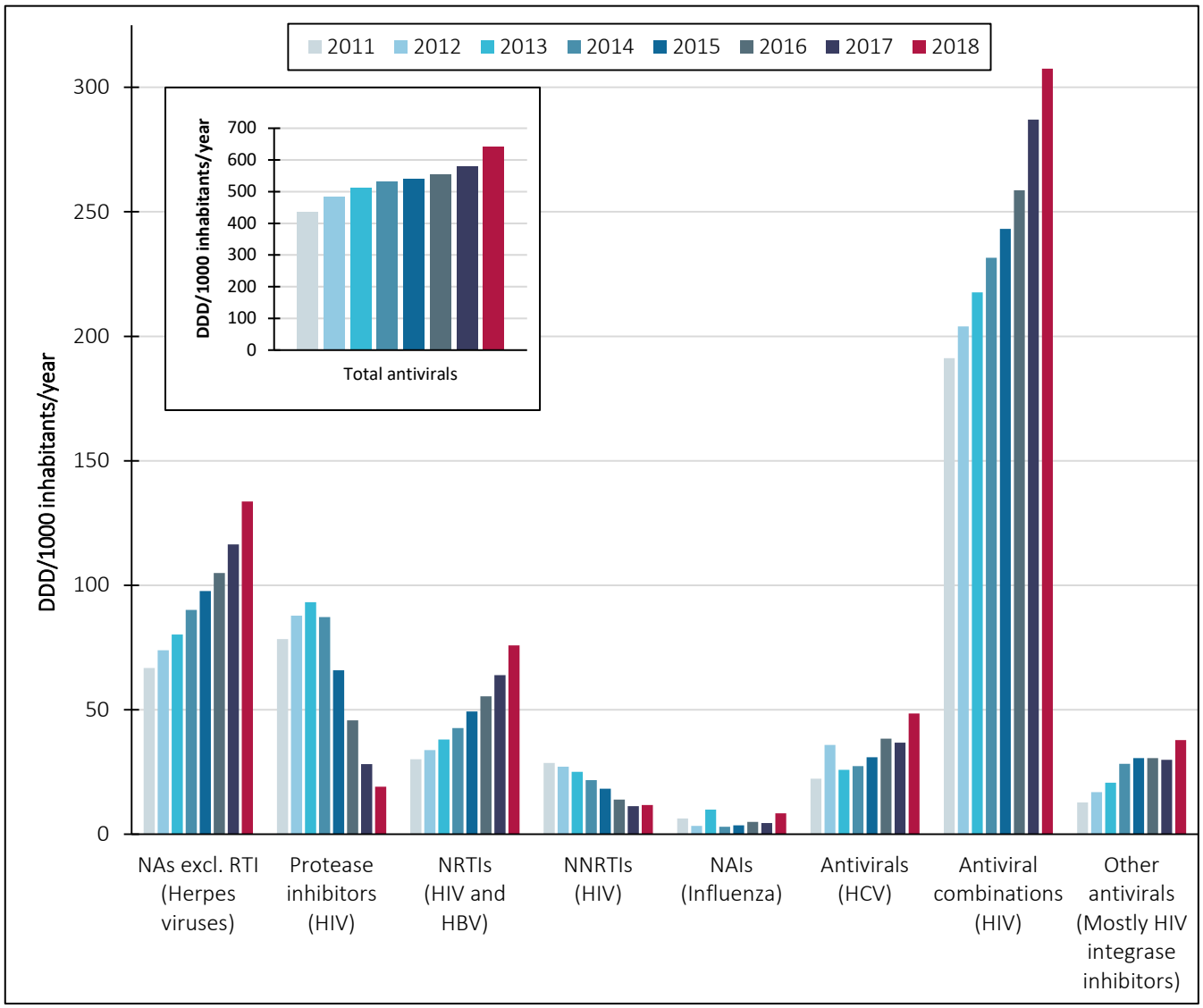

Figure 2.1: Sales of direct acting antiviral drugs for systemic use (ATC group J05A) for 2011- 2018 (3).

The figure shows the sales of direct acting antiviral groups over time. The numbers are given as defined daily doses (DDD) per 1000 inhabitants per year. NA excl. RTI: Nucleosides and nucleotides excl. reverse transcriptase inhibitors (J05AB); Protease inhibitors (J05AE); NRTIs: Nucleoside and nucleotide reverse transcriptase inhibitors (J05AF); NNRTIs: Non-nucleoside reverse transcriptase inhibitors (J05AG); NAIs: Neuraminidase inhibitors (J05AH); Antivirals, HCV: Antivirals for treatment of HCV infections (J05AP); Antiviral combinations, HIV: Antivirals for treatment of HIV infections, combinations (J05AR) and Other antivirals (J05AX). A plot illustrating the total sales of antivirals in ATC group J05A in Norway is inserted in the main plot. The total numbers also include phosphonic acid derivatives (J05AD) used against herpesviruses, due to low numbers this is not included in the main plot.

Number of persons treated with different DAAs has been increasing for all the different virus infections since 2011, except influenza (Figure 2.2). Antivirals used for treatment of HIV dominate when sales are measured in number of DDDs (Fig. 2.1), while DAAs against herpesviruses are by far the most used antivirals when measured in number of users.

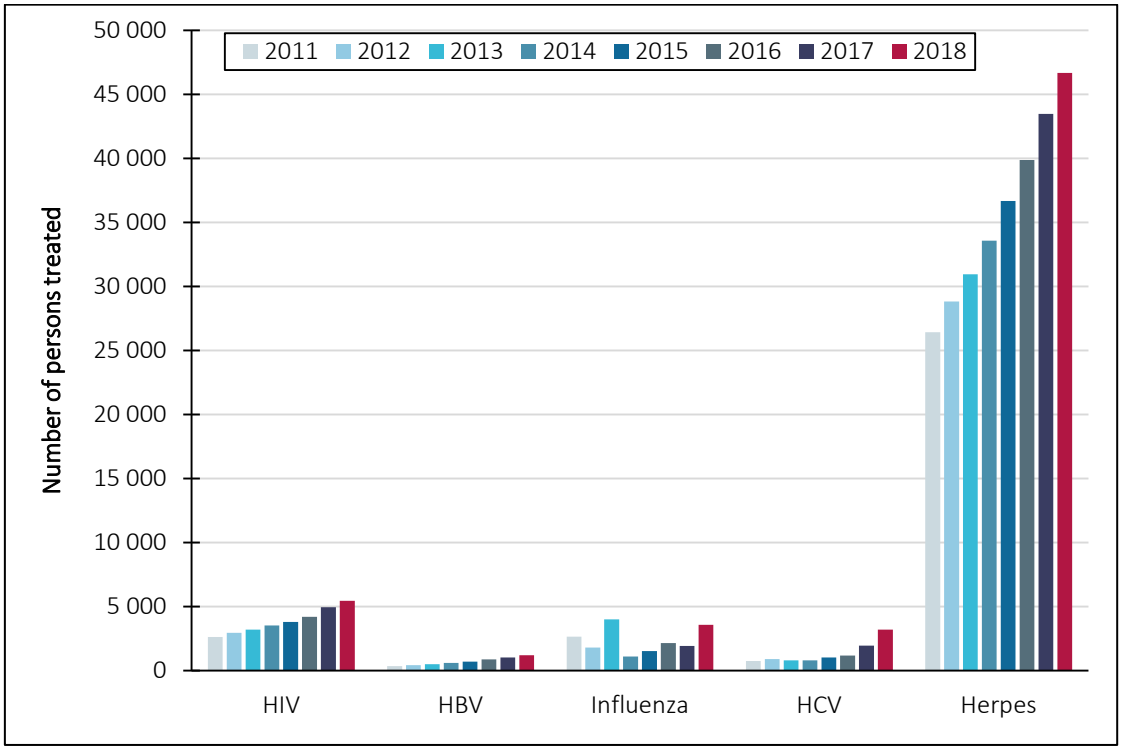

Figure 2.2: Trends in the use of direct acting antiviral drugs for systemic use (ATC group J05A) grouped by virus for 2011-2018 (3).

The figure shows the number of persons treated for different viruses with systemic direct acting antivirals over time. The number of persons treated is based on the number of patients given at least one prescription per year. HIV: All HIV pharmaceuticals (ATC-group J05AF05: Lamivudine, Zeffix is excluded); HBV: All HBV pharmaceuticals (ATC-group J05AF05: Lamivudine, Epivir is excluded). Single component drugs approved for both HBV and HIV are included in the HBV numbers only; Influenza: ATC-group J05AH: Neuraminidase inhibitors; HCV antivirals: ATC-group J05AP; Herpes: aciclovir (J05AB01), ganciclovir (J05AB06), famciclovir (J05AB09), valaciclovir (J05AB11), cidofovir (J05AB12) and foscarnet (J05AD01).

Influenza virus

The usage of antivirals for the treatment of influenza (ATC group J05AH), is shown in Table 2.1. The variations in the number of users of DAAs for treatment of influenza are probably related to the size and intensity of the seasonal influenza epidemic each year, the accuracy of the yearly influenza vaccine, and the proportion of the population vaccinated. Due to limited use, zanamivir was withdrawn from the marked in 2016; consequently, oseltamivir is the only neuraminidase inhibitor available for treatment of influenza in Norway.

Table 2.1: Number of individuals with at least one prescription of neuraminidase inhibitor (ATC group J05AH) per year (3)

	2014	2015	2016	2017	2018
Zanamivir	18	52	25		
Oseltamivir	1 080	1 477	2 129	1 923	3 571

Human immunodeficiency virus

There are currently 29 antiretrovirals, defined by 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 2014 to 2018. Figure 2.3 shows the trends in use measured in number of persons treated by the 10 most frequently used drugs in 2018.

During the whole period, almost 98% of treated persons received combination drugs containing more than one active substance. In 2018, more than 5400 persons in Norway retrieved prescriptions for a fixed combination drug. Single substance drugs can be given in addition to the fixed combination for some patients.

Tenofovir disoproxil, adefovir dipivoxil and emtricitabine are approved for treatment of both HIV and HBV infections, but the single substance drugs are rarely used for HIV therapy. The users of these drugs are therefore neither included in the total number of users of HIV treatment nor in the different groups in Figure 2.3. The sum of the patients using the different drugs is higher than the total number of patients treated with HIV drugs in Figure 2.2. This is because some patients receive more than one drug or may change treatment regimens during a year.

There is a range of drugs approved for treatment of HIV in Norway. The use of the different drugs has shifted in the last five-year period, as new drugs have been introduced and taken over for older drugs. An example of this is the newer integrase inhibitors replacing the older non nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors. This is illustrated in Figure 2.3 and Figure 2.4 The increased use of elvitegravir and dolutegravir, seems to correspond to a concurrent decrease of efavirenz, nevirapine, atazanavir, and lopinavir. Furthermore, several new fixed-dose combination drugs have been introduced, contributing to considerable changes in prescription patterns. It is expected that this trend will continue.

The fixed combination of emtricitabine and tenofovir disoproxil (TDF) has been the combination drug most used in recent years. A small decrease was seen in 2015 and 2016 before the use again increased in 2017. In 2016, this combination was approved as Pre-Exposure Prophylaxis (PrEP) to reduce the risk of sexually acquired HIV-1 infection in adults at high risk and given, with full reimbursement of the costs. This may explain the increased number of patients retrieving at least one prescription of this fixed combination in 2017. The fixed combination of emtricitabine and TDF is the only TDF-containing combination that was increasing in 2018, and 2130 persons were given at least one prescription of this combination this year. This corresponds to an increase of more than 30% compared to 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). A second 4-component combination (emtricitabine + TAF + darunavir + cobicistat) was introduced in 2018. TAF is given in lower doses and has a greater bioavailability in relevant body tissues than TDF. The increased use of the new TAF-containing combinations started in 2017 and has continued in 2018, while the use of TDF combinations other than the combination approved for PrEP, is reduced.

Since 2014, three new 3-component combinations and three 4-component combinations (emtricitabine + TDF + elvitegravir + cobicistat, emtricitabine + TAF + elvitegravir + cobicistat and emtricitabine + TAF + darunavir + cobicistat) have been introduced. Of the combinations introduced after 2013, the most commonly prescribed drug the last three years is the 3-component combination lamivudine + abacavir + dolutegravir, introduced in 2014.

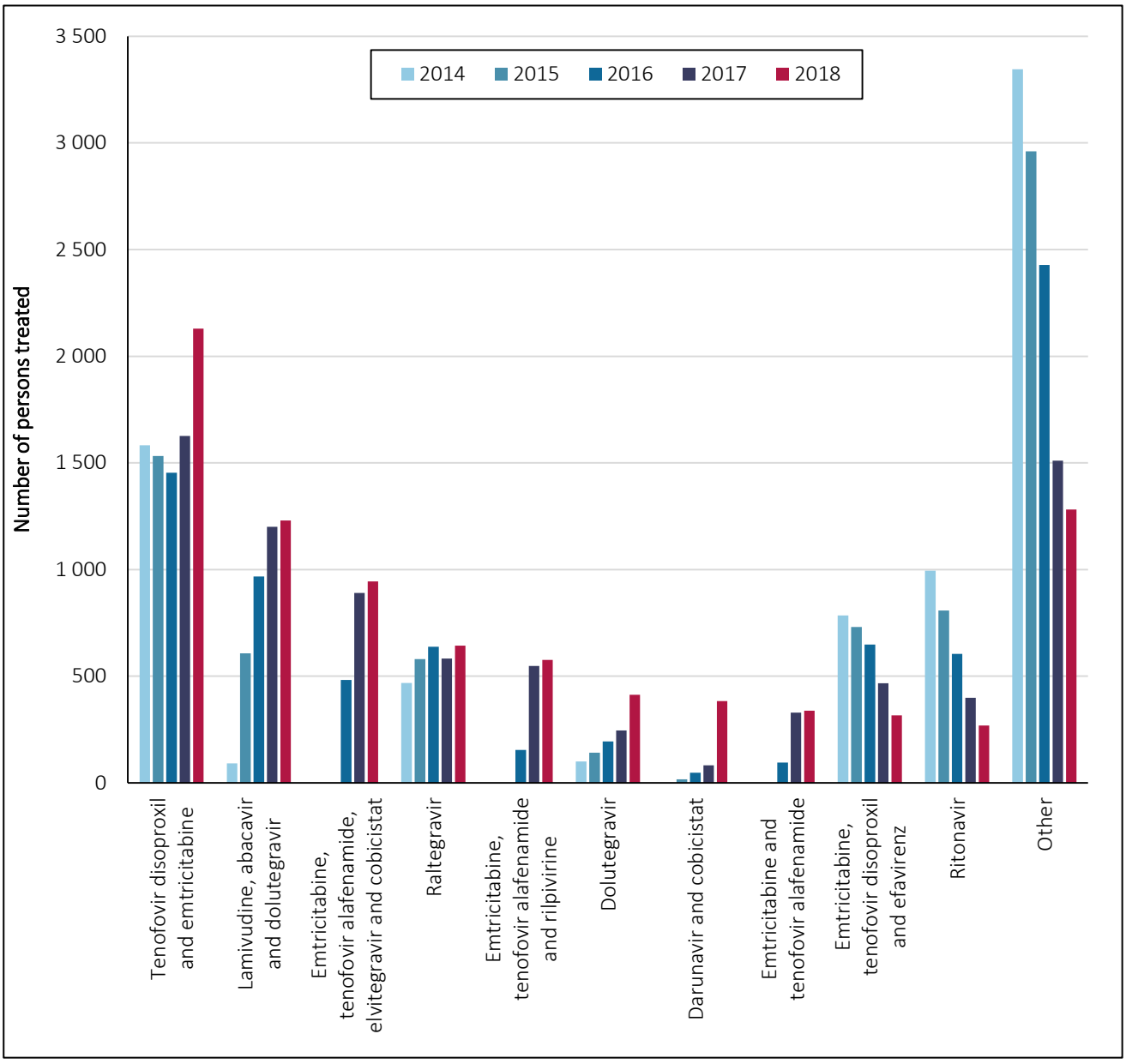

Figure 2.3: Trends in the use of antivirals for treatment of HIV in the period 2014-2018, number of persons treated (3).

The figure shows the trends in antiviral use for the treatment of HIV. The 10 most used drugs in 2018 are presented in the plot. The "other-group" is resolved in Figure 2.4. Number of persons treated is defined as the number of patients given at least one prescription per year.

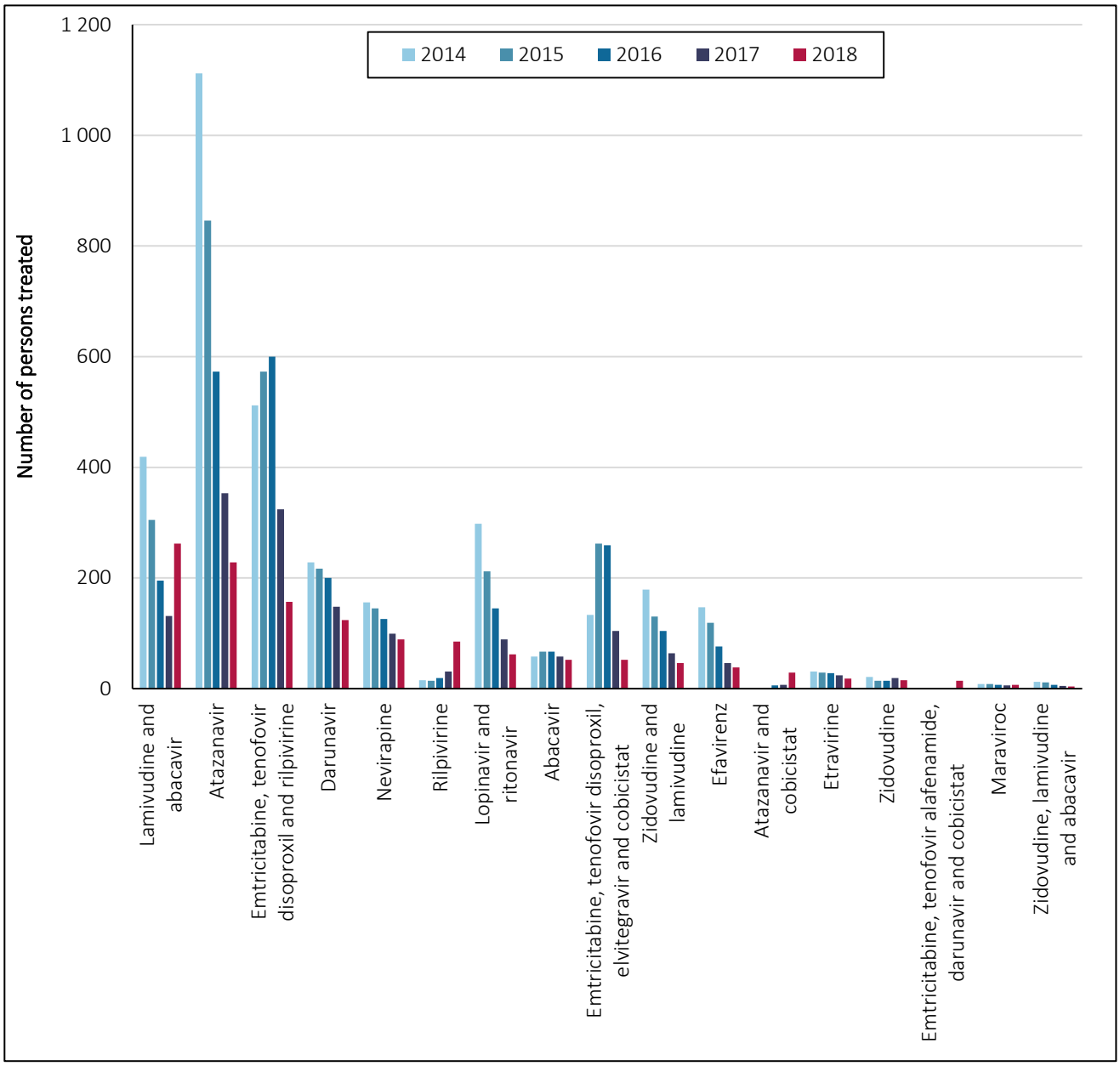

Figure 2.4: Trends in the use of antivirals for treatment of HIV in the period 2014-2018, number of persons treated, continued (3).

In this plot the "other-group" from Figure 2.3 is resolved. Saquinavir and didanosine were not prescribed in 2018 and are excluded from the figure.

Only 3 of the 10 most used drugs in Figure 2.3 are single component drugs while there is a range of different single component drugs included in the “other group” (Figure 2.4). The NNRTI rilpivirine and the integrase inhibitors dolutegravir and raltegravir are the only single component drugs that are increasingly used. Rilpivirine is also included in several fixed combinations. The use of the combination with tenofovir disoproxil and emtricitabine has decreased in the five-year period while the combination with tenofovir alafenamide and emtricitabine has taken over the market and is among the 10 most used drugs in 2018. Also, dolutegravir is increasingly used both as a single component drug and in the fixed combination with the nucleoside reverse transcriptase inhibitors (NRTI)

lamivudine and abacavir. Both drugs are among the 10 most used antiretroviral drugs in 2018 measured by the number of persons treated.

The number of prescriptions per active ingredient over time is shown in Figure 2.5.

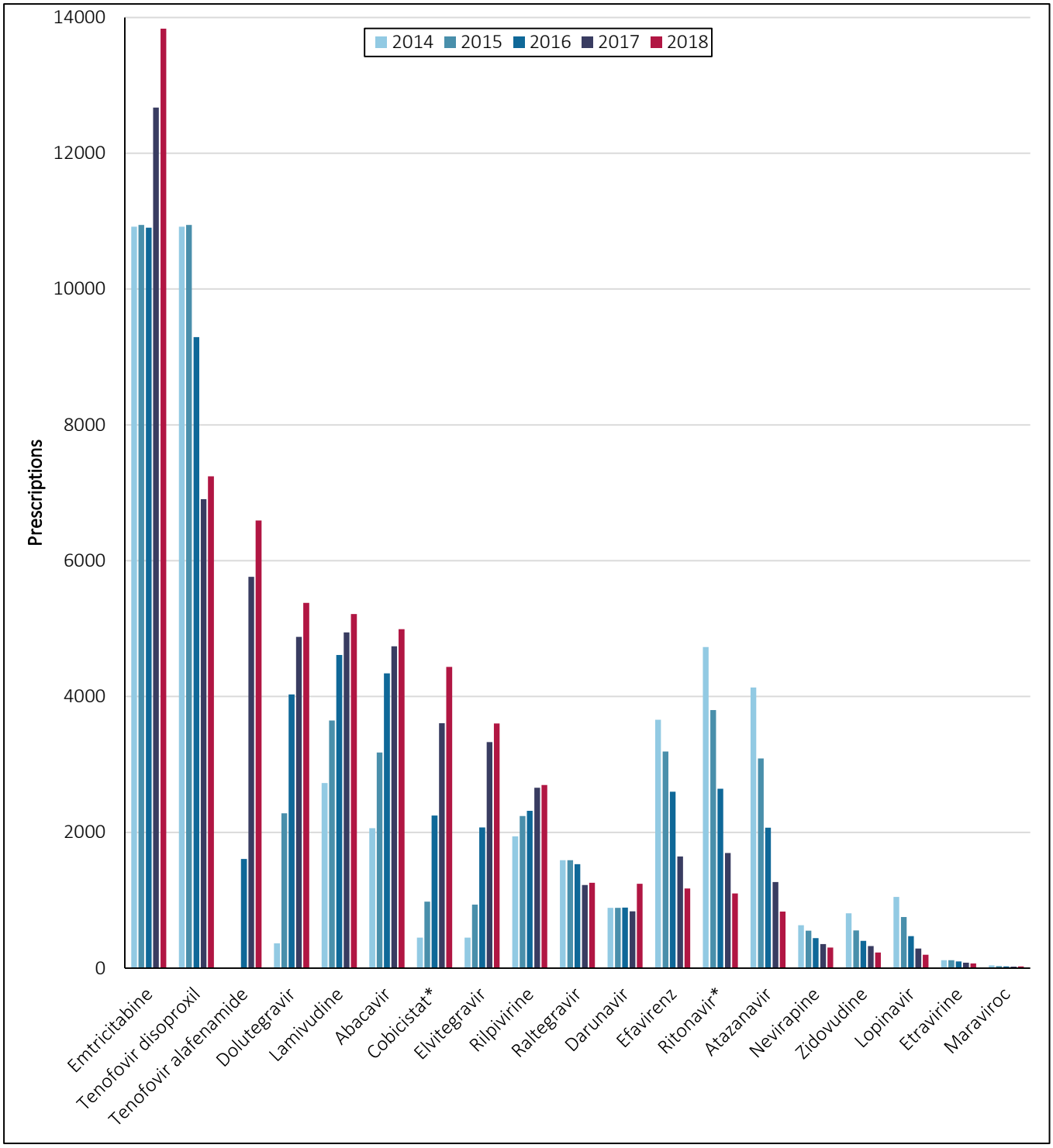

Figure 2.5: Number of prescriptions per active ingredient (3)

This plot shows number of prescriptions per active ingredient over time. Many prescriptions contain more than one active ingredient; these prescriptions are counted several times. Saquinavir and didanosine were not prescribed in 2018 and are excluded from the figure.

* Ritonavir and cobicistat are almost exclusively used as boosters for other antiretrovirals.

Hepatitis B virus

There are currently seven approved therapies for HBV infection including one interferon based and six nucleoside/nucleotide analogues (NAs). Treatment of HBV with antivirals is generally given as monotherapy. The use of the NAs is shown in Figure 2.6.

The data is based on the annual number of patients retrieving at least one prescription per year for the period 2014-2018. Lamivudine, adefovir dipivoxil, tenofovir disoproxil (TDF) and emtricitabine are approved for both HBV and HIV, while entecavir, telbuvidine (withdrawn in 2016) and tenofovir alafenamide (TAF), as a single substance drug, are approved for HBV only. An estimate of the number of patients treated with antivirals against HBV in Norway will therefore be in the range of 547-1198 in 2018. 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 six NAs (excluding lamivudine approved for HIV only).

The number of persons treated has been increasing during the last five 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 NAs, almost 99% received one of these three drugs in 2018. The introduction of TAF may further influence the pattern of use of anti-HBV drugs the next years.

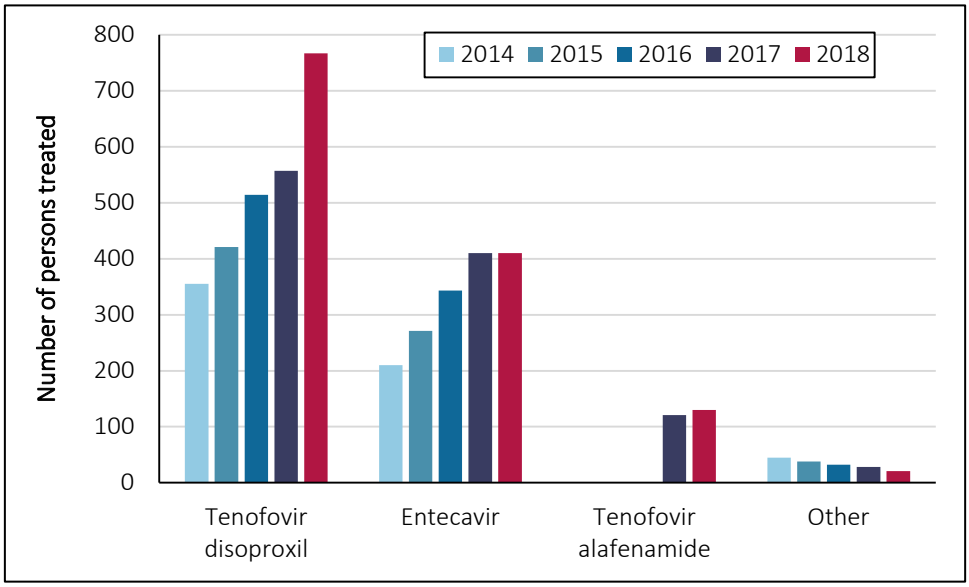

Figure 2.6: Trends in the use of antivirals for treatment of HBV for the period 2014-2018 (3)

This figure shows the trends in antiviral use for the treatment of HBV over time. Number of persons treated is defined as the number of patients given at least one prescription per year. Other: lamivudine (J05AF05), adefovir dipivoxil (J05AF08), emtricitabine (J05AF09) and telbivudine (J05AF11).

Human herpesviruses

Figure 2.7 shows the two most prescribed drugs for systemic use 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. Valaciclovir is the substance most commonly prescribed and the use of this drug is steadily increasing. The use of aciclovir has been stable during the five-year period. Ganciclovir and famciclovir have rarely been prescribed in this period.

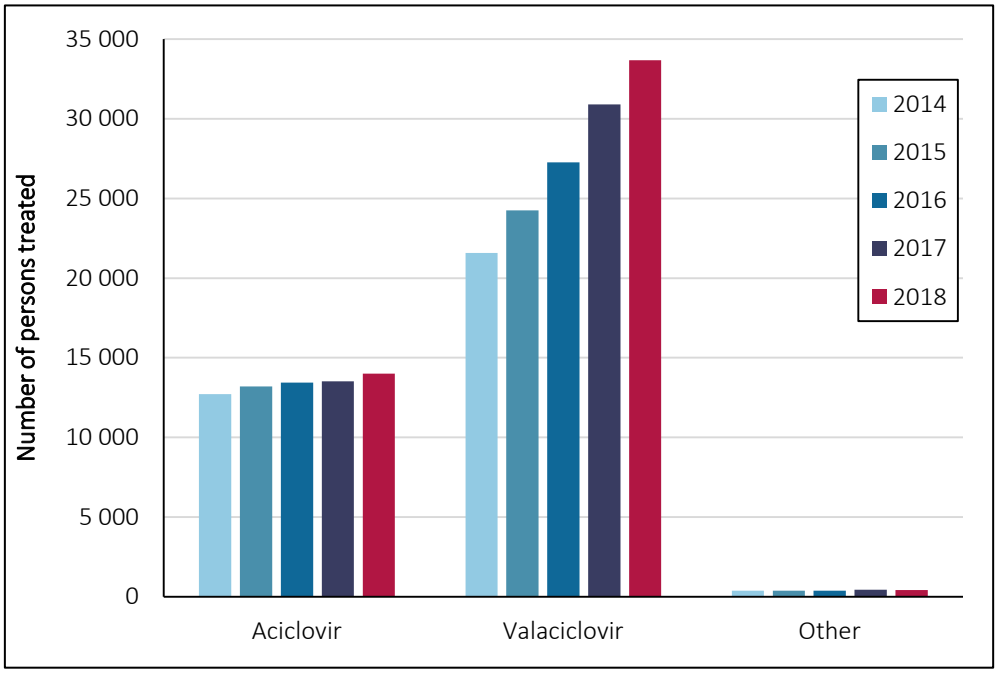

Figure 2.7: Trends in the use of antivirals for treatment of human herpes virus infection for the period 2014-2018 (3)

This figure shows the trends in direct acting antiviral use for treatment of human herpesviruses over time. Number of persons treated is defined as the number of patients given at least one prescription per year. Other: vidarabine (J05AB03), ganciclovir (J05AB06), famciclovir (J05AB09) and valganciclovir (J05AB14).

Creams for topical treatment of herpes simplex virus infections of the lips and face (herpes labialis) are available in Norway. Aciclovir and penciclovir are the active ingredients in these creams. Small packages of aciclovir cream were made available for over the counter sales in 2006. This resulted in a steep increase in use the next couple of years. Since then the consumption has been quite stable (Table 2.2).

Table 2.2: Sold packages of dermatological antivirals containing aciclovir (D06BB03) and penciclovir (D06BB06) (3)

Active ingredient	2014	2015	2016	2017	2018
Aciclovir	200 469	201 829	206 447	205 818	212 393
Penciclovir	29 809	27 726	30 122	24 062	18 957

Most packages contain 2 g of cream; the exception is a 5 g package with aciclovir as the active ingredient where prescription is needed. Approximately 90 % is nonprecribed medications.

Hepatitis C virus

The overall number of patients on treatment has increased during the last five years with the new drugs on the market. The number of persons who had at least one prescription for a HCV drug (except interferons) dispensed was 3189 in 2018, an increase by more than 63% from 2017. The general trend is that fixed combinations of two or more active ingredients replace single component drugs as shown in Figure 2.8. In 2018, 95% of the patients treated for HCV used fixed combination drugs, a significant increase from 27% in 2016, and none in 2014.

Recommended treatment protocols for HCV-infection depend on both genotype and stage of liver disease. Norwegian treatment guidelines HCV from the Norwegian Medical Association (NMA) have recently been updated, but the latest update was not yet available in 2018 (4). However, the recommendations from the Procurement services for Health Enterprises Ltd probably have an even greater impact on the choice of drugs for treatment of HCV (5). These recommendations are similar but not identical to the NMA guidelines.

The combination of sofosbuvir (SOF) (NS5B inhibitor) and velpatasvir (VEL) (NS5A inhibitor) is the most used drug in 2018. This was one of the combination therapies recommended by the Procurement services for Health Enterprises Ltd in 2018 and is one of the three pangenotypic fixed combinations with high treatment response. The others are the combination glecaprevir (protease inhibitor)/ pibretasvir (NS5A inhibitor) (GLE/PIB), and he triple combination SOF/VEL/voxilaprevir (VOX, protease inhibitor), both introduced in 2018. The second most used combination in 2018 was the fixed dose combination of elbasvir and grazoprevir. This was one of the combinations recommended by the 2018 procurement for treatment of most patients with HCV genotype 1. The trends of use shown in Figure 2.8 probably reflect the change in national recommendations for treatment of HCV in the five-year period, and the results of the procurement the last few years.

“The National strategy against hepatitis 2018-2023" (6) has two primary objectives: To reduce the prevalence of HCV by 90% by the end of 2023, and that no one in Norway should die or suffer serious illness caused by HCV. It will be interesting to follow the trend in the consumption of HCV drugs over the next few years, both with regard to number of persons treated and the agents used. The usage of DAAs is expected to change further in the coming years, both because new HCV medicines are likely to be authorized, and because of possible changes in treatment guidelines and reimbursement rules.

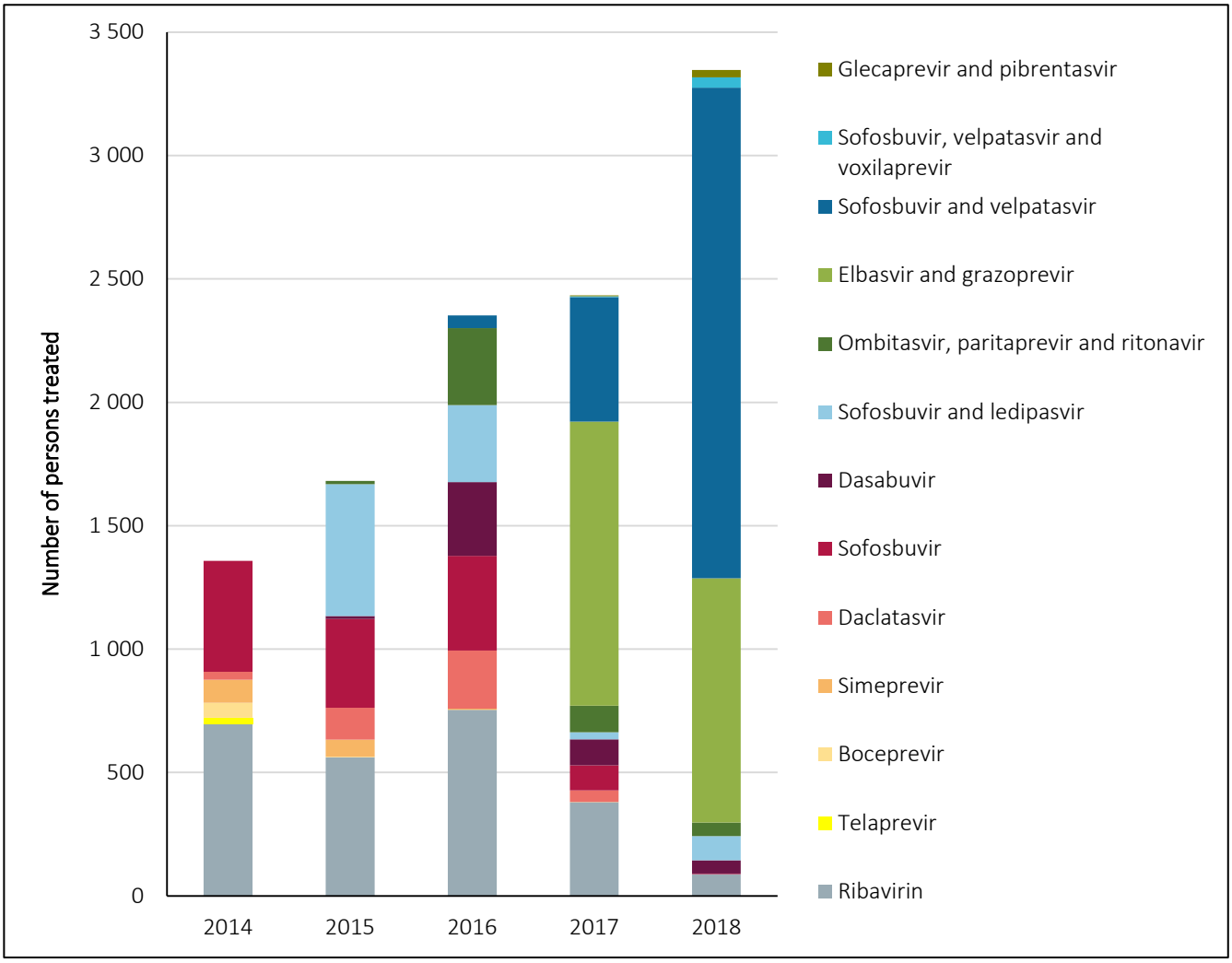

Figure 2.8: Trends in the use of antivirals for treatment of HCV for the period 2014-2018 (3)

This figure shows the trends in the use of direct acting antivirals for treatment of HCV over time. Number of persons treated is defined as the number of patients given at least one prescription per year.

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3 Influenza virus

Fact box: Influenza virus drug resistance	
Treatment	-Neuraminidase inhibitor: oseltamivir
Resistance testing method	Genotypic by pyrosequencing or Sanger sequencing Phenotypic by neuraminidase susceptibility assay (MUNANA) The WHO national reference laboratory for influenza, Norwegian Institute of Public Health, performs influenza drug resistance testing in Norway
Target gene	Neuraminidase (Because adamantanes are not used in Norway, the matrix gene is currently not regularly screened for resistance.)
Indication for resistance testing	- Patients treated with 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. - Surveillance
Surveillance	Screening for resistance as part of the national influenza surveillance program, which involves samples from both untreated and treated patients. There is currently no active systematic surveillance for treatment-induced resistance.

Surveillance methods

The WHO national reference laboratory for influenza in Norway is located at the Norwegian Institute of Public Health and monitors the occurrence of influenza viruses in Norway. A volunteer network of sentinel physicians in all parts of the country provide samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit a subset of confirmed influenza strains. Samples from both untreated and treated patients in the community are included. In order to facilitate detection of emergence and spread of viruses with resistance, there is a particular focus on samples from patients without known exposure to antiviral drugs.

Surveillance data 2018

Throughout the season (week 20 2018 to week 37 2019), 381 viruses have been analysed for resistance to the influenza antiviral drug oseltamivir and 215 for zanamivir. This represents in general 15% of all influenza positive specimens received by the influenza laboratory at NIPH for further analysis. No virus with resistance to neuraminidase inhibitors was detected this season. All circulating influenza virus groups are currently resistant to adamantanes, which are not used in treatment in Norway and most other

countries. Therefore, FHI has stopped testing routinely for adamantane resistance. Virus resistance to antiviral agents in Norway is reported by the WHO national reference laboratory for influenza, NIPH via the Global Influenza Surveillance and Response system (1) and ECDC / WHO. The most recent observation of virus with reduced susceptibility to neuraminidase inhibitors in Norway was a double-deletion B/Victoria-lineage virus from August 2018, B/Norway/3241/2018, harboring the substitution D197N in the neuraminidase gene.

Table 3.1: Norwegian influenza viruses resistant to the neuraminidase inhibitors oseltamivir and zanamivir and M2 blockers (adamantanes), during the influenza seasons 2005/06 through 2018/19. (n = number of samples tested)

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=120)	0% (n=66)	2.4%* (n=42)	0% (n=28)	0% (n=54)	0% (n=30)	100% (n=1)	100% (n=1)
2018/19	0% (n=247)	0% (n=108)	0% (n=26)	0% (n=82)	0% (n=107)	0% (n=26)	100% (n=1)	nd

nd= not done

* Updated October 2019 due to post-season analysis. Differs from the data presented in the 2018 RAVN report.

Conclusion

Antiviral drug resistance towards influenza remains low in Norway. Global estimates indicate that approximately 0.5% of all viruses tested have reduced susceptibility towards neuraminidase inhibitors (2) and this is expected to be similar for Europe. Continued monitoring is important both in samples from the community and in patients treated with antivirals.

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New flu antiviral drugs: potential for resistance

Karoline Bragstad, FHI

Vaccination is an important preventive measure against influenza. As the influenza virus is constantly changing and several different subtypes often co-circulate, the vaccine might not always match the circulating viruses as well as intended. In the case of a pandemic, vaccines might not be available or may be distributed too late to reduce the impact of the pandemic. In such a situation, antiviral drugs might be used in an attempt to delay the progression of the pandemic. They can also reduce the impact of an outbreak to some extent by easing symptoms, reducing the duration of hospitalisation, and preventing deaths. When antiviral resistance is low, antivirals are an important adjunct to influenza vaccine in the control of influenza. Antivirals are particularly important in the treatment of severely ill patients.

Currently, the neuraminidase inhibitor (NAI) oseltamivir (Tamiflu®) is the only antiviral drug for influenza on the market in Norway. Since the 2009-10 flu season, resistance to NAIs has been low. However, resistant subpopulations of the virus tend to occur after treatment with oseltamivir, especially in immunocompromised patients and children (1-3). Reduced sensitivity to one or more NAIs has been observed in 0.2% to 1.9% of viruses investigated globally since 2012/13 (4). In recent years viruses with reduced susceptibility to oseltamivir have occasionally caused small outbreaks in the community, also in Norway (5). Oseltamivir resistant viruses can also rapidly become dominant and spread globally only within one influenza season (see table 3.1: seasons 2007 to 2009) (6). Norway's reliance on a single influenza antiviral drug makes us especially vulnerable to such changes. Access to at least one additional drug is therefore urgently needed.

In comparison, three neuraminidase inhibitors are approved by the U.S. Food and Drug Administration (FDA), all with activity against both influenza A and B viruses; oral **oseltamivir phosphate** (available as a generic version or Tamiflu®), inhaled **zanamivir** (Relenza®), and intravenous **peramivir** (trade name Rapivab®). In addition a fourth drug, the endonuclease inhibitor baloxavir marboxil (Xofluza®), was approved by FDA in 2018 for those aged 12 years and older. All these drugs are also approved in Japan, in addition to a fourth NAI, laninamivir.

The NAIs currently authorised centrally via European Medicines Agency for use in all EU Member States, are oral capsules oseltamivir (Tamiflu®), and intravenous peramivir (Alpivab®). Furthermore, inhalation powder zanamivir (Relenza®) is authorised at national level in all EU Member States, while intravenous formulation zanamivir has been available in the EU since 2010 under a compassionate use programme for treating severely ill patients. Baloxavir marboxil is expected to be available on the European market in spring 2020 and will probably be the next influenza antiviral drug to be licensed in Norway.

Development and clinical application of new antivirals with different mechanisms of action is critically important. Numerous drugs with alternate modes of action and routes of administration are currently in phase III clinical trials. Most do not target the neuraminidase like oseltamivir, but instead target the heterotrimeric polymerase complex. The polymerase complex is composed of three highly conserved protein subunits that are essential for efficient viral replication and virulence. Three new antivirals, favipiravir, pimodivir, and baloxavir marboxil, target the polymerase basic (PB1 and PB2) and-acidic (PA) subunits, respectively (Table 3.2) (7). All three are inhibitory for influenza A viruses,

including those resistant to adamantanes and NAIs. Favipiravir and baloxavir also inhibit influenza B viruses. All are orally administered, and baloxavir marboxil offers single dose administration in uncomplicated influenza.

Baloxavir marboxil (Xofluza®) is an oral prodrug that is rapidly converted to its active form baloxavir acid, a potent inhibitor of influenza cap-dependent endonuclease function. It is administered orally in a single dose and is a long-acting endonuclease inhibitor, effective against influenza A and B viruses. Baloxavir marboxil is the first and only single-dose oral medicine approved for influenza treatment. During October 2018–February 2019, baloxavir was supplied to medical institutions serving approximately 5.6 million persons in Japan. In 2019, human-to-human transmission of a resistant virus in Japan was indicated when an infant without baloxavir exposure and a baloxavir-treated sibling carried the substitution I38T in the PA polymerase acidic gene of influenza (8;9). The same substitution has been found after treatment in phase 2 and 3 clinical trials of baloxavir, most frequently in H3N2 viruses (8). Surveillance data from the 2018-19 season showed that that 1.5% of H1N1 and 9.5% of H3N2 viruses from Japan possessed the PA I38T substitution. Nearly all cases were after baloxavir administration. In one clinical trial, baloxavir-resistant variant viruses were identified in 17% of treated children (10). Recent studies (11;12) indicate that the resistant viruses are as fit as the wildtype viruses, indicating high risk of transmission of resistant viruses. Also, the cost of treatment has been estimated to be three times than that of treatment with oseltamivir (13).

Pimodivir is an orally administered non-nucleoside PB2 inhibitor, which acts by occupying the 7-methyl GTP cap binding site (14). Pimodivir is effective against influenza A, but not influenza B viruses. A phase II clinical trial showed that resistance was identified in 10% of cases, caused by single point mutations in the PB2 protein (15). This drug has also been shown to work better in combination with oseltamivir (7). A pre-approval trial for treatment of patients with H7N9 infection has been allowed.

Favipiravir is a purine nucleoside analogue, with broad-spectrum antiviral activity. It was licensed in Japan in 2014 for use against novel influenza virus infections where existing antivirals are ineffective. Resistance to favipiravir has not been detected in treated patients to date, but passaging of influenza H1N1 in cell culture in the presence of favipiravir led to development of resistance, indicating that resistance towards favipiravir could occur naturally (16;17).

Table 3.2: Overview of polymerase inhibitors approved or in advanced clinical development (adapted from (7))

Feature	Favipiravir[a] (T-705)	Pimodivir (JNJ-63623872)	Boloxavir[b] (S-033188)
Influenza polymerase target	PB1	PB2	PA
Influenza virus-type spectrum	A, B, C	A	A, B
Inhibition of M2I and NAI-resistant viruses	Yes	Yes	Yes
In-vitro potency	µM	nM	nM
Synergy with NAIs for influenza A viruses	Yes	Yes	Yes
Route of dosing	Oral (Intravenous under development)	Oral (Intravenous under development)	Oral
Antiviral efficacy in uncomplicated influenza	Yes	Yes	Yes
Clinical efficacy in uncomplicated influenza	Variable	Not formally tested	Yes
Emergence of variants with decreased in-vitro susceptibility during monotherapy	Not to date	Yes, common	Yes, common

PA, polymerase acidic protein; PB, polymerase basic protein; NAI, neuraminidase inhibitor; M2I, M2 ion channel inhibitor.

[a] Approved for novel strains unresponsive to current antivirals in Japan in 2014 (trade name, Avigan).

[b] Approved for influenza treatment in 2018 in Japan and United States (trade name, Xofluza).

Other candidate drugs worth mentioning are the Das181 (Fludase) which removes sialic acid in the respiratory airways, arbidol (Umifenovir) which is a broad-spectrum drug that makes haemagglutinin resistant to conformational changes triggered by pH, and Ingavirin which interacts with the influenza nucleoprotein and inhibits viral genome release (18). Ingavirin is approved in Russia. Monoclonal antibodies targeting the conserved region of haemagglutinin have been tested and some have reached phase II. They were all found to be safe and well tolerated and no mutated viruses were detected after monoclonal antibody administration (19). Nitazoxanide, originally an antiprotozoal agent, has shown efficacy against influenza A in vitro, and phase II and III clinical trials are recently completed (20). The drug blocks the final assembly and folding of haemagglutinin (21). Resistance was not identified following serial passage of influenza viruses in increasing concentrations of the drug (22;23).

The role of combination therapy with NAIs and the polymerase inhibitors is currently being investigated in phase III clinical trials. Both baloxavir marboxil and pimodivir monotherapy are associated with high frequencies of resistance, but combination with oseltamivir reduces the risk. The polymerase inhibitors show synergistic interactions with NAIs in preclinical models of influenza A virus infection, and combinations of oseltamivir and these agents are advancing in clinical testing in hospitalised influenza patients (7). Wider availability of one or more polymerase inhibitors would provide important therapeutic options. Combination therapy should increase antiviral potency and reduce the risk of antiviral resistance emergence.

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4 Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV) drug resistance	
Treatment	Antiretroviral treatment (ART) of HIV-infection is always given as a combination of drugs from at least two of the five different classes: - Nucleoside reverse transcriptase inhibitors (NRTI) - Non-nucleoside reverse transcriptase inhibitors (NNRTI) - Integrase strand transfer inhibitors (INSTI) - Protease inhibitors (PI) - Entry inhibitors (CCR5 antagonists and fusion inhibitors)
Resistance testing method	Genotypic assays based on Sanger sequencing of target genes, and identification of mutations associated with drug resistance. Plasma viral load > 500 copies/mL is usually required. In Norway, all HIV-1 drug resistance tests are performed at the National Reference laboratory for HIV 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	The national surveillance program for HIV-1 monitor primary drug resistance against protease inhibitors (PI) and reverse transcriptase inhibitors (NNRTI and NRTI). Samples from all patients with newly diagnosed HIV-1 infections are tested for resistance mutations in the protease and reverse transcriptase genes.

Surveillance methods

The Norwegian surveillance data is based on resistance testing of samples taken from newly diagnosed patients in Norway. Although some of these patients may be previously exposed to antiretroviral drugs, most are treatment naïve, and the data may serve as a marker of transmitted drug resistance. Although resistance testing is recommended for all newly diagnosed patients, not all are included in the surveillance system. This could be because the sample was not submitted, the MSIS report number was missing from the referral form, or the viral load was suppressed (because treatment was started before arrival to Norway, or for some other reason).

The World Health Organization (WHO) recommends the use of a consensus genotypic definition of transmitted HIV-1 drug resistance to compare estimates of transmitted drug resistance rates across geographic regions, and over time (1;2). A standard list of surveillance drug-resistance mutations (SDRMs) was published in 2009, based on a set of

criteria to ensure that the mutations included are nonpolymorphic, applicable to the most common subtypes, and in fact contributing to resistance (1;2). The SDRM list is not designed for individual patient management as it excludes several clinically relevant drug resistance mutations, and may include certain mutations with less clinical relevance for current regimens. They are however robust markers of temporal trends in transmitted drug resistance. The monitoring in Norway is based on the WHOs SDRM-list from 2009 and analyzed using the Calibrated Population Resistance (CPR) tool at Stanford HIV Drug Resistance Database (1-3).

There has been an increase in the use of integrase strand-transfer inhibitors (INSTIs) in first line regimens, but resistance mutations affecting these compounds are still rare in treatment naïve patients. Baseline testing of resistance to integrase inhibitors is therefore not yet recommended (4), and there is no surveillance of primary resistance to INSTIs in Norway.

Surveillance data 2018

A total of 119 samples from newly diagnosed cases of HIV-1 in Norway were analyzed for primary HIV-1 drug resistance in 2018, which equals 62% of the 191 cases reported to MSIS in 2018. The corresponding rates of sequenced samples for the years 2010-2018 are shown in Figure 4.1.

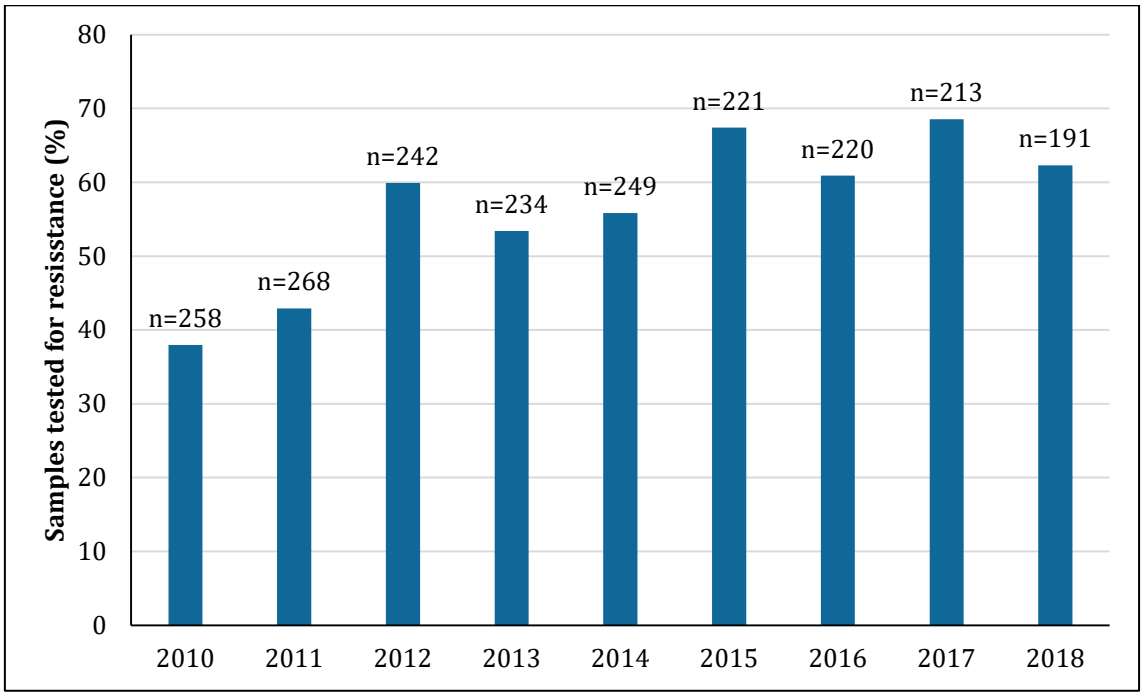

Figure 4.1: Samples tested for resistance (2010-2018).

Data shown as percentage of newly diagnosed cases of HIV-1 infection according to MSIS (5). n = number of cases reported to MSIS.

In 2018, surveillance drug-resistance mutations from the WHO list were detected in 9.2% of the analyzed sequences. The frequencies of SDRMs are presented in Figure 4.2, showing the percentage of sequences with detected SDRMs during each year of surveillance. Of the analyzed sequences, 5.9 % had SDRMs associated with non-nucleoside reverse transcriptase inhibitors (NNRTI), and 5.0 % with nucleoside reverse transcriptase

inhibitors (NRTI). In 2018, none of the sequences had SDRMs associated with protease inhibitors (PI). The individual mutations are specified in Table 4.1.

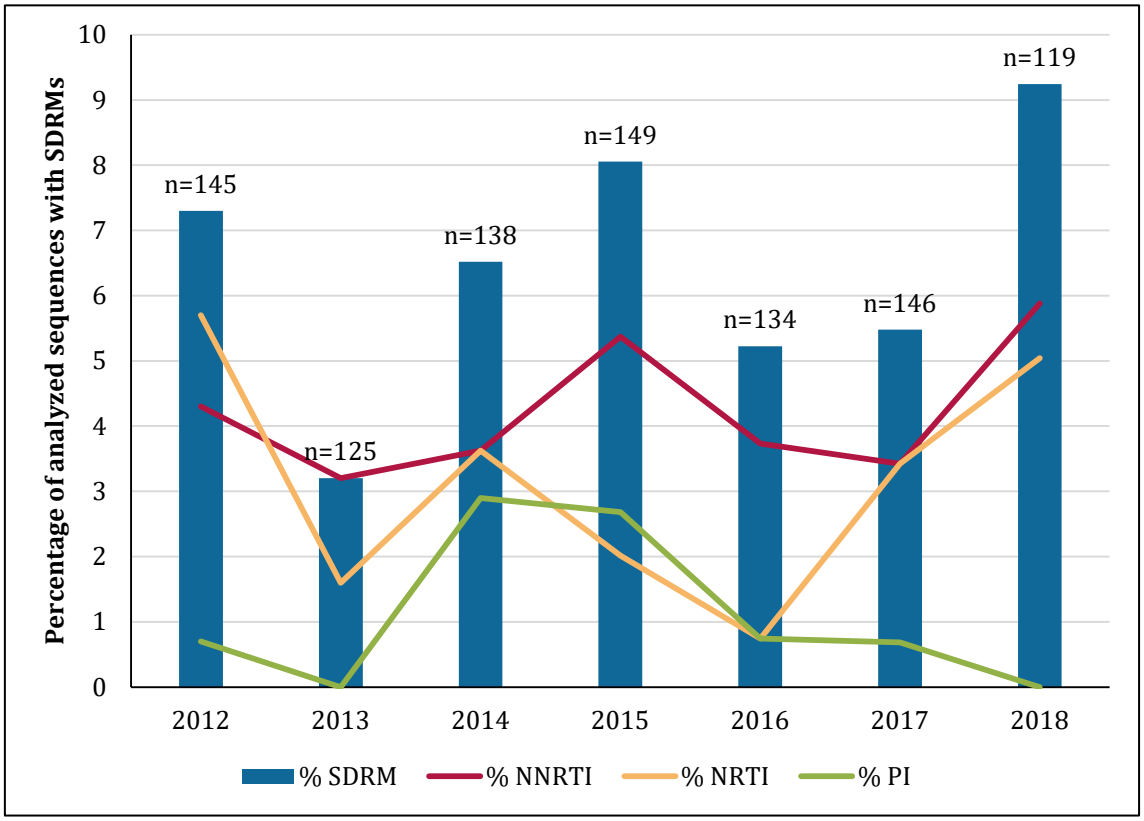

Figure 4.2: Sequences with detected surveillance drug resistance mutations (SDRMs).

Percentages of the analyzed sequences containing one or more SDRMs through the years 2012-2018 are shown as blue columns Percentage of mutations affecting the individual drug classes are shown as colored lines; non-nucleoside reverse transcriptase inhibitors (NNRTI) in red, nucleoside reverse transcriptase inhibitors (NRTI) in yellow, and protease inhibitors (PI) in green. n = number of sequences analyzed for primary resistance. There may be several SDRMs per sequence.

Table 4.1: Specification of the surveillance drug resistance mutations (SDRMs) detected in 2018.

Sequence ID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	K219Q	None	None
2	M184V	K103N	None
3	M41L	None	None
4	M41L	None	None
5	M41L	None	None
6	T215C	Y181C	None
7	None	G190A	None
8	None	K101E	None
9	None	K101E	None
10	None	K103N	None
11	None	K103N	None

SDRM: surveillance drug resistance mutations; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

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 (2). The data include patients infected abroad, both Norwegian residents infected while travelling, and immigrants infected before arrival in Norway. Furthermore, some of the latter may have received treatment in their home countries. Thus, the data does not reflect the risk of being infected in Norway with a drug resistant strain of HIV-1.

SDRMs were detected in 9.2% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2018, while the corresponding number for 2017 was 5.6%. It is important to notice that although there seems to be an increase in percentage of sequences with SDRMs, the absolute numbers were not that different. In 2017, there were five sequences with NRTI- and five with NNRTI-associated mutations, while in 2018 the corresponding numbers were six and seven, respectively. However, both the total number of new HIV-infections in Norway, and the number of samples analyzed for primary drug resistance, were lower in 2018 compared to 2017. Continued surveillance is necessary to decide whether there is indeed an increasing trend in transmitted drug resistance in Norway. In a currently ongoing collaboration study between the NIPH and the National reference laboratory for HIV at OUH, resistance data is being cross-referenced to epidemiological data from MSIS. Data from this study will enable a more detailed analysis of the trends in prevalence of transmitted drug resistance in Norway by facilitating measurement of the prevalence of SDRMs in different subgroups, such as risk groups or country of infection.

Conclusions

Surveillance drug-resistance mutations were detected in 9.2% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2018. These were mutations associated with resistance against NRTI and NNRTI, and no mutations affecting PI were found. The prevalence of transmitted drug resistance has been stable in recent years. The introduction of pre- and post exposure prophylactic treatment (PrEP and PEP, respectively) could challenge this situation, and particularly the recent implementation of PrEP calls for intensified surveillance. Surveillance of HIV-1 resistance over time is important in order to make informed decisions on implementation of preventive measures to control dissemination of resistant HIV-1 strains.

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National tenders on HIV drugs: Perspectives on antiretroviral drug resistance

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Treatment of HIV with a combination of antiretroviral drugs has had dramatic impact on HIV-related morbidity and mortality. The drugs are expensive, and treatment is fully reimbursed by the national health authorities. There are several different combinations of drugs available that are almost equally effective, and deciding which regimen to offer each patient should ideally be based on individual considerations of different aspects, such as side effects, toxicity, co-morbidities, genetic barrier, dosage and pill burden, the patients daily routines, life situation, and adherence.

In 2018, a tender on HIV medicines was issued. Clinicians are instructed to evaluate all patients for the drug regimens according to a prioritized list. The drug combination listed first is the preferred combination, followed by nine other combinations. All patients, both patients that have been treated for a long time, as well as treatment naïve patients starting their first regimen, should be considered for the drug combination listed as number one after the tender. If other combinations are chosen, clinicians are required to justify the decision in the patient's medical records.

The process is administered by the Norwegian health authorities via the procurement organization Sykehusinnkjøp HF. All pharmaceutical companies selling antiretroviral drugs in Norway are invited to bid on a tender for drug combinations recommended in the Norwegian National HIV guidelines. Thereafter a committee with representatives from the health authorities and the hospitals, together with several infectious disease specialists, makes a prioritized list. The only factor objectively differentiating the recommended alternatives to make a prioritized list, is the price. However, the prices and the premises for the decisions are confidential, and not open to the public. The decision is valid for a maximum of two years, and then a new procurement will be announced. Thus, after one to two years, all patients may again be considered for changing their therapy to the new preferred combination. This process has been criticized by clinicians (1), and by the Norwegian Society for Infectious diseases (2): "The idea of trying to reduce the cost is of course well understood. However, choosing an antiviral combination therapy for an individual patient must be based on individual preferences by the treating physician in dialogue with the patient, and cannot be changed every year or two."

In 2017, a similar tender was implemented on antiviral drugs used for treatment of chronic hepatitis C virus infection (HCV). Over the last years, new and highly effective drugs have become available, but the prices have been high. Implementation of the tender has resulted in significantly reduced costs for treatment of HCV. There are however major differences between the treatments of HCV and HIV. Treatment of HCV with direct acting antivirals is administered as a cure lasting 8-12 weeks, e.g. the drugs are used for a limited time period. The experiences and outcome of the HCV tender do not transfer directly to HIV, which requires life-long treatment, entailing greater risks for antiviral resistance, harmful side effects and treatment compliance problems.

The focus on the tender for HIV medication is on drug expenses, but a number of other relevant associated costs also need to be taken into consideration: Intensified follow-up due to frequent change of drug regimens will lead to additional consultations at the hospital, extra blood sampling, and costly laboratory analyses. There are ongoing debates discussing the political, clinical, personal, and economic consequences of the implementation of this tender. In this report, we focus on the concerns regarding antiretroviral drug resistance.

Today, most of the treated patients obtain fully suppressed viral load, and clinically relevant drug resistance is rarely seen. This fact has several reasons. Highly efficient drugs with simplified dosing regimens and less side effects compared to older drugs have been developed. Furthermore, the larger number of drug classes and vast number of possible drug combinations makes it easy to adapt the therapy to the individual needs for each patient; avoiding drug interactions and adapting to the patient's lifestyle and food preferences. Although treatment failure with acquired drug resistance is no longer a major issue in western countries (3), history has shown that drug resistance is easily selected during ongoing viral replication under subtherapeutic drug levels. HIV has a high capacity for genetic adaptation in response to a changing selection pressure, and patient adherence to therapy is crucial for maintaining full suppression of viral replication. For patients stable on efficient ART, a yearly follow-up with clinical examination and blood samples is sufficient. A change of treatment is always considered if the current regimen seems suboptimal, either due to side effects, low tolerance, virological failure, or other reasons. After such a switch, the patients are monitored more closely.

Treatment of HIV-infection is life-long, and with the highly effective regimens currently available, life expectancy of a patient with chronic HIV-infection does not differ very much from the rest of the population. Thus, a young person diagnosed with HIV, may expect to use antiretroviral drugs for the next 40-60 years. With a new tender for HIV medicines every second year, well treated patients may be experiencing up to 30 possible changes of drug regimens. For most patients with fully suppressed viral load, a switch to a new regimen is uncomplicated. For every switch, however, there is a small risk of complications that could affect drug resistance, and the total risk will accumulate with frequent changes.

First, there are biological aspects that may lead to subtherapeutic drug-levels, such as different half-life of drugs, gastrointestinal side effects affecting absorption of the drug, or the risk of drug interactions. There is also a risk of preexisting resistance mutations that might be selected after a switch of therapy.

Second, and maybe of more concern, there are behavioral aspects. Side effects or toxicity may lead to reduced adherence. In addition, the exhaustion of continuously changing regimens, or reduced trust in the doctor that keeps proposing new regimens, also need to be considered as risk factors for reduced adherence. Furthermore, confusion with altered regimens, misunderstandings, communication difficulties, or risk of forgetting doses when routines are changed, may lead to accidental noncompliance.

All of the above are factors that would lead to lower drug levels and thus, increased risk of developing resistance. This calls for closer management and follow-up of patients that otherwise would be stable on a regimen with regular follow-up every 12 months. In addition to clinical and biochemical monitoring of toxicity and side effects of every new regimen given to a patient, there will be need for more frequent blood sampling and viral load measurements for monitoring the efficacy and potential development of drug resistance. Furthermore, a detectable viral load will trigger further testing including expensive and laborious drug resistance testing.

The consequences of reduced adherence to therapy are considerable, as development of drug resistance will hamper future treatment options. Although the probability of an individual well-controlled patient developing drug resistance in association with changing the treatment regimen is low, the cumulative risk for hundreds of patients changing treatment every second year for 50 years will be considerable. Early signs of subsequently

increased drug resistance will be difficult to detect through the current surveillance system, which is directed towards transmitted drug resistance.

Although the tender has worked out well for HCV, there are some major concerns that ought to affect how this should be implemented for HIV. From a virological perspective, we would prefer a modification of the tender to concern only patients starting their first regimen and patients considering switch of therapy, and not patients stable on an effective antiretroviral regimen. By encouraging frequent changes of treatment regimens, we risk that the sums we may be saving on reduced prices for the drugs, are by far exceeded by the price paid in terms of increased risk of drug resistance that may hamper future treatment options. We would welcome a thorough health economic analysis that takes these perspectives into consideration.

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5 Hepatitis B virus

Fact box: Hepatitis B virus (HBV) drug resistance	
Treatment	Treatment of HBV infection with antivirals is generally given as monotherapy: - Nucleoside/nucleotide analogues, usually entecavir, tenofovir disoproxil, or tenofovir alafenamide
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 Institute of Informatics. A plasma viral load > 1000 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
Indication for resistance testing	Virological failure/breakthrough on antiviral treatment.
Surveillance	Surveillance of both treatment experienced and treatment naïve patients: 1) Patient samples submitted for drug resistance testing mostly in relation to breakthrough on antiviral treatment 2) Population-level surveillance of a treatment naive population conducted on samples submitted for genotyping

Surveillance method

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. Monitoring of the latter population can therefore be regarded as surveillance of primary resistance. Mutations altering specific amino acid positions within the polymerase gene can give rise to resistance to the different approved antivirals for the treatment of HBV.

Surveillance data 2018

The resistance mutations detected in Norway between 2011 and 2018 are presented in Table 5.1.

Table 5.1: HBV antiviral resistance in samples submitted for drug resistance testing in 2011-18.

HBV-variants resistant to antivirals	2011	2012	2013	2014	2015	2016	2017	2018
Total analysed	14	3	9	17	10	23	23	20
Wild type	11	2	8	15	8	17	21	16
M204I	1		1	1	1	1		
L180M + M204I/V	1	1		1		1	1	
L180M + M204V + S202G/S						1	1	2
L180M + M204V + T184I + S202G						1		
L180M + M204V + M250V						1		
I169T + L180M + T184A + M204V								1
L180M + M204V + S202G + T184A								1[a]
A181T + N236T	1							
Uncharacterized mutation*						1[b]	2[c]	4[d]

*Uncharacterized mutation: new mutation of undetermined significance in a position associated with resistance.

[a] The uncharacterized mutation V173M was also detected in this sample.

[b] V173M and N236D

[c] One sample with I169L and one sample with M250L.

[d] One sample with L80S, one sample with A181S and two samples with V173M.

In 2018, 20 patient samples were submitted for HBV drug resistance testing. Entecavir drug resistance was detected in four samples (Table 5.2), all with mutation L180M and M204V, as well as mutation S202G and/or T184A and/or I169T. All had been on treatment with ETV. According to EASL-guidelines (1) a switch to tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) therapy is recommended in case of treatment failure.

No drug resistance mutations were detected in patient samples submitted for HBV-genotyping (N=245) only.

Table 5.2: Resistance mutations detected in samples from 2018

Sample	Resistance mutations detected	Treatment*	Resistance				
			LAM	TBV	ETV	ADV	TDF/TAF
1	L180M + M204V + S202G/S	ETV	R	R	R	S	S
2	L180M + M204V + S202G/S	ETV	R	R	R	S	S
3	I169T + L180M + T184A + M204V	ETV	R	R	R	S	S
4	L180M + M204V + S202G + T184A	ETV	R	R	R	S	S

LAM: lamivudine; TBV: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate ; TAF: tenofovir alafenamide; R: resistant; S: sensitive.

* Treatment specified at the time of resistance testing.

Conclusion

Entecavir drug resistance mutations was detected in 4 of 20 samples tested for drug resistance mutations. No HBV drug resistance was detected among the 245 samples submitted for genotyping.

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6 Human herpes viruses

Surveillance of cytomegalovirus drug resistance

Fact box: Human cytomegalovirus (CMV) drug resistance	
Treatment	- Nucleoside/nucleotide analogues: ganciclovir/valganciclovir (first choice), cidofovir and foscarnet (second choice)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analyzed for amino acid substitutions 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 the Oslo University Hospital, Rikshospitalet.
Target genes	CMV phosphotransferase (UL97) and DNA polymerase (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.

Surveillance method

The antiviral drug resistance has been characterized by comparing phenotypic and genotypic test results. For routine testing only genotypic tests, looking for known resistance mutations, are applicable. Resistance to ganciclovir develops by mutations in the viral phosphotransferase CMV UL97-and/or the DNA polymerase CMV UL54 gene. Normally resistance mutations in the CMV UL97 gene precede mutations in the CMV UL54 gene, because ganciclovir is first choice treatment, and the fitness cost of mutations in CMV UL54 is higher (1). Foscarnet and cidofovir resistance is conferred by mutations in the UL54 gene.

There is no population level surveillance of CMV drug resistance, and the surveillance is based on samples from patients with persistent high viral load despite ongoing therapy. Immunocompromised patients are more prone to develop drug resistance.

Surveillance data 2018

In 2018, 27 specimens from 22 patients were received for genotypic analysis of CMV resistance mutations. Six specimens were not analyzed because the amount of CMV DNA was insufficient. In the 21 remaining specimens, CMV resistance mutations were recorded in four samples (Table 6.1) from two different patients. The mutations detected are listed in Table 6.2.

Table 6.1: Number of samples analyzed for anti-CMV drug resistance mutations and number of samples in which CMV drug resistance mutations were detected.

Year	Number of samples tested	Number of samples with CMV resistance mutations (percentage of tested samples)
2017	30	7 (23 %)
2018	21	4 (19 %)
Sum	51	11 (22 %)

Table 6.2: CMV resistance mutations recorded in samples tested in 2018

Patient	Sample	UL97 mutations	UL54 mutations
1	**1, 2**	E756EK[1]	
2	**3, 4**		L595S[2]

UL97 encodes the viral phosphotransferase. UL54 encodes the viral DNA polymerase.

[1] Foscarnet moderate and ganciclovir/ cidofovir low grade resistance. Two samples from the same patient

[2] Ganciclovir moderate resistance. Two samples from the same patient

Conclusion

Despite increasing consumption of ganciclovir for therapeutic and prophylactic treatment, drug resistance is infrequent.

Surveillance of herpes simplex virus drug resistance

Fact box: Herpes simplex virus (HSV) drug resistance	
Treatment	- Nucleoside/nucleotide analogues: ganciclovir/valganciclovir (first choice), cidofovir and foscarnet (second choice)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analyzed for amino acid mutations associated with drug resistance. Until March 2018, all HSV drug resistance tests for Norway were performed at Folkhälsomyndigheten, Stockholm. From 1. March 2018 the resistance testing for HSV is performed at Sahlgrenska University Hospital, Gothenburg
Target gene	HSV thymidine kinase (HSV-TK)
Indication for resistance testing	Persistent HSV-infection despite ongoing therapy
Surveillance	Population-level surveillance is currently not necessary

Surveillance method

The surveillance is based on samples from patients with persistent HSV-infection despite ongoing therapy. There is no population level surveillance of HSV resistance. Immunocompromised patients are more prone to development of drug resistance, but information on the patients' immune status is not available for surveillance purposes. For routine testing, only genotypic tests are applicable.

Surveillance data 2018

In 2018, seven samples from Norway were submitted for HSV resistance analysis. Due to insufficient amounts of HSV DNA, two of the specimens could not be analyzed. In the five remaining specimens, a known resistance mutation was recorded in one sample and a possible resistance mutation was recorded in another, see Table 6.3.

Table 6.3: HSV resistance associated mutations

Sample	HSV-type	TK-mutations	Aciclovir susceptibility
1	HSV-2	280delG	resistant
2	HSV-2	Ins 1125	unknown

Conclusion

Despite an increasing consumption of aciclovir for both therapeutic and prophylactic treatment, treatment failure is rare, and very few samples are submitted for resistance testing. Thus, the numbers are small, but this may indicate an overall low frequency of resistance to aciclovir.

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7 Hepatitis C virus

Fact box: Hepatitis C virus (HCV) drug resistance	
Treatment	Antiviral treatment of HCV-infection consist of a combination of drugs from at least two of the four different classes: - Nucleoside analogue polymerase inhibitors (NS5B) - Non-nucleoside analogue polymerase inhibitors (NS5B) - Protease inhibitors (NS3/4A) - NS5A inhibitors Direct acting antivirals may be supplemented with ribavirin. Treatment protocols depend on genotype and stage of liver disease.
Resistance testing method	Sequencing of relevant genes and/or the complete HCV genome Routine resistance testing is currently not available in Norway, but a next generation sequencing-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 Baseline testing of patients with HCV genotype 1a and high viral load (>800 000 IU/ml) considered for treatment with elbasvir + grazoprevir Baseline testing of cirrhotic genotype 3 patients
Surveillance	Currently no surveillance. Will include resistance testing from clinical routine samples or surveillance projects

Routine resistance testing is currently not available in Norway, but a method for HCV whole genome analysis is currently under the final phase of validation at the NIPH. The method is based on next generation sequencing with Illumina MiSeq technology. For the detection of genotypes and resistance-associated mutations (RAS) the HCV-Glue database and analysis tools is used (1;2). The method is able to detect all recognized RAS in all target genes, as well as HCV genotypes and HCV co-infections, all in one analysis.

The method has been tested on clinical hepatitis C samples with viral loads down to 1000 IU/ml. For genotyping of HCV a viral load above 2200 IU/ml is sufficient for successful analysis. For high quality deep sequencing of the complete genome including detection of all RAS, a viral load of >10 000 IU/ml is required.

The HCV resistance analysis will be available as part of the national viral hepatitis reference laboratory analysis repertoire by the end of 2019, please check our webpage for updates on release date (3).

Samples gathered through the reference laboratory or specific HCV projects will be analysed by this method and used for surveillance of HCV RAS in Norway.

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