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FROM THE EDITORIAL BOARD

Dear readers,

With pride I hereby present you our twelfth edition of RAMS. This is the second edition that has been released during our lustrum year, after working hard for so many months. As the new chair of this year's editorial board, I hope with all my heart that the passion for (bio)medical sciences will be awakened while reading this edition.

The academic year is almost halfway done, but for me it feels just like the beginning of everything when working on a new edition. RAMS has been active for five great years now. Each year, new ideas led to new rubrics and this year is no different. In this edition, we introduce to you our newest rubric: *time for innovation*! Innovation can be defined simply as 'new idea'. We as human beings tend to always do things the same way every time. As Albert Einstein said: the only source of knowledge is experience. So trying new ideas, which lead to new experiences, will bring you loads of knowledge! Hopefully, our new rubric will give you a lot of new knowledge too.

Besides our new rubric, this year we focus not only on bio-medical students but also on other students from other faculties. On top of that, we want to include more students from the first to third year. I believe that students from all years have their own ideas and knowledge based on their experiences in the biomedical field. We can all learn so much from each other, whether you are on the verge of graduating or starting your first year. After all, parents learn a lot from their children too.

In this newest edition, we present you various articles about different topics. We discuss whether light products can lead to losing weight or not. Moreover, the spreading of resistant bacteria is also argued in this edition. Our *zebra* describes ichthyoses, a skin disorders which resembles the scales of fish. Last but not least, parasomnias and the scary effects are argued, which almost fits a Halloween theme.

I wish you lots of joy reading this edition of RAMS. Hopefully, this edition will keep you warm during the winter days. At last, try not to forget to try something new after reading this edition during the cold days. Who knows what knowledge you will gain after your new experience!

Yours faithfully,

Naaz Shareef
Chair of the Editorial Board

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LIVING THE DREAM: PARASOMNIAS

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Insights

People do bizarre things while asleep, varying from innocent examples such as mumbling to more harmful examples. In 1987, Kenneth Parks, a 23 year old Canadian, drove 14 miles to the home of his in-laws and stabbed his mother-in-law to death. Within an hour after this event he turned himself in. He claimed he did not know what happened and that he woke up with a bloody knife in his hand [1]. In 2008, Brian Thomas, a 59-year-old Welshmen, strangled his wife of almost 40 years to death. He called the alarm number and told the operator he dreamed that he was strangling a burglar, but woke up to see that he actually strangled his wife [2]. Both men claimed that they were innocent and said they suffered from so-called parasomnias. Opinions differed on the truth of these statements. In the end, they were both declared innocent [1,2].

Introduction

With an estimated lifetime prevalence of seven percent, it is likely that you know someone who is or used to be a sleepwalker [3]. Perhaps you have suffered from this condition yourself. The act of sleepwalking often leads to entertaining stories, for example of someone who plunders the entire refrigerator during the night and wakes up with their face covered in chocolate [4]. The situations caused by sleep disorders are not always comical. Sleeping can unfortunately also coexist with violence. In literature, this phenomenon is known as sleep-related violent behaviour. This includes self-inflicted injury and injury to others [5]. Like the murder cases mentioned above, shocking examples of sleep-related violent behaviour draw a lot of media attention. Because of its sensation value, it is also the topic of multiple books and movies, both fiction and nonfiction [6, 7]. Some case reports have also been written about this topic [5]. These cases, however, although sensational, are believed to be extremely rare [5].

Various conditions can cause someone to display behaviour such as walking, eating or acting violent [8]. These include epilepsy and certain psychiatric diseases, such as dementia. Symptoms of these conditions, however, are not exclusively bound to being asleep. The most common cause of sleep-related violence are parasomnias [8]. Parasomnias are undesirable events or experiences that occur during sleep. Over 60% of people have experienced at least one parasomnia at some point in their life [9]. Since parasomnias are not uncommon and are often put in a bad light by the media, it is important that more is known about these sleep-bound diseases. In this article parasomnias are further investigated.

What is sleep and how does it work?

In order to understand parasomnias it is important that we first learn more about the circumstances in which they occur: while asleep, entry into sleep or during arousals of sleep [11]. Sleep is a naturally recurring state characterised by altered consciousness, reduced interactions with surroundings, automatic eye movements and inhibition of nearly all voluntary muscles [10]. To objectively distinguish sleep from wakefulness, a polysomnography is often performed. This multi-parametric test monitors brain activity, eye movements, muscle activity, heart rhythm and breathing parameters while asleep.

The exact function of sleep is unknown, but it is expected that sleep does not serve solely one physiological role. Sleep seems to be crucial for many vital functions including energy conservation, development, modulation of immune responses, cognition and psychological state. Sleep plays an important role in memory consolidation [12]. The importance of sleep is apparent from animal studies. In rats for example, chronic sleep deprivation leads to severe consequences even with death as result [13]. But how can just laying down with your eyes closed be that efficient? It turns out that sleeping is not as monotonous as it seems. Human sleep consists of five sleep cycles that take 90 minutes each. During a sleep cycle two different phases occur; non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. An essential difference between these two phases is that during REM sleep, muscle inhibition arises. NREM sleep can be further classified into NREM stage I (light sleep), II (deeper sleep) and III (deep sleep). The ratio in time between NREM and REM varies with each cycle. The duration of NREM sleep gets smaller during each cycle, while those of REM sleep gets longer [14]. The sleep cycle is further illustrated in figure 1.

Since sleep is a complicated process, it is not difficult to imagine it can also go wrong. Roughly 20% of the population suffers from one or more of the 80 sleep disorders described by the International Classification of Sleep Disorders (ICSD-3) [15]. Common examples of these are insomnia, sleep apnea and the aforementioned parasomnias.

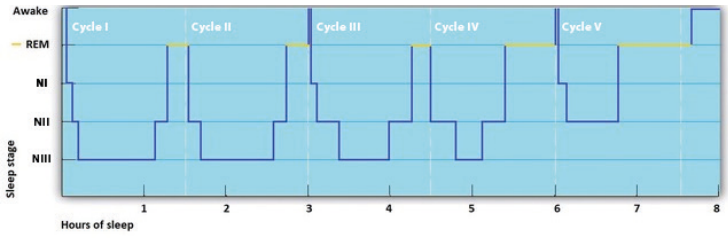

Figure 1: Sleep cycles and its stages.
In this figure, a hypnogram is displayed which is a graph that represents the stages of sleep as a function of time. A hypnogram is also used in polysomnography to recognise the different phases of sleep: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. NREM sleep can be further classified into NREM stage I (light sleep), II (deeper sleep) and III (deep sleep). This is indicated in the figure as respectively NI, NII and NIII. In this hypnogram five sleep cycles are displayed, which is the average number of cycles a human experiences during sleep [14, 15]. All sleep cycles start with NREM sleep (the first 60 minutes) and end with REM sleep (the last 30 minutes). The ratio in time between NREM and REM varies with each cycle. The duration of NREM gets shorter as the night progresses, while the duration of REM gets longer.

Parasomnias

Frenchman Henri Roger was the first to use the term parasomnia in 1932 to describe the odd phenomenon of a boy frequently screaming in his sleep. He derived this term from the words *para* meaning 'beside' in Greek and *somnus* meaning 'sleep' in Latin [16]. Doing the name justice, parasomnias are currently described as a category of sleep disorders defined as undesirable motor, behavioural or sensory experiences that exclusively occur during sleep. Parasomnias occur because of an error in the regulation of sleep, which means that one or more of the aforementioned characteristic symptoms of sleep do not go as planned [16].

As mentioned before, many people have experienced some form of parasomnia at least once in their life. Recurring parasomnias occur most often in pre-adolescent children, with a prevalence of 15%, but can occur at any age. The prevalence amongst adults is estimated to be around four percent [17]. Genetic factors seem to play an important role in the pathophysiology of most parasomnias, as often several family members suffer from it. Parasomnias also occur in close association with other sleep disorders. Furthermore, they are also more common in certain comorbidities, such as Parkinson's disease [17]. The ICSD-3 lists ten core categories of parasomnias, eight of which are described in table 1 and 2 [18]. NREM and REM sleep disorders will be further discussed.

NREM parasomnias

NREM parasomnias are also known as disorders of arousal, meaning they often result from an incomplete transition out of deep NREM sleep. Since these parasomnias exclusively occur during deep sleep, which is part of NREM sleep, they are more common in children, because their deep sleep cycle lasts longer [17]. NREM parasomnias have certain key characteristics which are described in table 1.

The diagnosis of NREM parasomnias is based on clinical symptoms. Usually no further polysomnography is necessary, because most individuals who suffer from these parasomnias only occasionally show symptoms. One-time polysomnography during a night, therefore, hardly contributes to the diagnosis. A polysomnography can, however, be used to exclude underlying sleep disorders, such as sleep apnea. NREM parasomnias are usually outgrown in early childhood and hardly ever require treatment [19]. If deemed necessary, however, there are some lifestyle and pharmacological treatment options, as described in table 1 [17, 19].

The ICSD-3 distinguishes five NREM parasomnias: disorders of arousal (which is a term for NREM parasomnias not otherwise classified), confusional arousals, sleep terrors, sleep-related eating disorder and sleepwalking [17]. Of these parasomnias, confusional arousals is the most common and least complex type. Confusional arousals are characterised by a short episode in which the individual seems awake, yet absent and oblivious to their surroundings. These events can be accompanied by automatic behaviours, which are mostly purposeless and uncontrolled, such as kicking [17]. Sleep terrors are another common parasomnia in children. Like confusional arousals, the person seems unaware of his or her surroundings, however, instead of coming across as being confused, they seem terrified. Events of sleep terrors are characterised by screaming and by display of autonomic arousal, such as tachycardia and rapid breathing [17]. Both of these parasomnias can evolve into the most complex NREM parasomnias, known as sleepwalking. Sleepwalking, also known as somnambulism, consists of multiple increasingly more complex behaviours that finally result in walking. Events of sleepwalking typically last less than 15 minutes, but there are reports of events that lasted multiple hours. During these sleepwalking events, the individual exhibits decreased awareness and impaired responsiveness to their surroundings. More complex behaviours can be made alongside walking, such as eating or driving, but in almost all cases individuals do not leave their room [17, 19]. During these actions the sleepwalker appears uncoordinated and prone to self-inflicted injuries. Attempts to wake up a sleepwalker will usually fail [17].

Although extremely rare, some cases of homicidal somnambulism have been reported, including the case of Kenneth Parks who claimed to have killed his mother-in-law in his sleep [1]. His wife and close relatives confirmed he was a known sleepwalker. At the time of his case, polysomnography was a new test and was, therefore, not performed on Parks [21]. Doctors were, however, already convinced of Parks' innocence, because he cut several of his fingers during the murder, injuring tendons and nerves, without noticing. The lack of pain seen during sleepwalking is known as somnambulistic dissociative analgesia [22]. After long deliberation by the jury, Parks was acquitted [1].

Table 1: NREM Parasomnias.

In this table the subtypes, characteristics, provoking factors, diagnosis and treatment options of NREM parasomnias are described. The five subtypes of NREM described in this table are listed in the International Classification of Sleep Disorders (ICSD-3) [19].

NREM parasomnias				
Subtypes	Characteristics	Provoking factors	Diagnosis	Treatment
(1) Disorders of arousal (2) Confusional arousals (3) Sleepwalking (4) Sleep terrors (5) Sleep-related eating disorder	More common in young children An individual can alternate between subtypes Individuals have their eyes open during an episode Individuals cannot be woken up during an episode Individuals have amnesia of the event	Stress Insufficient sleep Medications that deepen sleep and alcohol Disorders that result in arousals (including pain and sleep apnea)	Clinical picture (see characteristics) Polysomnography	Create a safe living environment Prevention of stress and sleep deprivation Treatment of underlying sleep disorders Low-dose benzodiazepines or anti-depressants

REM parasomnias

REM parasomnias, which take place during REM sleep, have little in common with each other. In fact, the only thing they have in common is their initiation during REM sleep. This sleep phase is characterised by electroencephalography (EEG) waves similar to those observed during waking. Paradoxically to these EEG waves only the brain appears active during REM sleep. This sleep phase is accompanied by paralysis of nearly all muscles. Only the muscles that allow breathing and control eye movements remain active [17]. Other key characteristics of REM parasomnias are described in table 2. REM parasomnia is a clinical diagnosis almost always supported by video material and polysomnography. There are multiple treatment options for this disorder as described in table 2 [17].

The ICSD-3 distinguishes three REM parasomnias: sleep paralysis, nightmare disorder and REM sleep behaviour disorder (RBD) [19]. Sleep paralysis happens in the sleep-to-wake transition and is thought to be caused by a delay in lifting up of voluntary muscle activity. Although fully conscious, individuals are not able to control the muscles of their body [17]. Nightmare disorder is characterised by frequent nightmares in which the content most frequently involves imminent danger. Nightmares are a normal part of sleep, as 40% of children and 20% of adults experience at least one nightmare per week. It is called a disorder, however, when the nightmares influence the person's daily life, either due to a high frequency of occurrence or because of the content [17].

Although very unpleasant, both aforementioned REM parasomnias cannot lead to violence. This is in contrast to RBD, in which cases of violence are described. This parasomnia occurs due to a faulty mechanism of muscle paralysis. Because there is no paralysis, affected individuals act out their dreams. Typically, patients with RBD have unpleasant, often even frightful dreams in which fighting plays an important role [23]. Studies have shown that induced bilateral brainstem lesions in cats result in RBD-like behaviour, but it is not known if the same applies to humans [17]. This would explain, however, why RBD is far more prevalent in individuals who suffer from synucleinopathies, of which Lewy Bodies and Parkinson's are examples [23]. Synucleinopathies are disorders characterised by accumulation of aggregates of alpha-synuclein protein in the brain. Interestingly, RBD can also proceed these synucleinopathies by many years and will not always lead to these disorders. This indicates that the relationship between RBD and synycleinopathies is not as simple as once believed [23]. Like sleepwalking, RBD hardly ever requires treatment, especially because during episodes of RBD individuals rarely leave their bed. If necessary, it can be treated with clonazepam. This benzodiazepine is effective in reducing both problematic behaviours and threatening dreams. Another treatment option is melatonin, a hormone that affects the sleep-wake cycle [17].

Cases of homicide in RBD are even less common than homicidal somnambulism. The most probable reason for this is that the prevalence of RBD is less than a half percent while the prevalence of somnambulism is seven percent [3, 24]. A few cases of homicide due to RBD, hyped by the media, have been described. Brian Thomas strangled his wife of 40 years while on holiday. He recalled the event as a dream in which he was saving his wife from an intruder [2]. Thomas was not diagnosed with RBD beforehand. However, a few days prior to their holiday he stopped taking his antidepressants [25]. Certain drugs, including antidepressants and hypnotics are known to suppress REMS. Withdrawal from these drugs can, therefore, trigger REM parasomnias [17]. Like Parks, Thomas was also acquitted [2].

Table 2: REM Parasomnias.

In this table the subtypes, characteristics, provoking factors, diagnosis and treatment options of REM parasomnias are described. The three subtypes of REM described in this table are listed in the International Classification of Sleep Disorders (ICSD-3) [19].

REM parasomnias				
Subtypes	Characteristics	Provoking factors	Diagnosis	Treatment
(1) REM sleep behaviour disorder (RBD) (2) Sleep paralysis (3) Nightmare disorder	More common in adult men (RBD) An individual cannot alternate between subtypes Individuals have their eyes closed during an episode Individuals can be woken up during an episode Individuals remember most of the event	Synucleinopathies (such as Parkinsons) (RBD) REM suppressant drugs (including antidepressants and hypnotics) Mental disorders (sleep paralysis and nightmare disorder) Insufficient sleep (sleep paralysis)	Clinical picture (see characteristics) Polysomnography Video material (RBD)	Create a safe living environment Prevention of insufficient sleep (sleep paralysis) and use of REM suppressant drugs Treatment of underlying disorders Clonazepam (RBD) and cyproheptadine (Nightmare disorder)

Conclusion

Parasomnias are a type of sleep disorder often negatively portrayed in the media, because of the rare association between this disorder and violence. Murder, although extremely rare, especially draws a lot of attention. There are ten core parasomnias, but only two potentially lead to violence, namely somnambulism (a NREM parasomnia) and RBD (a REM parasomnia). Even in these two disorders violent behaviour hardly ever occurs. Diagnosis of these disorders is mostly clinical, because individuals with these disorders only sporadically show symptoms, and a polysomnography is not required. There are multiple treatment options for parasomnias, but these are often unnecessary, because of their harmless character. As can be drawn from the above, parasomnias are not nearly as dangerous as is displayed by the media. So, if you happen to know someone with a parasomnia, or suffer from this disorder yourself, you should not lose sleep over it.

Acknowledgements

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EXAM QUESTIONS

As RAMS aims to enlighten both students and professionals, we would like to present you two exam questions. Find out if you can remember what you have learned during your bachelor's!

We challenge you!

Question 1

Different resistance mechanisms exist against antibiotics. What is the most predominant resistance mechanism that bacteria use against tetracyclines?

A. Efflux
B. Enzymatic changes
C. Higher production of target binding sites
D. Lower membrane permeability

(Topic from Q4 MGZ Attack and Defence, 2017)

Question 2

People without a spleen are more vulnerable to sepsis caused by encapsulated bacteria. This is because the spleen normally ...

A. Produces complement factors that lead to lysis of the encapsulated bacteria
B. Filters encapsulated bacteria from the blood circulation
C. Contains NK-cells that kill encapsulated bacteria

(Topic from Q4 MGZ Attack and Defence, 2017)

The answers to these questions can be found on page 10 in this journal.

STANDING AT THE DAWN OF A POST-ANTIBIOTIC ERA

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Perspective

Notes from the field hospitals at the time of World War I (1914-1918) reveal the horrors of an era without antibiotics. The discovery of penicillin by Alexander Fleming would happen a decade later. "The surgeon's one aim was to open up and clean out the wound, or to cut off the mortifying limb before the dread gangrene had tracked its way into the vital parts of the body" (Crofton E.1997, *p. 270-271*) [1]. Of course, this was also due to the high number of severe casualties and wounds contaminated with soil and shrapnel. Although systemic treatment was not an option, doctors were still very resourceful, and wounds were treated with local antiseptics. "I remember one particular case that had multiple injuries... it involved the genital organs and the anus. That was a very bad case and of course, it turned septic like everything else. We had to, in the end, put him in a bath, slung in a bath of potassium permanganate and kept him in that all the time. Just lifted him out to clean it up and put him back in again. The wound was permanently underneath, immersed in this solution" (Major General Escritt,1979, *letter, the Liddle collection*) [1]. The modern era of antibiotics has transformed medicine and saved millions of lives. However, the effectiveness of antibiotics is being endangered by the rapid emergence and spreading of resistant bacteria.

Introduction

In 1928 Alexander Fleming looked at a Petri dish, contaminated with mould, to see that it killed the bacteria he was examining. This event started the development of penicillin, which would be first prescribed to treat infections around 1940. A recent report from the United States Centers for Disease Control and Prevention illustrates that resistance is not a recent problem [2]. Within the year of the introduction of penicillin, a resistant strain of *Staphylococcus* was already identified. The occurrence of resistance stimulated the pharmaceutical industry to introduce new antibiotics [3]. Unfortunately, bacteria eventually seem to be able to form resistance to nearly all antibiotics that have been developed [3]. Within two years after introduction of methicillin, a methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in 1962 [2]. Vancomycin, a second-line antibiotic, seemed untouched for almost two decades until resistance was documented in 1988 (Vancomycin-resistant *Enterococcus*; VRE) [2]. Around the same time, bacteria started producing extended-spectrum β-lactamases (ESBL), which makes them resistant to most penicillins and cephalosporins [2, 3]. The latter class of antibiotics is an important class for treatment of severe infections in hospitals. Shortly after, resistance was seen to carbapenems (Carbapenemase-producing *Enterobacteriaceae*; CPE), considered a last resort antibiotic, making CPE resistant to nearly all antibiotics [2, 3]. This article will further address the magnitude of the problem today, describing the importance of surveillance and give an overview of what could be expected in the future.

A Global Problem

There are several factors contributing to the emergence of resistant microorganisms. There is a clear relationship between overuse of antibiotics and dissemination of resistant strains [3]. Antibiotics decimate the susceptible microorganisms in a microbiome (i.e. the gut or skin flora) leaving the resistant strains to reproduce. Genes encoding the bacteria's resistance can also be transferred horizontally among both different and related species, using a 'mobile genetic' carriage system called plasmid. Prescribing the wrong type of antibiotic further promotes resistance [3]. Also, incorrect dosage or duration could promote gene alteration, due to subtherapeutic concentrations.

The World Health Organisation (WHO) identified all the actors at play and calls for worldwide action [4]. Every individual can help reduce infection load by following doctor's prescriptions closely (i.e. no 'over-the-counter' use), preventing infection through hygiene measures (especially healthcare workers) and keeping up-to-date with vaccinations. Policymakers should play their part in creating national plans, including financing systems for investment in new antibiotics, vaccines and diagnostic tools, and contribute to international programmes to tackle antibiotic resistance. Finally, the agriculture sector should cut back on its extensive antibiotic use in the veterinary sector and the spraying of crops. An astonishing 80% of antibiotics sold in the United States (U.S.) are being used in agriculture as it yields higher production [3]. The Netherlands already cut back extensively by reducing its use with 63% since 2009. Moreover, antibiotic residues, found in soil, wastewater and manure, originating from its use in agriculture, also exert a selective pressure in favour of resistant strains in nature's microbiome [5]. In the end, these resistant bacteria can find their way to humans. Access to preventive measures (proper sanitation, vaccines and disinfectants) and diagnostics tool for targeted treatment is more scarce in lower income countries. Overviewing the requirements to combat this crisis, it could well be argued that antibiotic resistance is also a problem of poverty [6].

To illustrate the need for global action, reports are published to raise awareness. It is estimated that 700,000 people worldwide die annually from resistant strains of bacteria, human immunodeficiency virus, tuberculosis and malaria put together [6]. For the U.S. and Europe the combined estimated annual number of deaths due to resistant bacteria is around 50,000 (e.g. a fully booked Johan Cruijff ArenA) [2, 6]. A meta-analysis of English literature between 1980 and 2000 found that the risk of death due to MRSA is 40% higher compared to its non-resistant form [7]. An increasing number of countries adhere to evidence-based guidelines annually. But, there is still a lot of room for improvement as only 19 countries (including the Netherlands) are currently at level five (the highest level) of their national antimicrobial action plan, called for by the WHO [8].

The A-team

The situation in the Netherlands is not as dire as it is assumed from these international numbers. Still, the Netherlands is not untouched, but we are mainly dealing with longer hospitalisation. This is explained by the need for relative toxic antibiotics, some of which can only be administered intravenously, in patients with infections with resistant bacteria [9]. CPE is rarely seen in the Netherlands, but in 2015 there was an outbreak of

29 CPE positive patients of unknown origin in a Dutch teaching hospital [10]. Hospitalisation abroad is the biggest risk factor for CPE carriage, emphasising the need for surveillance. Also, an astonishing 75% of Dutch travellers will be colonised with ESBL, for a median length of 30 days, after visiting India, revealing the price we pay for the current level of globalisation [11]. Therefore, a big portion of antibiotic resistance prevention in the Netherlands is focused on 'keeping the bad bugs out'.

Our own antimicrobial stewardship is covered by the so-called 'A-teams' operating within all Dutch hospitals, since 2014 [9]. They play a key role through pro-active surveillance of antibiotics use. In the Radboudumc, the A-team monitors the use of antibiotics, promotes the switch from intravenous to oral submission and warrants therapeutic drug levels [9]. Current evidence shows that this kind of antimicrobial stewardship decreases the length of hospitalisation [12].

New Therapies

New antibiotics are needed to tackle the current crisis. However, less than five percent of pharmaceutical investment is used for antimicrobial development [6]. It seems unprofitable to invest in a market when there is a worldwide call to cut back on its product. Therefore, it is important to create a new system in which developers are properly rewarded for their product [6]. On the other hand, we can look at alternatives to antibiotics. A recent review in The Lancet identified 19 'candidates', meaning they mostly still require basic research and translation in clinical trials, in various degrees [13]. We will now further look into a few promising alternatives.

Antibodies are widely used in medicine, because of their specific binding properties. This exploitation of the natural human immune system goes for treating autoimmune diseases, cancer and skin disorders among others, but could also further aid in treating infectious diseases when administered passively. For example, when bowel bacteria are decimated by antibiotics, *Clostridium difficile* sometimes grows in pathogenic proportions. Bezlotoxumab (a registered engineered antibody) binds and neutralises the toxins produced by *Clostridium difficile*, providing temporary passive immunity against infection

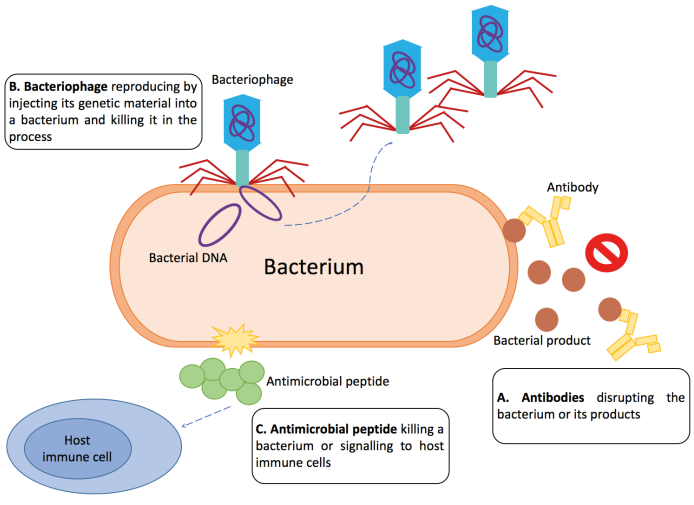

Figure 1: An overview of possible alternatives to antibiotics.

A: *Antibodies can be used to disrupt the bacterium in its environment (e.g. communication) or by binding to its products (e.g. toxins), amongst others.* ***B:*** *A bacteriophage infects a bacterium by injecting its own genetic material. The reproduction of new bacteriophages will eventually kill the bacterium as they burst out to find new bacteria to infect.* ***C:*** *These complex peptides, called antimicrobial peptides, naturally occur in our bodies and those of other multi-cell organisms. They can both modulate immune response (e.g. chemotaxis) and have bactericidal properties, meaning they can kill the bacterium. Unlike bacteriophages, they have very low specificity.*

caused by these toxins [14]. Another strategy, proven beneficial in a mouse model, is the disruption of communication between bacteria using antibodies causing less severe infection (figure 1A) [15].

Bacteriophages are another popular mentioned alternative to antibiotics. These so-called bacteria infecting-viruses occur throughout nature. In contrast to antibiotics, they tend to be very specific for the species as well as the strain of bacteria (figure 1B). Thereby, it is a potentially useful strategy if the bacterium causing an infection is identified, but imposes a problem if not, because then one would require a cocktail of bacteriophages [16]. Other challenges like rapid elimination by the body or development of resistance could be overcome by genetically engineering bacteriophages [13]. More research is needed to better understand the interaction between bacteriophage, human host (pharmacokinetics) and microbiome before large-scale implementation [16]. The earliest anticipated registration for clinical use is around 2023 in the U.S. and Western Europe [13]. Another take on fighting bacteria in this area of research is using just the purified lysins, which are the bactericidal enzymes produced by bacteriophages [13, 16]. *For more information take a look at our news item on the 4th of February 2018 on www.ramsresearch.nl*

Another promising area of research is the use of antimicrobial peptides. These peptides are naturally occurring in numerous multi-cell organisms aiding in host defence by binding to the plasma membrane of susceptible microbes. Apart from bactericidal properties, they are also able to modulate host immune response (figure 1C). Moreover, they are effective at very low concentrations and have a very broad spectrum of activity. Alike other alternatives, this group imposes many obstacles if used systemically, namely, proteolysis in the digestive tract, toxicity against red blood cells (and other host membranes) and high costs [17].

Most likely, alternatives to antibiotics will not serve as monotherapy right away, but rather as adjuvants in their first clinical trials, and prove their efficacy. Some alternatives could aid in preventing bacterial infections themselves, like vaccines. In the long run, combinations of alternatives or even monotherapy could be effective to combat pathogenic bacteria [13]. The need for new antibiotics and alternatives in the Netherlands right now is not pressingly urgent (in contrast to other parts in the world), but with a sustained rising number of resistant bacteria, we will eventually need them in the future [18]. Until then, it is important to preserve our current antibiotic effectiveness as much as possible for many years to come.

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Conclusions

With the emergence of CPE, which is resistant to almost all antibiotics, one could argue that we are standing at the dawn of a post-antibiotic era. In the Netherlands, the current antibiotics have still got a lot of life in them, by means of careful prescription and good surveillance. Still, with modern levels of globalisation this is a global problem and it is top priority for the WHO. New antibiotics are needed and, therefore, a new investment system is needed. Also, extensive research has provided a few alternatives to traditional antibiotics and some are expected to be used in clinics within a decade. Most promising alternatives include antibody therapy, bacteriophages and antimicrobial peptides, but most are still in a pre-clinical phase, making current antibiotics still the backbone of combating bacteria.

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CORRECT ANSWERS TO THE EXAM QUESTIONS

Answer question 1:
A. Efflux

The efflux mechanism entails resistance towards tetracyclines, which is often plasmid-mediated and attributable either to active efflux pumps. These are generally specific for tetracyclines or proteins that protect the ribosome from tetracycline action. With this mechanism, tetracyclines are removed from the bacterial cell by increased expression of efflux pumps. Enzymatic changes as a resistance mechanism can occur in antibiotics such as fosfomycin and aminoglycosides. Higher production of target binding sites as a resistance mechanism occurs in glycopeptides and lipoglycopeptides, while lower membrane permeability occurs in β-lactams.

For further reading: Hooper, D.C. Bacterial Resistance to Antimicrobial Agents in Harrison's Principles of Internal Medicine, 20e, Vol. (McGraw-Hill Education, New York, NY, 2018)

During the exam, 35% of the participants answered this question correctly.

Answer question 2:
B. Filters encapsulated bacteria from the blood circulation

In the absence of a spleen (asplenia), the natural filtration of microbes in the blood does not take place and leads to a predisposition to fulminant infections by encapsulated bacteria. Asplenia leads to lower production of antibodies since the proliferation and differentiation of antibody-producing plasma cells take place in the spleen and lymph nodes. Complement factors are specifically synthesised by the liver, not the spleen. Also, the complement system is responsible for opsonisation by phagocytosing cells, not lysis. Splenic macrophages and dendritic cells are responsible for the uptake of microorganisms and microbial products in the blood. The macrophages and dendritic cells then stimulate the B and T cells that arrive in the spleen from the blood. Natural killer cells can kill encapsulated bacteria when these are opsonised with antibodies (antibody-dependent cell-mediated cytotoxicity), but this is not specific to the spleen.

For further reading: Fischer, A. Primary Immune Deficiency Diseases in Harrison's Principles of Internal Medicine, 20e, Vol. (McGraw-Hill Education, New York, NY, 2018) and Parham, P. Elements of the Immune System and their Roles in Defense in The Immune System, 4e (Garland Science, New York, NY, 2014)

During the exam, 48% of the participants answered this question correctly.

The exam questions can be found back on page 7 in this journal.

MYTH OR SCIENCE?
LOSING WEIGHT WITH LIGHT PRODUCTS

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Critical Appraisal

Nowadays, almost every product in the supermarket also has a light version or is even replaced by it. These products become more and more popular and, in the Netherlands, there is even a plan in which these products will get a subsidy [1]. The reason for this is the fact that overweight and obesity are becoming a serious health problem with a rising incidence each year [2]. Being overweight is a problem for about one-third of the European population, who in general have a calorie-dense diet and a relative lack of exercise [2]. This results in a net positive energy balance and increases the risk of getting overweight or obese [3]. The long-term-associated health outcomes include dyslipidaemia (an abnormal amount of lipids in the blood), coronary artery disease, type 2 diabetes mellitus, hepatic steatosis (fat accumulation in liver cells), cerebrovascular disease, and insulin resistance [3]. Stimulation of using light products might contribute to losing weight [1]. However, some theories believe that light products are no better for your weight than the normal products and that they might even be unhealthy or harmful. Are these theories myth or science?

Introduction

About 86% of the Americans use light products, according to a survey from the Calorie Control Council, but what is the difference compared to the normal product [4]? The definition of a light product is determined by law; it contains at least 30% less energy (kcal), fat or sugars compared to the regular product [5]. A product that contains at least 30% less fat, contains, in general, more sugar than the normal product [6]. Therefore, it contains the same number of calories or even more [6]. In a product that contains more than 30% less sugar, these sugars are in general replaced by low energy sweeteners (LES) [4]. These are synthetic or natural chemical compounds that stimulate the sweet taste receptors on the tongue [4]. LES contain (almost) no calories and are hundreds to thousands times sweeter than normal sugar [4]. In Europe, there are nine approved LES and those all have an E-number, which means that they have been checked by the European Food Safety Authority and are considered to be safe [7]. One of the most commonly known and used LES is aspartame, also known as E951 [7]. It is added to over 6,000 products like light drinks, chewing gum, yoghurt and toothpaste [7].

Light products and weight loss

In the hope of losing weight, many people that are overweight choose to consume light products containing LES instead of products containing sugar. In theory, this would be a good choice. There is no doubt about the fact that LES reduce the energy density of foods and beverages [8]. Therefore, this can contribute to a reduced energy intake and caloric deficit over the day with subsequent weight loss in the long-term [8]. However, some theories suggest that the consumption of LES would contribute to overeating and/or increased energy intake and therefore weight gain.

First, consuming light products would reflect a type of cognitive process in which a light product is perceived to be 'healthy' and that this grants permission to overconsume this product or other 'non-healthy' foods [8-11]. However, the consumption of beverages sweetened with LES has no effect on total daily energy intake [12]. Also, LES consumption is not significantly related to later energy intake [9,13-16]. It does not matter whether the participants were blinded for the intervention or not [9]. Another mechanism that would increase energy intake is the relationship between the caloric density of food and sweet taste changing due to LES consumption [9,17-20]. For people consuming LES, the validity of a sweet taste as a signal for caloric food would be weakened and the ability to evoke responses that maintain energy homeostasis would be degraded [8,9,18-20]. However, a large number of studies report only limited effects of various LES on glycaemic, insulinemic and gut hormone responses [9,12,21-23]. Also, studies comparing LES to unsweetened products, water or nothing suggest that the exposure to sweetness itself is not a significant stimulus for later energy intake [9,13-16].

Another theory, commonly spread via social media and food bloggers, is that LES would have an effect on the cephalic phase insulin response [24]. Consumption of LES would increase the insulin release due to the sweet taste [24]. Contradictory to the intake of glucose, there will be no increase in sugar in the blood which would result in a low blood glucose level [24]. Therefore, this would lead to overeating, increased energy intake and weight gain [24]. However, a recent randomised controlled trial, published in 2018, with a sample size three to nine times greater than previous similar trials, found no effect on the insulin concentration for daily aspartame consumption for 12 weeks [25]. Also, another randomised controlled trial found no effects in glucose and insulin response on the day of LES consumption [12]. In addition, multiple other studies showed no acute changes in the release of insulin after consuming LES [8,21,22,26,27]. Therefore, there is a lack of evidence to confirm the theory that LES would not increase the insulin release, neither direct after consumption, nor after long-term use.

In 2016, a systematic review of the evidence from human and animal studies on LES consumption and body weight was performed, including meta-analyses [9]. In total, 141 articles reporting 335 studies or comparisons were included; these were animal studies, observational (prospective cohort) studies, short-term intervention studies and sustained intervention studies [9]. The main conclusion of this systematic review is that the consumption of products containing LES instead of sugar leads to reduced energy intake and body weight, in children and adults [9,13-16,28-30]. Therefore, this systematic review supports the disempowerment of the aforementioned theories that LES consumption would contribute to overeating and/or an increased energy intake and therefore weight gain.

Light products and health effects

In light products, most discussion is about the safety of LES, especially about aspartame and its metabolites [4]. Aspartame has an acceptable daily intake (ADI) level of 40 mg/kg/day, which is equal to 2.5 litres cola light [4]. This level is determined using the "no observed adverse effect level" with daily exposure [4,31]. Intake below the ADI level is, therefore, considered to be safe. In most European countries the ADI level is not exceeded, even not in worst-case scenarios [32]. After the intake of aspartame, it is hydrolysed by esterases and peptidases in the gastrointestinal tract [31]. Aspartame is broken down into methanol and two amino acids: aspartic acid and phenylalanine [31].

An extreme excess of aspartic acid would cause hyperexcitability of neurons and lead to the degeneration of astrocytes and neurons since it is a precursor of the amino acid glutamate [31]. Very high concentrations of phenylalanine would contribute to reduced levels of dopamine, serotonin and noradrenaline in the brain [31,33]. This would be due to an extreme excess of phenylalanine that would block the neutral amino acid transporters (NAAT) [31]. These transporters are used to transport various amino acids over the blood brain barrier which are important for neurotransmitters' synthesis [31]. For adults, daily intake of aspartame is about 2-10 mg/kg/day and 50% of this is transformed into phenylalanine [34]. For a typical person weighing 75 kilograms and a consumption of 10 mg/kg/day, this would be 375 milligrams g of phenylalanine a day. However, normal daily intake from the diet of the essential amino acid phenylalanine from protein-rich products like milk, eggs and meat is about 2.5-3 grams [35]. Therefore, even when the ADI of 40 mg/kg/day is reached, only 1.5 grams of phenylalanine would be formed from aspartame against a normal daily intake of 2.5-3 grams (figure 1). In addition, phenylalanine is an essential amino acid and it is important in the structure and function of many proteins and enzymes [35]. A recent study even found an inverse association between a higher dietary intake of phenylalanine and obesity risk [35]. Only 40% of aspartame is transformed in the amino acid aspartic acid. Aspartame intake of the ADI for a typical person weighing 75 kilograms, would then lead to 1.2 grams against a normal daily consumption for adults from the diet of about 5 grams (figure 1) [35]. Normal aspartame consumption (below the ADI) will, therefore, never lead to aspartic acid and phenylalanine concentrations high enough to cause effects in the brain [32]. Only in people with the metabolic disease phenylketonuria, phenylalanine will lead to negative effects in the brain. This is because they are not able to break down phenylalanine, which will accumulate [36].

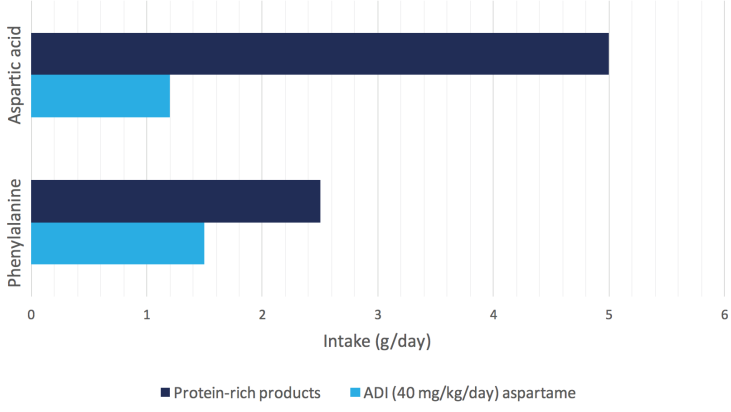

Figure 1: Comparison of phenylalanine and aspartic acid intake from protein-rich products and aspartame consumption.

After aspartame consumption, it is transformed in phenylalanine (50%) and aspartic acid (40%). These amino acids would actually have effects in the brain. However, for a typical person, weighing 75 kilograms, an aspartame intake of the ADI (40 mg/kg/day) is equal to only 1.5 grams of phenylalanine per day. On the other hand, the normal daily intake of phenylalanine from protein-rich products is about 2.5-3 grams per day. An aspartame intake of the ADI is equal to 1.2 grams of aspartic acid per day. The normal daily intake of aspartic acid from protein-rich products, on the other hand, is about 5 grams per day. Therefore, phenylalanine and aspartic acid concentrations due to the consumption of aspartame will never reach concentrations high enough to cause effects in the brain. In addition, daily intake of aspartame almost never reaches the ADI; normal daily intake is only 2-10 mg/kg/day, resulting in even lower phenylalanine and aspartic acid concentrations because of aspartame consumption.

Another commonly heard claim is that aspartame would be carcinogenic and genotoxic. There is some *in vitro* evidence for induction of chromosomal damage, however, this is probably not due to primary DNA damage but to a secondary effect of cytotoxicity [37]. DNA repair assays did not show any DNA-damaging properties for aspartame [38]. Also, some other studies found no *in vitro* and *in vivo* induction of gene mutations [37,39,40]. In general, there is limited evidence to suggest that consumption of aspartame may increase the risk of certain cancers [41]. For normal consumption of all LES, the possible risk to induce cancer seems to be negligible [38].

Diet can influence the gut microbiota profile and analysis in mice and humans suggest that LES would disrupt the balance and diversity of gut bacteria [42]. The gut bacteria have an important role because specific enzymes that are not encoded in the human genome are employed by the microorganisms in the gut [43]. These enzymes enable the host to extract calories from otherwise indigestible foods [43]. Altered microbiota, due to consumption of LES, would increase hepatic lipogenesis in adipocytes, thereby promoting storage of calories harvested from the diet into fat [44]. Moreover, this altered microbiota would also be correlated with disease markers linked to obesity; impaired glucose tolerance and elevated fasting blood-glucose levels [45]. However, the gut microbiota is very complex and there is no conclusive evidence on this topic [42]. This might be because of individual variability, interactions, unique chemical composition and metabolism of different LES and the dose that is consumed [42].

Conclusions

Some theories suggest that inclusion of light products containing LES in the diet promotes energy intake. This would be due to several mechanisms caused by LES like a disbalance in energy homeostasis, overeating of other 'non-healthy' foods due to cognitive processes and an effect on the cephalic phase insulin response. Therefore, the addition of these products would also lead to an increase in body weight. However, several studies, including a recent very extensive systematic review, conclude the opposite; products containing LES contribute to a reduced energy intake and body weight.

The second finding of this article is that consumption of LES below the ADI is considered to be safe. For example, genotoxic and carcinogenic health effects of LES are in general negligible. Also, the theory that aspartame and its metabolites would have some health effects in the brain is invalidated. The metabolites aspartic acid and phenylalanine would lead to the degeneration of astrocytes and neurons, respectively and to decreased levels of serotonin, dopamine and noradrenaline in the brain. However, normal consumption of aspartame below the ADI will never reach to concentrations high enough to cause these effects. Even an intake higher than the ADI will lead to phenylalanine and aspartic acid concentrations much lower than the normal daily intake from protein-rich products. Last, there is no conclusive evidence that LES would change the composition of gut bacteria and the possible consequences

of this. In conclusion, products containing LES contribute to a reduced energy intake and body weight and as long as there is no excessive use of these products there are no health consequences.

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ZEBRAS OF MEDICINE
COMPARING HARLEQUIN ICHTHYOSIS AND ICHTYOSIS VULGARIS

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Abstract

Review

BACKGROUND: Ichthyoses are a group of cutaneous disorders that are characterised by cutaneous scaling. Ichthyosis vulgaris is relatively common, and most of these patients have a mild phenotype. Harlequin ichthyosis is a rare disorder, its presentation is impressive, and this disease has a high mortality rate.
OBJECTIVE: This article aims to introduce ichthyoses as a group of cutaneous disorders and to highlight ichthyosis vulgaris with harlequin ichthyosis.
CLINCAL PRESENTATION: Ichthyosis vulgaris is caused by loss-of-function mutations in the *FLG gene*. Children born with ichthyosis vulgaris do not show any signs of disease at birth. It is often sufficient to treat these patients with moisturising ointments and keratolytics.
Harlequin ichthyosis is caused by loss-of-function mutations in the *ABCA12* gene. In contrast to ichtyosis vulgaris, children born with harlequin ichthyosis typically present with extreme hyperkeratosis. The barrier function of the skin is impaired due to scaling, which can result in a high risk of infection and other negative effects. Therefore, the mortality rate in the neonatal period is high, which is why the newborn should be treated in a neonatal intensive care unit. In a later stage in life, ointment needs to applied on the child's skin multiple times a day and frequent baths need to be taken to wash off excess skin.
CONCLUSIONS: There are multiple mutations identified that cause a specific type of ichthyosis. Phenotypes can be widely varying in severity of disease.

KEYWORDS: cutaneous disorders, hyperkeratosis, *FLG gene*

Introduction

To understand cutaneous disorders like ichthyosis, it is first important to know more about the different layers of the skin. The skin has a total of three layers: the epidermis, the dermis and the hypodermis. The epidermis is the outer layer, and it has three functions: producing new epithelial cells (keratinocytes), expressing skin tone through melanocytes and providing a waterproof barrier to the outside world [1]. The epidermis itself is also made of layers, mentioned here from top to bottom: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. New keratinocytes are produced in the stratum basale. While these new cells are produced, old cells are pushed up to the stratum corneum. The upper layer, the stratum corneum consists of 15-30 layers of dead keratinocytes, and these slowly shed to make room for new cells. In a healthy skin, the stratum corneum is replaced entirely in about four weeks. The dermis is the middle layer of the skin and is composed of connective tissue. It contains a lot of structures, such as hair follicles, sweat glands, nerves, blood and lymph vessels. The hypodermis is the last layer of the skin and connects the dermis with the underlying fascia of bones and muscles. The hypodermis consists of loose connective tissue and fat, providing fat storage and insulation [1, 2].

Ichthyoses are a heterogeneous group of cutaneous disorders that are characterised by abnormal epidermal differentiation caused by genetic mutations. If a patient has ichthyosis, the process of renewing keratinocytes and transporting them from the stratum basale to stratum corneum is disturbed. A minor genetic error can cause a skin with an impaired barrier function and/or hyperkeratosis and scaling, as seen in ichthyosis [3]. Examples of these errors are: abnormal lipid composition, absence of lamellar membranes and incomplete differentiation of keratinocytes.

The word ichthyosis is derived from the Greek word *ichthys*, which means fish. It refers to the cutaneous scaling seen in this disease that resembles scales of a fish. This scaling can be localised or generalised [4]. Some of the disorders within this group are rare, others more common. Ichthyosis vulgaris, whereby most patients have a mild phenotype, has an estimated incidence of one in 250 live births [5]. Variants that are rare, such as lamellar and harlequin ichthyosis, have an estimated incidence of one in 200,000 live births. In this article, we will highlight ichthyosis vulgaris with harlequin ichthyosis. Furthermore, we will discuss its presentation, the first care of the newborn, diagnosis and further treatment options.

Clinical presentation

Children born with ichthyosis vulgaris do not show any signs of the disease at birth. During their first year of life, fine white to grey scaling will appear. The scaling is most prominent on the abdomen and extensor surfaces of the extremities, while flexures and face are spared.

Children born with harlequin ichthyosis typically present with extreme hyperkeratosis and already fissured skin, resembling a knight wearing a body armour. Commonly present is eversion of the eyelids (ectropion), eversion of the lips (eclabium) and deformation of the ear. These newborns often have respiratory distress at birth due to restricted chest wall expansion. After seeing a newborn with harlequin ichthyosis, one will remember it for a lifetime. Sometimes children born with severe forms of ichthyosis, whether it is ichthyosis vulgaris or harlequin ichthyosis, manifest at birth with a collodion membrane. This thin transparent membrane covers the newborn's body for his first few weeks of life, resembling plastic foil. The collodion membrane slowly disappears with the application of ointment, and will sometimes be replaced by hyperkeratotic skin with dark scaling. A self-healing collodion baby also exists, whereby the membrane slowly disappears and is replaced by healthy skin [6].

First care for the newborn

Newborns affected by severe forms of ichthyosis are exposed to various risks. They need supportive care to prevent infection, dehydration and starvation. The barrier function of the skin is impaired due to scaling and

impaired barrier function of the skin, which can result in transepidermal water loss and increased heat loss. Therefore, the newborn should be treated in a neonatal intensive care unit and his vital signs should be closely monitored. An incubator with increased humidity around 50-70% is ideal, as the newborn will dehydrate quickly. Furthermore, a calorie-dense diet should be offered because of the high metabolic rate of these newborns. Currently the mortality rate in the neonatal period for harlequin ichthyosis is around 50%, but improved neonatal intensive care and oral retinoids may increase survival [7]. The use of oral retinoids will be explained later in this article.

Diagnosis

Diagnosis of ichthyosis is a combination of clinical findings and histopathologic findings. The physician first examines the skin phenotype and pays attention to the following details: scale pattern, skin colour, presence of a collodion membrane, erythroderma, erosions or blistering, photosensitivity and hair abnormalities. Parents are asked about the time of onset, development over time and the family history regarding ichthyosis. A skin biopsy can provide additional information to find the diagnosis. Genetic testing is more specific, as multiple genetic mutations have been identified that cause syndromes associated with ichthyosis, such as the Kallmann syndrome and Netheron syndrome.

Ichthyosis vulgaris is caused by loss-of-function mutations in the *FLG* gene and is autosomal semi-dominantly inherited [8]. Patients with one mutated allele have a mild phenotype, patients with two mutated alleles have a more severe phenotype. Still, the phenotype varies considerably. Histologically, ichthyosis vulgaris is characterised by a reduction of keratohyalin granules or an absence of the stratum granulosum in electron microscopy.

Harlequin ichthyosis is caused by loss-of-function mutations in the *ABCA12* gene [9]. *ABCA12* encodes for an ATP-binding cassette transporter, which is a lipid transporter in keratinocytes. These mutations result in a loss of the skin lipid barrier. Histologically, harlequin ichthyosis is characterised by extreme hyperkeratosis, follicular plugging and the absence of lamellar bodies and lipid bilayers in electron microscopy [10].

Further treatment

Patients with ichthyosis vulgaris often experience seasonal variation in severity. The condition of the skin improves in warm and sunny weather with a high degree of humidity and worsens in dry and cold weather. Moisturising ointments and keratolytics are often sufficient as treatment. Patients with ichthyosis vulgaris have an increased risk for atopy, especially atopic eczema because of the impaired skin barrier and should be checked for asthma and allergies

Patients with harlequin ichthyosis that survive the neonatal period need lifelong treatment. Ichthyosis is a chronic skin disorder that takes a lot of time to manage, especially for the parents or caretakers. Ointment needs to applied on the child's skin multiple times a day and frequent baths need to be taken to wash of excess skin. Monitoring vitamin D levels is also important, as children with harlequin Ichthyosis tend to get limited sun exposure [11]. Intermittent use of oral retinoids such as acitretin is often prescribed. Oral retinoids are frequently used in keratinisation disorders of the skin, such as psoriasis or ichthyosis. Retinoids normalise epidermal cell proliferation, differentiation and cornification [12]. They also accelerate shedding of hyperkeratotic skin [13]. Futhermore, they help to reduce ectropion of the eyelids without needing surgical procedures [12, 14]. However, there are some serious side effects associated with the use of retinoids, which includes teratogenicity, dryness and irritation of mucous membranes, thinning of the skin, hair loss, photosensitivity, hepatotoxicity, hyperlipidaemia and skeletal abnormalities [13].

Furthermore, psychological aspects of this disease should not be forgotten; support from other patients and a psychologist could be helpful. However, there is very little scientific research available about the quality of life of patients with ichthyosis. A specific quality of life questionnaire has been developed for families of patients with ichthyosis, but no other studies have been published using this questionnaire [15]. *Readers who are interested in stories from a patient's perspective, can visit the website of the Foundation for Ichthyosis and Related Skin Types at http://www.firstskinfoundation.org.*

Conclusion

Ichthyoses are a heterogeneous group of cutaneous disorders. There are multiple mutations identified that cause a specific type of ichthyosis. Phenotypes can be widely varying in severity of disease. Newborns with harlequin ichthyosis need to be admitted to a neonatal intensive care unit, but unfortunately the mortality rate is around 50%, whereas patients with ichthyosis vulgaris are born healthy and only show symptoms later in life.

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SCREENING FOR BREAST CANCER: WHAT DOES THE FUTURE BRING?

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Abstract

Practice Innovations

BACKGROUND: Breast cancer has become an enormous burden on society due to the daunting consequences on physical- and emotional health, as well as the effects on healthcare expenditures. Since breast cancer incidence is at the top of cancer incidences in females, a screening program is crucial to detect early cases of breast cancer and subsequently improve breast cancer prognosis. Although the benefits caused by the current mammography screening program outweighs the harms, the high numbers of screened individuals necessitate the development of alternative techniques to reduce the total burden caused by misdiagnosis and over-treatment.
OBJECTIVE: In this review, new approaches are discussed which show high potential for future screening programs. The first approach that is discussed is molecular imaging, followed by breath- and urine analysis.
CURRENT RESEARCH AND FUTURE DIAGNOSTICS: Current research focuses on the molecular mechanisms of disease, as well as biomarkers. These approaches (molecular imaging, breath- and urine analysis) show high sensitivity and specificity with regards to breast cancer detection. Although the initial results are very promising, further research must be performed in order to prove their clinical relevance for the application in a screening program for breast cancer screening.
CONCLUSION: Several approaches are currently researched and are tweaked in order to gain a higher sensitivity and specificity for the application as a breast cancer screening program.

Introduction

Consequences of breast cancer cast an enormous burden on human health, healthcare costs and emotional suffering at a population-wide level [1]. From data between 2005 and 2009, a one in eight probability of developing breast cancer during a lifetime has been estimated [2]. Breast cancer incidence in the Netherlands is estimated to be a devastating 18,000 new cases in 2017 and the incidence is increasing every year [2]. These numbers make breast cancer the most common form of newly detected cancer in females with a frightening 30% of all female cancer incidences [1]. Furthermore, with a 14% mortality rate, breast cancer is the second leading cause of female death due to cancer [1]. Since female breast cancer is considered a major health concern, screening programs are implemented for the reason that early diagnosis improves breast cancer prognosis, resulting in an estimated 20% reduction of breast cancer mortality [3]. This review first sets out the limitations of current practice as a population-based screening program. Then, it will introduce the ideal characteristics of future diagnostics and lastly, it will introduce promising techniques that are currently under development to inform you what the future of breast cancer screening might bring.

Current practice

Currently, screening programs mainly utilise X-ray mammogram technology in order to detect breast cancer at an early stage [3]. However, alongside the harms caused by mammography (e.g. exposing women to low doses of radiation, which may cause cancer), a screening program has a serious impact on screened persons such as increased anxiety, discomfort and more serious: over-diagnosis [3, 4]. Over-diagnosis is the detection of cancers due to screening which would otherwise not have gained clinical relevance, which is estimated at approximately three percent in the Netherlands [3, 5]. Furthermore, mammography has limitations when it comes to breast cancer screening, such as the detection of breast cancer in females who have high density breast tissue (due to the lack of X-ray contrast in these tissues). However, these women are individuals with a markedly higher risk of developing breast cancer [6]. Also, breast cancer can remain undetected due to overlapping breast tissue, which obscures the tumor, and leads to misinterpretation of mammograms [7, 8]. Including the aforementioned limitations of mammography, other limitations resulting in false-positive and -negative results contribute to the four billion overspent dollars (in the United States) due to under- and overdiagnosis [9]. Although these benefits are considered to outweigh the harms, other methods are necessary in order to diminish harms and increase the benefits of breast cancer screening.

Future diagnostics

When mammography was implemented as a screening method, an alternative was the well-known and appreciated MRI-technique. Utilising MRI in screening programs mainly increases the diagnostic value in hard to evaluate, dense breast tissue, as well as occult breast cancer with metastasis at other cancer sites [10]. Unfortunately, the main reason why utilisation of MRI is not as feasible as a breast cancer screening program is the drawback of high costs that come with the use of MRI [10]. Therefore, in order to improve the current breast cancer screening program, future diagnostic techniques should minimise harms, increase benefits (such as MRI) and suppress healthcare expenditure. In the next sections, the following three promising diagnostic tests, which are currently under development are briefly summarised: molecular imaging, breath- and urine biomarker analysis [4, 11].

Researchers are currently exploring molecular imaging as an approach to perform breast cancer screening. Despite the almost ancient theory (year 1929) [12], this technique only recently cleared the road for a more patient acceptable diagnostic technique, in contrast to mammography, by oral administration of a pill. This pill contains a label that can be detected using near-infrared (NIR) fluorescence (light with a wavelength between 650-900 nm) [4]. The fluorescent label (fluorophore) is coupled with a targeting ligand, the part of the agent that can specifically bind the diseased site [4]. After ingestion of the pill and absorption by the intestines, the targeting ligands move toward the highly expressed integrin $\alpha v\beta 3$ receptors of tumor cells and can be identified using both spatial (3D imaging) and molecular information due to the coupled NIR fluorophores (Figure 1) [4]. Even though this method of cancer screening is expected to be theoretically superior to current practice, since it results in a feasible, safe and cost-effective screening approach, several obstacles have to be overcome in order for this method to be applicable in a clinical setting [4]. First, the pharmacokinetic (movement of the drug through the body) properties of the labels need to be optimised for human application with regards to human variability. Individuals that only absorb a low amount of the drug have a lower contrast between tumor specific fluorescence

and non-specific fluorescence coming in the body, which complicates interpretation for clinicians [4]. Furthermore, one single biomarker is not able to differentiate all tumors of the breast or metastasised tumors. Therefore, additional biomarkers with a different targeting ligand, which is detected at different wavelengths, should be combined in order to establish a more specific screening method [4]. Although this 'easy' applicable method looks promising, aforementioned barriers need to be overcome. After overcoming the barriers, clinical trials need to be performed in order to determine efficacy and safety in humans before its application as a population-based screening method [4].

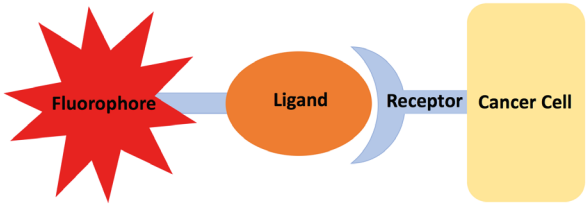

Figure 1: Principle of molecular targeting.
A fluorophore is bound to a targeting ligand that specifically binds avβ3 receptors of cancer.

In extension to the molecular imaging technique, this section elucidates on breath analysis as a breast cancer detection technique. Breast cancer is usually accompanied by oxidative stress alongside the induction of specific hepatic enzymes (polymorphic mixed oxidase Cytochrome P450 enzymes) [13]. Both the aforementioned processes affect the amount of outgassed volatile organic compounds (VOCs), which are hydrocarbons that originate from within the body. These outgassed VOCs mainly consist of (methyl-) alkanes, which are produced during lipid degradation of cell membranes [13, 14]. This origin nominates them as suitable biomarkers for breast cancer detection [13]. Exhaled VOCs are measured using a two-minute breath test and are subsequently quantified by means of gas chromatography [11, 14]. Data from the measured gasses of each individual patient are put into a predictive model for breast cancer, which has shown superior sensitivity and specificity in comparison with the current practice, but larger studies need to be performed to validate these findings [14].

Alongside breath exhaled-biomarkers, a similar technique utilises the measurement and evaluation of microRNAs (miRNAs) in urine samples as breast cancer biomarkers [11, 15]. MiRNAs are small non-coding RNAs which exhibit important post-transcriptional regulating properties, such as the modulation of tumor suppressor genes and oncogenes [15]. Aberrant miRNAs are involved in cancer progression as a consequence of (1) epigenetic mechanisms, (2) genetic alterations, (3) defects in the miRNA biogenesis pathways and (4) transcriptional repression [16, 17]. Analysis of miRNAs in urine samples enables tumor detection, estimation of tumor progression and tumor drug resistance [18, 19].

The approach of utilising biomarkers (VOCs and miRNAs) to detect breast cancer might allow us to specifically detect breast cancer with a high sensitivity and specificity. Furthermore, this approach would be a very rapid diagnostic test and cost-effective.

Conclusion

To summarise, multiple promising screening methods for breast cancer detection are currently under development that might overcome the limitations that come with mammography. Molecular imaging has gained high expectations with regards to feasibility, safety and cost-effectiveness, but several barriers have to be overcome such as the pharmacokinetics and technical interpretation before clinical application is possible. Regarding biomarker approaches utilising breath- and urine analysis, breast cancer biomarkers may contribute to early detection of breast cancer and might enhance prediction of tumor progression and identification, although more research is necessary in order to evaluate its value for clinical application with regards to a population-wide specificity, sensitivity and economical cost-effectiveness. All three methods are still far from implementation, but they have reached high expectations this far and might be what the future will bring us.

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RECENT HIGH-IMPACT PAPERS FROM RADBOUDUMC RESEARCHERS

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Summary

With over 3,000 publications per year, scientific research is a cornerstone of the Radboud university medical center [1]. In this section, recent high-impact papers with an impact factor higher than 5 – published by researchers from the Radboudumc – will be discussed.

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Rare immune cells kick-start anti-cancer immune response

To reduce the mortality rates from cancer, there is need for new treatments. The past years, a lot of research is being done in immunotherapy, specifically on a vaccination that enhances immune cells to clear cancer cells [2]. This article from Nature Communications (impact factor 12) focusses on dendritic cells in this subject. Dendritic cells (DCs) are a rare type of immune cells that scan the body for infectious invaders and also cancers, which make them important for immunotherapy. Type I interferon (IFN) is a messenger molecule that plays a central role in the initiation of immune responses and that is the key driver of immunity to infections and cancer. Plasmacytoid DCs (pDCs) are a certain type of DCs that produce large quantities of type I IFN. The mechanisms that control this process are key to more effective treatment for cancer but are poorly understood. To shed some light on these processes, researchers from Radboudumc and Eindhoven University of Technology developed a nano lab to better understand cell-cell variability and the fundamentals of cellular communication. In the developed device, millions of droplets containing a single cell were produced. In this way, the type I IFN response could be studied at the single-cell level within a tunable microenvironment. The research team of Florian Wimmers (from the Radboudumc research group Dendritic Cell Therapy) tested thousands of single pDCs for their ability to produce type I IFN. They discovered that only a subset of pDCs produces type I IFN and that the pDCs collaborate to amplify their activity and generate population-driven type I IFN responses. Developing future interventions that target pDCs should take into account that not all pDCs are the same and that their activity is dependent on their local density.

Influence of breast compression pressure in mammography screening

In mammography, breast compression is applied to reduce the thickness of the breast to ensure acceptable image quality. A parameter indicating the amount of compression is not available and little is known about the relationship between the amount of breast compression and breast cancer screening outcomes. The research of Katharina Holland, Nico Karssemeijer and Ritse Mann from the Department of Radiology and Nuclear Medicine of the Radboudumc found results that indicate that when high pressure is applied during mammography, this may reduce sensitivity [3]. Sensitivity was computed by the number of screen-detected cancers and interval cancers diagnosed between two screening rounds. Results showed a decrease in sensitivity between the groups with lower and higher forces. The cause for this reduction in sensitivity is not clear. In contrast, if pressure is low this may decrease specificity. More research into this subject and more attention to a meaningful standardisation of compression levels might improve the quality of mammography in the future. This article was published in Breast Cancer Research (impact factor 6).

Thermogels to the rescue

The changing of wound covers can cause pain and possibly disrupts wound repair. Novel synthetic thermosensitive hydrogels based on polyisocyanopeptide (PIC) may offer a solution. The architecture and mechanical properties of PIC gels closely resemble collagen and fibrin, which are essential for wound repair. The gels are liquid below 16 °C and become gels beyond room temperature. This reversible thermoresponsive behaviour facilitates that PIC gels are easy to apply and remove. Adding arginyl-glycyl-aspartic acid (abbreviated to RGD-peptide, which is a ligand for integrins) to the PIC gel may increase biocompatibility due to enhanced cell binding capacities. Roel op 't Veld from the Department of Biomaterials of the Radboud Institute for Molecular Life Sciences studied the use of PIC gels in mice wounds together with a multidisciplinary team from the Radboudumc (Dentistry; theme Reconstructive and regenerative medicine), Radboud University (Molecular Materials), and the Association of Dutch Burn Centres [4]. Full-thickness dorsal skin wound models were compared to wounds treated with PIC gel and PIC-RGD gel for three and seven days. No foreign body reactions and similar wound closure rates were found in all groups. Granulocyte populations (indicating an immune response against bacteria) in the wound decreased significantly in the PIC-gel groups, probably because bacteria could not penetrate the gel. RGD-peptides did not improve the effect observed for the PIC gel. In short, the PIC gels did not show adverse effects, were easy to apply and their possibilities for bio-functionalisation make them suitable for further development into wound dressings. The article was published in Biomaterials (impact factor 8).

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RAMS

A Word from the Board of RAMS

Dear reader,

I hope you enjoyed reading the twelfth edition of RAMS, which is also the first edition that was put together by the new editorial board. I would like to take this opportunity to compliment our fifth editorial board for this accomplishment.

Coming back to the edition, the number twelve is not only famous for its classical biblical meaning of perfection but is also famous in modern science. For example, the human body has twelve cranial nerves that are important for most of our perception and make communication with others possible.
Another example of the number twelve in medicine is the duodenum, the first part of the small intestine. The etymological origin of the word 'duodenum' is the Latin word duodecim, translating into 'twelve'. In Dutch and German this part of the bowel is called 'twaalfvingerige darm' and 'Zwölffingerdarm', respectively, describing its length which was originally measured by using the widths of one's fingers. Nowadays, the etymological meaning of the duodenum is still valid as the duodenum's length of twelve fingers is also approximately twelve inches.

Last but not least, a part of you, our readers who are medical students, will study for approximately twelve semesters during which you will learn about much more than the twelve cranial nerves and facts about the duodenum. We, as RAMS, also try to impart knowledge to students and I want to invite you to help us broaden the students' knowledge.

On behalf of the board of RAMS,

Leonhard K. Neumann
Vice-chair of RAMS 2018-2019

General Board

RAMS is directed by the general board, which consists of five (bio)medical students. As members of the board they frequently meet to make sure all activities run smoothly. Moreover, they are in close contact with the supervisory board and the editorial staff. If you have any questions on general, promotional or financial subjects, please contact the general board of RAMS via voorzitter.rams@ru.nl.

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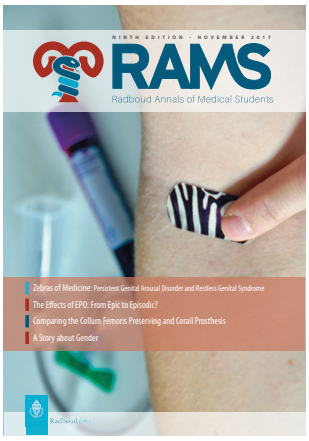

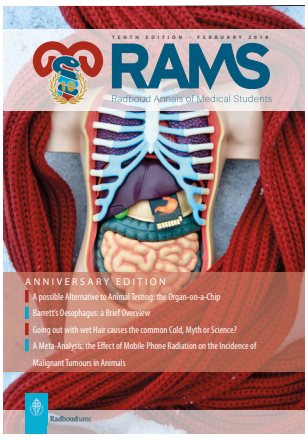

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