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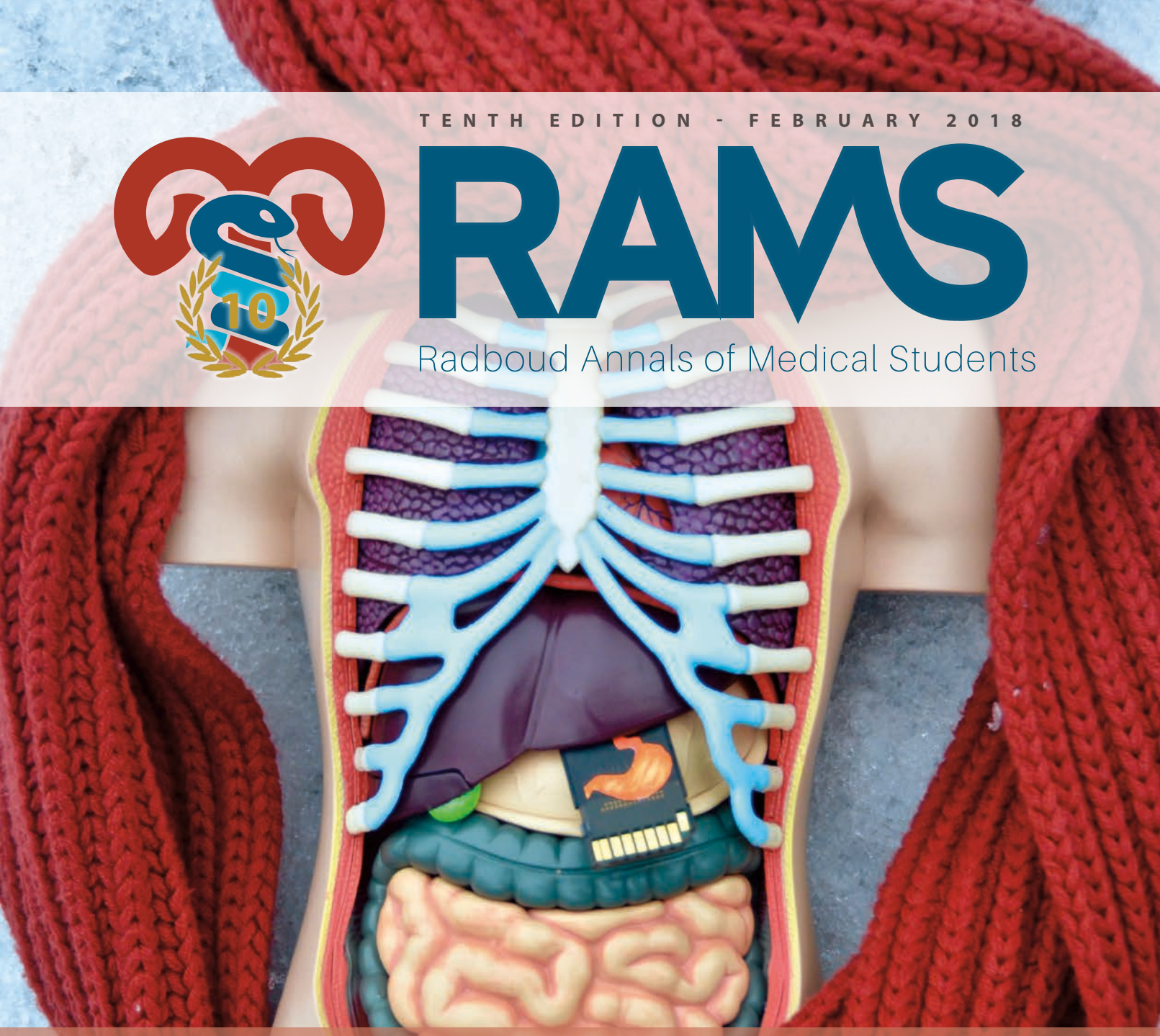

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FROM THE EDITORIAL BOARD

Dear readers,

It is my pleasure to present you the tenth edition of RAMS. This is a good moment to remember where and how it all started and to envision what we want to accomplish in the future. Especially for this edition, we asked the founders of RAMS to write about their experience in creating RAMS and what they think of the current status of RAMS. I also want to point out that we keep exploring new possibilities to improve RAMS. For example, quality and validity of the articles are major topics for RAMS. Therefore, from this edition forward, not only the scientific but also all editorial articles published in RAMS will be checked by a specialist in that specific field. This will not only provide us with more learning experiences but also increases the validity of all articles.

As Scientific Editor-in-Chief, I want to emphasize once more the importance of staying up-to-date and practising according to the evidence and not just 'common sense'. Sometimes, we may think something is true and we can substantiate it with logical reasoning, but evidence proves us wrong. For example, in one of my internships, I encountered a patient with liver cirrhosis who needed pain medication. Logically, I did not want to prescribe paracetamol, since paracetamol can damage the liver, and wanted to resort to non-steroidal anti-inflammatory drugs (NSAIDs). However, after an extensive literature search, evidence pointed out that paracetamol in normal dosage is not dangerous at all for patients with liver cirrhosis, but NSAIDs can possibly be fatal. This knowledge was tested in general practitioners and internists. It turns out that 95% of the general practitioners and 70% of internists prescribed, incorrectly, NSAIDs over paracetamol in patients with liver cirrhosis. So, even though we think something is logical, we still need to follow the evidence as the basis of our practice.

In this edition, you can read evidence in the form of a meta-analysis on a long-debated subject, namely if mobile phone radiation can cause tumours. Furthermore, you can read about whether wet hair makes you more susceptible to catch a common cold, or if this is just a myth. Moreover, in this edition is discussed how technology can affect biomedical research in an article about organ-on-a-chip. We also present you two articles about the upper gastrointestinal tract. One is a review on Barrett's oesophagus and another is the recently created recurrent "Zebras of Medicine", which will focus on how to differentiate between gastroesophageal reflux disease and achalasia. Furthermore, our Chair of the Editorial Board and last year's Vice-Chair went to the Congress of the Nederlandse Vereniging voor Medisch Onderwijs (NVMO) to give a roundtable discussion wherein was spoken about the ins and outs of a scientific medical journal for medical students. You will find a short report of this experience of the Congress on page 17.

Yours faithfully,

Joost Kools
Scientific Editor-in-Chief

INDEX

WORD FROM THE FIRST BOARD

Dear readers,

Thank you for reading this anniversary edition of RAMS. It might be hard to imagine nowadays (hopefully), but not too long ago RAMS did not yet exist.

About four years ago, two fellow medical students (Michiel Schoenaker and Rick Verstegen) came up with the idea of creating a medical journal aimed at and made by students of our medical faculty. I vividly remember my own application interview for a position on the first board of RAMS. It must have been the end of 2013. It was so exciting to be involved in all the first steps, together with fellow enthusiastic board members and editors.

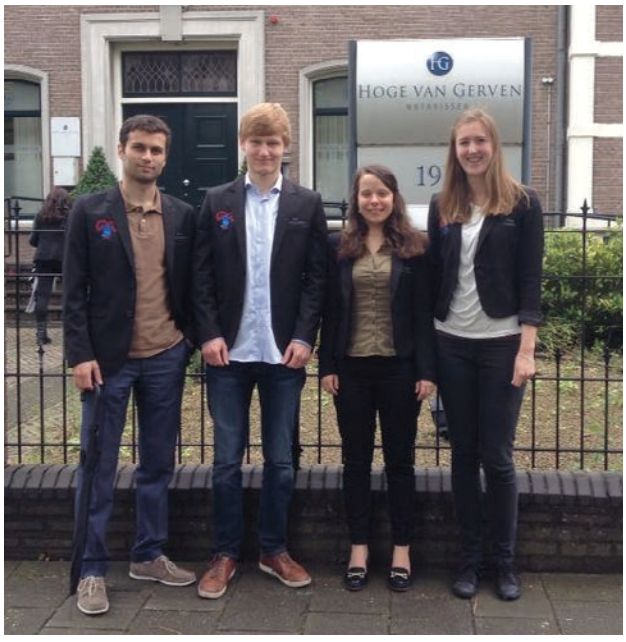
All aspects had to be covered. From designing a logo to coming up with an alternative website address (rams.nl was already taken...) and from optimizing our internal structure to organising masterclasses for all new editorial members and reviewers. One of the highlights of that first period was the establishment of the associated foundation. I still remember that moment with my fellow board members, all suited up, at the notary office. Another highlight surely was our presentation for the board of directors of the Radboudumc, in which we pitched the idea of RAMS. After they pledged their moral (and not unimportant: financial) support, the real work on the first trial edition of RAMS started. I still remember the hunt for the first article in RAMS and the excitement of the first paper that was submitted to us.

So much development has taken place since that first trial edition. RAMS has grown out to be one of the main journals of our faculty. Meanwhile, I have graduated, but every time I return to the Radboudumc or faculty I am so proud to see yet another new edition of RAMS on display.

I would like to thank the current members of the (editorial) board for the opportunity to contribute to RAMS one more time. I hope you all enjoyed making this tenth (!) edition of RAMS just as much as we did in creating the first.

Enjoy RAMS!

Tessa Schoot
First Chair of RAMS



Picture 1: *The corresponding foundation of RAMS, Stichting Medisch Wetenschappelijk Studenten Tijdschrift Radboud universitair medisch centrum, was established on June 4th, 2014 by Barov Sanaan, Lars Gallée, Josianne Luijten and Tessa Schoot (here shown from left to right).*

Picture 2: *Cover of the pilot edition of RAMS, published in June 2014.*

GOING OUT WITH WET HAIR CAUSES THE COMMON COLD, MYTH OR SCIENCE?

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Introduction

Opinion

We have all heard our mother or grandmother say: "Do not go out with your hair all wet like that, you will catch a cold!". Just like me, most have never paid much attention to this well-meant warning and dismissed it as if it was nothing. Nonetheless, it has been a much-debated topic for centuries. It is a common folklore that exposure to a cold environment can be associated with the development of the common cold. The theory is that wet hair, clothes and feet can lead to acute cooling of the body surface which in turn can cause symptoms of a common cold. But, as we all know as (bio)medical students, the common cold is caused by a virus. Is it possible that there is a link between viral infections and a cold environment? Is there some truth in this theory linking being cold and the common cold, as it is such a widespread belief?

The common cold

Before we look further into the question if going outside with wet hair can lead to the common cold, it is important to know what this common cold entails. First of all, the most common symptoms of the common cold are nasal stuffiness and discharge, sneezing, a sore throat and often coughing as well. We, as (bio)medical students, would refer to it as a harmless upper respiratory tract infection caused by a virus infection, most often with a rhinovirus. Although the common cold is a self-limiting illness in most of the cases, the viral infection can spread, which leads to more severe complications such as sinusitis and pneumonia in some cases. In addition, the common cold is an enormous burden on society since it often leads to absences from work [1]. In 2016, according to the Centraal Bureau voor de Statistiek (CBS), (2017), over 41% of the Dutch population reported to have experienced the common cold. The flu and common cold are in every age category the biggest reason for school and workplace absenteeism in the Netherlands. So, even though the common cold is harmless in most cases, it would be beneficial to prevent it. If indeed a relation between acute body cooling and the common cold exists, this could pave the way for preventive measures.

Viral behaviour

During the winter months, half of the of Dutch young adults and adults experience the common cold, the flu and/or tonsillitis. While in the summertime, it is only one third, according to the CBS (2010). An explanation for this is that viruses spread easier during the winter months because people stay indoors and people are in more close contact with each other as a result. Another contributing factor, in this case, is the increase of indoor heating levels during winter time. There is a continuously recirculated body of air which has a very low humidity, making it easy for a virus to spread. It is also hypothesized that decreased ambient temperature increases physiological stress, consuming more energy for thermoregulation. This effort can, in turn, weaken the immune system, which can lead to an increased susceptibility to infection [2]. In summary, there are plenty of reasonable arguments on why only acute body cooling will not lead to a cold, but is this enough proof?

Old wife's tale...

An argument that is often used against the so-called "myth", is that the common cold is caused by a virus and you cannot get a virus from just being cold. A virus can be detected in approximately 80% of the cases of the common cold, most often a rhinovirus [3]. It is thought that in the other 20% of the cases, detection of a virus is not yet possible because the virus causing the common cold, has not been identified yet [1].
Many articles on the internet state that it is a myth that you can catch a cold from being cold or going out in the cold without wearing a warm jacket. However, most of these articles are not supported by scientific proof, they simply call it an old wives tale. Although research on this topic has been conducted, most studies neither confirm nor deny that there might be a connection. Decades ago, in 1967, an article by Douglas, Couch and Lindgren was published, titled: "Cold does not affect the 'common cold' in study of rhinovirus infections". In their study, different doses of rhinoviruses were used to inoculate volunteers who were free of detectable antibodies to this virus. The test subjects were either placed in a cold room of 4°C or in a water bath of 33°C for up to 2,5 hours. All the test and control subjects who received the highest dose of the rhinovirus became infected, opposed to none of the subjects who received the lowest dose. Based on these results, Douglas et al. concluded that 'cold' in the common cold is something of a misnomer [4].

… or scientifically proven?

Interesting research concerning the underlying causes of the common cold has been conducted by Ronald Eccles, former director of the Common Cold Centre in Cardiff, Wales. In 2002, he published an article in which he stated a new hypothesis regarding the effects of acute body cooling based on previous research. According to Eccles, acute cooling of the body surface causes vasoconstriction, also in the nose and upper airways. This vasoconstriction leads to inhibition of the local respiratory defences and a subclinical infection can convert into a clinical infection. These local respiratory defences consist out of a non-specific immune response. Because of the vasoconstriction, this non-specific immune response becomes less effective. This reduced effectiveness results from the reduction in blood flow to the airway epithelium, reducing the supply of nutrients and leukocytes to the site of infection. In addition, the temperature of the airway epithelium will drop because of the reduced supply of warm blood. On top of that, cooler temperatures enable replication of the common cold virus by diminishing the immune response [5]. Not everyone infected with a virus will show symptoms, but all the factors stated above can converse a subclinical infection into a clinical infection [6].

Eccles and his colleague Johnson decided to set up a study in which they wanted to determine if acute chilling caused common cold symptoms. They randomized 180 healthy study subjects to receive either a foot chill or control procedure. The group that was assigned to the chilling procedure was asked to take their shoes and socks off and place their

bare feet in a bowl containing up to 10 litres of water. The subjects kept their feet in the 10°C water for twenty minutes. The group allocated to the control procedure was asked to keep their socks and shoes on and they had to place their feet in an empty bowl for the same twenty minutes. Immediately after the procedures, the subjects had to score common cold symptoms. In addition to this, they had to score the same symptoms twice a day for 4 to 5 days. While there was no immediate difference between the two groups, there was a delayed effect of the chilling. The total symptom scores for days 1-4/5 following the chilling procedure were significantly higher than the symptom scores for these days in the control group. Out of the 90 chilled subjects, 26 (28.8%) were suffering from a cold, as opposed to 8 out of 90 (8.8%) control subjects. These results suggest that there is an association between acute cooling of the body surface and the onset of common cold symptoms. Although these results are promising, further research is necessary to determine if the development of these common cold symptoms following exposure to cold is associated with infection [7].

Current research at the Radboudumc

Solid evidence is hard to find. Luckily, there is still research being conducted on this matter, also here at the Radboudumc. PhD students Charlotte de Bree, Marlies Noz, Rob ter Horst and Anne Jansen from the Department of Experimental Internal Medicine are currently working on a project to learn more about the influence of cold weather on the response of the immune system. For this project, they and their colleagues collected data from 200 healthy volunteers at the Lowlands festival this summer. Their test subjects took a cold bath of 16°C for 4 minutes. Before and after the cold bath, a bit of blood was drawn from the subjects. With this research, these Radboudumc researchers hope to elucidate the effect of acute cooling on the immune system by testing the susceptibility of isolated cells for the flu virus.

All in all, there might be some truth in the advice given by my mother not to go out with wet hair. Although it is not the reason for giving you a cold, it might trigger one. In the vast majority of common cold cases, a virus can be detected. Centuries ago, Douglas et al. came to the conclusion that the 'cold' is not a factor in the development of the common cold, but that a virus is. New research has been conducted on this topic since, revising this conclusion. Eccles et al. proved that there is an association between acute cooling of the body surface and the onset of common cold symptoms, but further research is necessary to give a definitive conclusion.

In the context of better safe than sorry even when I am in a hurry, I will dry my hair before I hop on my bike to get to class.

Acknowledgements

RAMS would like to thank Marlies Noz, MD, and Rob ter Horst, MSc, from the Department of Experimental Internal Medicine of the Radboud university medical centre Nijmegen for supplying the author with insight regarding this topic.

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BARRETT'S OESOPHAGUS: A BRIEF OVERVIEW

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Introduction

Mini Review

Barrett's oesophagus (BO) is a condition in which the normal oesophageal squamous epithelium is replaced by columnar epithelium. This process is called metaplasia. BO is considered as a benign pre-stage of distal oesophageal adenocarcinoma and occurs as a result of prolonged gastro-oesophageal reflux, which also causes symptoms of heartburn.

Epidemiology and risk factors

The occurrence of BO differs worldwide with a prevalence of 1.6% in Sweden [1] and 5.6-6.8% in The United States [2]. These percentages are likely underestimated due to the lack of symptoms related to BO. Gastro-oesophageal reflux is the main risk factor to develop BO, yet only 7.8% have symptoms of heartburn [3]. Patients older than 65 years have a higher prevalence of BO with a prevalence of 19.8% and 14.9%, respectively, in patients with and without symptoms of heartburn [4]. Besides reflux and age, other risk factors for the development of BO are central obesity (OR 1.98; 95%-CI 1.52-2.57) [5], male gender (OR 2.16; 95%-CI 1.84–2.53) [6], increased BO segment length (OR 1.25; 95%-CI 1.16–1.36), and the presence of a hiatal hernia, which is present in 76.9% of the patients with BO [7]. Additionally, BO is more frequent in patients who have ever smoked cigarettes (OR 1.67; 95%-CI 1.04-2.67) [8].

Malignant progression

During the last decades, the number of patients with adenocarcinoma has been rising and the incidence has increased sixfold [9]. In patients with BO, the risk of progression to adenocarcinoma is 0.25-0.70% per year, which is 24 times higher than in the general population [10-13]. This risk is higher in men and in patients with long-segment BO. If oesophageal adenocarcinoma has developed, the 1-year and 5-year survival are 50% and 20% respectively, but these rates get better if the cancer is recognised in an early stage [14]. The prognosis of patients with adenocarcinoma is dismal, American Cancer Society brought out the first estimates for 2017; 16,940 new oesophageal cancer cases and 15,690 deaths from oesophageal cancer [15]. To prevent malignant progression, intensive surveillance programs are offered in patients with BO (see paragraph Prevention and Surveillance).

Pathobiology

The oesophageal wall is originally covered by squamous epithelium. In patients with BO, this squamous lining is replaced by columnar epithelium. Gastro-oesophageal reflux leads to inflammation of the oesophageal wall (i.e. reflux oesophagitis). Prolonged oesophageal reflux may alter oesophagitis into BO, followed sequentially by low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually oesophageal adenocarcinoma. Specifically, reflux of bile can lead to oxidative stress and is associated with carcinogenesis [16]. Regularly, one BO segment comprises multiple different islands (which coexist like mosaic); one island could contain LGD, while the other contains HGD [17].

Three types of columnar cells are found in a BO segment; 1. the junctional or cardiac type (which is generally located at the gastroesophageal junction), 2. the gastric type, 3. the intestinal type. Mainly the intestinal type is known to predispose malignant progression [18]. Some guidelines advocate that intestinal metaplasia (IM; the replacement of squamous cells by intestinal type cells) is required for BO diagnosis, but other guidelines fear underdiagnosis if replacement by the cardiac or gastric type is not detected [19-21].

Helicobacter pylori (H. pylori) is associated with symptoms of heartburn, chronic gastritis, peptic ulcer disease and IM of the gastric epithelium. However, it is thought that *H. pylori* plays a protective role against BO and the development of adenocarcinoma (OR 0.50) [22].

Symptoms

Metaplasia of the distal oesophagus (BO) itself does not cause any problems. However, gastro-oesophageal reflux disease (GORD) is a major risk factor and has the following symptoms: regurgitation, heartburn and dysphagia [19].

Diagnosis

The healthy oesophageal mucosa has a pale colour, in contrast to BO, which is recognised by bright salmon-coloured mucosa extending above the gastro-oesophageal junction (Figure 1). The gastro-oesophageal junction is defined as the transition zone between the stomach and the oesophagus, which can be recognised as the proximal end of the gastric folds. For diagnosis, histologic confirmation (by taking a biopsy) and a segment of more than 1 cm are required [23]. Another reason of taking biopsies is to rule out coexisting HGD or adenocarcinoma. These biopsies are obtained during gastroesophageal endoscopy according to the Seattle protocol, which comprises targeted tissue sampling of visible nodules and four-quadrant random biopsies (i.e. 12, 3, 6 and 9 o'clock) with 2 cm intervals up to the proximal end of the Barrett's segment. If the segment is shorter than 2 cm, at least four biopsies should be obtained [24].

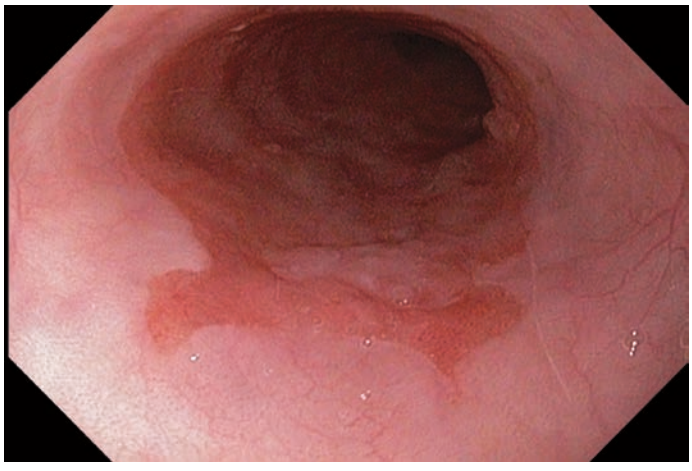

Figure 1: *Endoscopic view of a Barrett's oesophagus segment.*

During endoscopy, the Barrett segment is described with the Prague C&M classification by assessing the circumferential (C) and the maximum (M) length of the salmon-coloured mucosa in centimeters (Figure 2) [25,26]. Histologic analysis according to the Seattle protocol has several drawbacks: 1. it prolongs the procedure time, 2. the adherence to the protocol by the endoscopist is reduced for patients with longer Prague segments 3. this biopsy method often only samples 4-6% of the whole salmon-coloured surface [27], 4. the interobserver agreement between pathologists is often low. During the last decades, new techniques have been developed (e.g. Narrow Band Imaging (NBI), Volumetric Laser Endomicroscopy (VLE), Confocal Laser Endoscopy (CLE), WATS3D) to address this problem [28].

A frequently used technique during endoscopy is NBI. NBI uses high-intensity blue light to enhance capillaries in the mucosa and the mucosal patterns. An irregular mucosal pattern with increased vascularity is suspicious for HGD [29].

Treatment

Acid suppression
Proton-pump inhibition (PPI) is the treatment of choice in patients with BO. This agent suppresses acid production by the inhibition of H+/K+ ATPase of the gastric parietal cells in the fundus and the corpus of the stomach. Hillman et al [30] found a hazard ratio of 20.9 for developing HGD or adenocarcinoma in patients who did not receive PPI-treatment. However, this effect has never been proven in prospective trials.

An alternative to the pharmaceutical approach is to create a mechanical barrier against acid reflux. One example of anti-reflux surgery is Nissen fundoplication; this technique aims to wrap the gastric fundus around the distal oesophagus and narrows oesophageal hiatus with stitches. Various studies have compared the pharmaceutical and surgical approach for anti-reflux therapy, but a statistically significant difference has not been found [31,32]. Yet anti-reflux surgery should be considered in treatment-resistant patients.

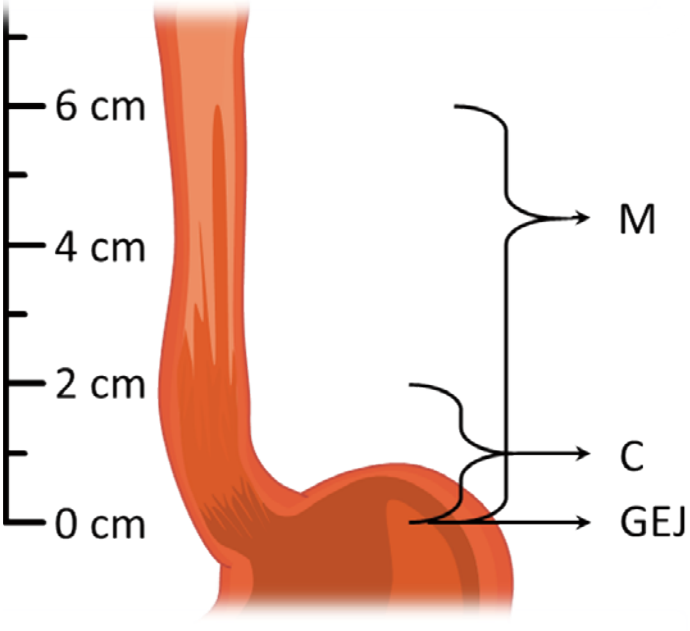

Figure 2: *The C&M Prague criteria. 'C' represents the circumferential Barrett's Oesophageal length in cm measured from the gastroesophgeal junction (GEJ), 'M' represents the maximal extent of the metaplasia in cm (C2M6) measured from the GEJ.*

Endoscopic treatment
Most Barrett's lesions can be treated endoscopically. In case of flat BO (without nodules), the abnormal mucosa is treated with ablative therapy. The approach that is frequently used is Radiofrequency Ablation (RFA), this technique eradicates the superficial layers of the oesophageal wall with high frequency energy. The device is passed through the biopsy channel and can eradicate large areas at once. Orman et al. [33] performed a large meta-analysis and showed complete eradication of IM and dysplasia in 78% and 91% of the cases, respectively. One drawback is stricture formation, which occurs in 5.6% of the cases [33], resulting in dysphagia. The RFA technique reduces the risk of progression to HGD or adenocarcinoma with 25% [34].

In case of nodular disease, endoscopic mucosal resection (EMR) is applied. It can be used prior to RFA or individually. The response rate is high (96.6%), but so is the stricture rate (37-88%) [35].

Oesophagectomy
In case of multifocal dysplastic lesions, oesophagectomy is considered, in which the entire oesophagus is surgically removed. This can be performed 'trans-hiatal', in which the oesophagus is approached from the abdomen through the oesophageal hiatus) or 'trans-thoracic' (e.g. Ivor Lewis procedure with an upper abdominal incision and a posterolateral thoracotomy). Williams et al. [36] studied the histology of oesophagectomy specimens in 38 patients with HGD, in 29% of the cases occult EAC was found. In case of only HGD in the pathology analysis, lymphadenectomy is not required [37,38].

Prevention and Surveillance
Secondary prevention focuses on the detection of a disease in a subclinical stage to treat in an early stage, which is related to better survival rates. Although the risk of oesophageal adenocarcinoma in patients with BO is relatively low [10-13], the high mortality, related to adenocarcinoma, calls for surveillance [14]. A Dutch BO expert panel recommends the following in patients with non-dysplastic BO [39] (see Figure 2 for the Prague C&M classification):

- No follow-up in case of a Prague length (M) of < 1cm
- Follow-up after 5 years in case of a Prague length (M) of 1-3 cm
- Follow-up after 3 years in case of a Prague length (M) of 3-10 cm
- Reference to a BO expert centrum in case of a Prague length (M) of >10 cm

Frequent surveillance in patients without dysplasia, elderly (>75 years) and patients with significant comorbidity is discouraged by recent Dutch (concept) guidelines. Patients with LGD should undergo treatment (e.g. RFA), since the risk that it also harbours HGD or adenocarcinoma is 14% [39] and if left untreated, 13% develops HGD or adenocarcinoma [40]. In case of HGD or adenocarcinoma, there should be a second evaluation by a pathologist experienced with BO. In case of HGD or adenocarcinoma, it is recommended (in the Netherlands) to refer the patient to one of the eight BO expert centres and let a pathologist, experienced with BO, do a second evaluation [39] .

Acknowledgements

I would like to thank Dr. Wallace and Dr. Wolfsen of the Department of Gastroenterology and Hepatology of the Mayo Clinic in Jacksonville (FL, USA) for sharing their knowledge about Barrett's Oesophagus and providing the image of the Barrett's segment, obtained during an endoscopic procedure.

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A POSSIBLE ALTERNATIVE TO ANIMAL TESTING: THE ORGAN-ON-A-CHIP

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Introduction

Perspective

New innovations are popping up everywhere, from daily life to science. In this article, we aim to shed some light on an innovation aimed at reducing the usage of animals while not compromising the goal of making research to human disease more translational: the so-called organ-on-a-chip models. Here, we will explain what organ-on-a-chip models are, the advantages and disadvantages of using them, their current applications and their future possibilities.

Background

For the past centuries, men improved and replaced many old techniques. Horse and carriage were replaced by the car, the bow and arrow were replaced by the revolver and the cassette tapes were replaced by the CD. The world of medicine does not differ from this cycle of replacement and innovation, thinking of the imaging techniques, immunotherapy, prostheses created with computer-aided design and so on. Despite all the great innovations, however, we are still confined to one very old part of medicine and science: testing on animals. Even though animal testing is being regulated more and more, we are still dependent on it. The conditions in which animal testing is allowed have been evolving over the years. For example, there are strict guidelines and it is forbidden to test cosmetics on animals. In the Netherlands, research institutions need a permit for every single project from the central committee of animal research (CCD) [1]. The permit is only given when the usefulness and necessity of trials sufficiently outweigh animal suffering, and when there are no alternatives available, like research on tissues. Because of these strict guidelines, researchers have to be very specific about what they want to investigate, which feels like a burden by most researchers. These strict conditions do not take away our dependence on animal research. Most of the animals are used for applied and translational research, legislation required toxicity and safety tests, and fundamental scientific research. In 2015 the Netherlands used over 500.000 animals for animal testing [2]. But in line with the cycle of replacement and innovation, could this number not be different? The goals are to reduce, refine and hopefully replace animal studies, also known as the 3Rs [3]. But how can something as complex as an animal be replaced? Organ-on-a-chip models might be the answer for that!

What is an organ-on-a-chip?

An organ-on-a-chip is a 3D cell culture device reproducing a microenvironment that mimics the activity, mechanics and physiological responses of an organ or organ system from the body. Simple organ-on-a-chip devices consist of a single compartment, using a single cell type cultured directly on a channel's surface. These cells can be exposed to fluid shear stress, which is physical stress acting on the luminal surface of cells in the direction of the fluid flow, thus creating a frictional force, comparable to the *in vivo* situation in tissues. More complex devices contain multiple compartments divided by (semi)permeable membranes to allow for transcellular transport. In order to study cell-cell interactions, different cell types can be used in the different compartments of the device. Creating a chip that mimics tissue environments is thus achieved by structural architecture, mechanical forces and co-culture with multiple cell types [4].

A multitude of different organ-on-a-chip devices resembling single organs exist. Some examples of these are the liver-on-a-chip, skin-on-a-chip and kidney-on-a-chip. The latter is an *in vitro* recreation of the microenvironment of the renal tube. For kidney cells, the 3D structure is essential for its function, because it creates the barrier between the blood and pre-urine. When this matrix is disturbed, the kidney no longer retains its function. A kidney-on-a-chip can be comprised of two layers of silicon. This creates two channels, which are then separated by a porous membrane coated with extracellular matrix components. Proximal tubule cells are most often cultured in 3D channels (Figure 1). This site in the kidney is of special interest because it is the primary site of drug clearance and reabsorption. Lastly, a physiological level of flow is applied to create fluid shear stress, which seems to be especially important for the right level of transporter expression, such as Na/K-ATPase and aquaporin-1. Consequently, this gives the epithelial cells more height and a polarity that more closely resembles the situation *in vivo* [5].

Current limitations

Though organ-on-a-chip systems seem promising, there are still some limitations to overcome before implementation can take place.

The biggest limitation of many organ-on-a-chip models is the fact that no primary cells, which represent the *in vivo* situation best, are used in experiments using the devices. Primary cells are difficult to culture and generally have a short life-span, making them less convenient for longitudinal usage [6]. However, using primary cells also has the advantage of being more translational.

Another limiting factor regarding organ-on-a-chip models is that they need to be reproducible in order for the results to be valuable and useful. Therefore, the system has to be validated with established assays with approved read-outs before broad implementation. Reproducibility of these models, both technically as well as biologically, is still a challenge due to the fact that there is no standardisation for important properties such as flow and pressure fluctuations. Furthermore, the sensitivity and specificity of the 3D model have to be compared to existing *in vitro*, animal and clinical data to make sure that the organ-on-a-chip models have the same responses as the organs in our bodies [7]. It thus can be said that the organ-on-a-chip models still need improving, but as these models were only coined a few years ago, they already came a long way.

Current applicability of the organ-on-a-chip

Not only are many researchers trying to perfect their organ-on-a-chip models, but they are also performing experiments to unravel mechanisms of disease and pharmacotoxicology with them.

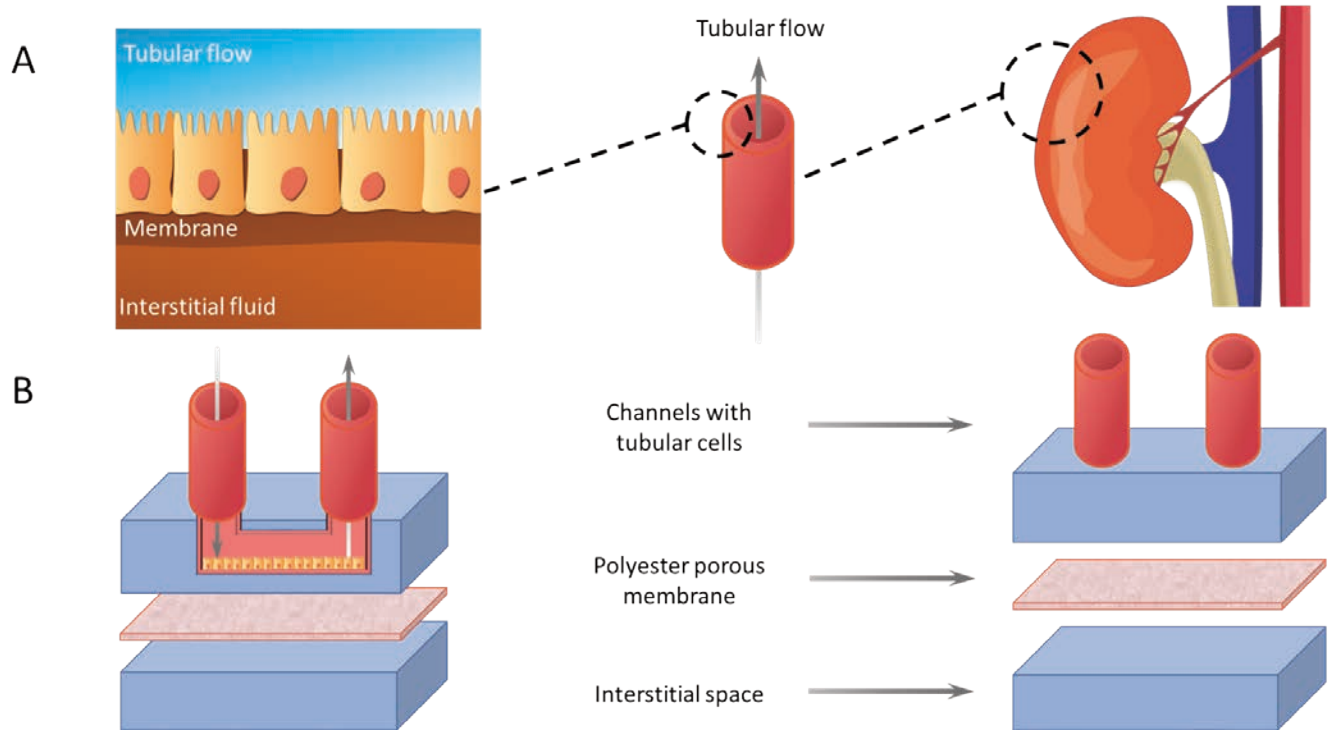

Figure 1: *The kidney-on-a-chip. The microfluidic device consists of two polymeric channels, resembling the proximal tubule and interstitial space, separated by a porous membrane coated with extracellular matrix components. Cells are cultured on top of the membrane, in the presence of a physiological level of flow. (b) Device assembly: The upper layer, polyester porous membrane, and lower layer are bonded together through surface plasma treatment.*

Wang et al. aimed to elucidate the mechanism underlying kidney damage in relation to exposure to high glucose levels, as seen in diabetic nephropathy, using a kidney-on-a-chip [8]. The first sign of diabetic nephropathy is commonly proteinuria. In proteinuria, patients lose albumin and other proteins through the kidney due to damage to the glomerular filtration barrier. Wang et al. used a kidney-on-a-chip that mimics this glomerular filtration barrier containing glomerular endothelial cells, basement membrane and podocytes. The artificial glomerulus was exposed to high concentrations of glucose in the blood compartment to mimic the pathological responses as seen in patients with diabetes mellitus. They found an increased barrier permeability to albumin caused by the high concentrations of glucose, which shows that the artificial glomerulus shows a similar response to high glucose levels as seen in patients. These results reveal that hyperglycemia plays a crucial role in the development of increased barrier permeability to albumin and thereby glomerular dysfunction leading to proteinuria. Moreover, the kidney-on-a-chip mimics diabetic nephropathy that has not been possible by cell-based and animal models, which makes it a useful platform for studying the mechanism of diabetic nephropathy and developing an effective therapy in glomerular diseases.

Kim et al. used the kidney-on-a-chip model to investigate which pharmacokinetic profile of a drug would result in the least nephrotoxicity [9]. Kidneys, together with the liver, are the most important organs for the metabolization and elimination of drugs. Research dedicated to determining the fate of substances in the body, called pharmacokinetics, thus often focuses on the kidneys and liver. However, being part of the pharmacokinetics of potentially very toxic drugs comes with a price for these organs: the kidney and the liver can be severely damaged. In most cases, it is necessary to use animal models to examine this so-called nephrotoxicity and hepatotoxicity, but animals and humans are not the same. The metabolism at cell-level is different, which makes animal studies less reliable. With the rise of organ-on-a-chip models, new opportunities arise. Kim et al. use a microfluidic kidney model containing epithelial cells to examine the nephrotoxicity of the antibiotic gentamicin. Gentamicin is mainly metabolized by the kidneys and is known for its nephrotoxicity and neurotoxicity [10]. The epithelial cells of the microfluidic kidney model were exposed to gentamicin using two different pharmacokinetic profiles: bolus injection and continuous infusion. The researchers concluded that gentamicin bolus injection causes less nephrotoxicity in their model compared to a continuous infusion regimen. For the use of organ-on-a-chip models, it means that the organ-on-a-chip is suitable because it seems to have the same reactions as the organ would have in the body. These studies show that even though the organ-on-a-chip models are still in development, they could already have an important role in refining toxicology studies in animal models.

Multi-organ systems

Diseases and toxicity seldom restrict themselves to a single organ but tend to disrupt homeostasis across multiple organ systems. With an animal model, this could be assessed because it is a multi-organ system by itself. Therefore, to simulate homeostasis disruption *in vitro*, a more challenging system with two or more organ chips is needed. With such a system one could study multi-organ physiological functions and pathophysiology in human cell lines directly [12].

Bauer et al. have given an example of a pathophysiological study using multiple organ-on-a-chip models. They developed a two-organ-chip model to study the interaction between human liver- and pancreatic islet cells. Type 2 diabetes mellitus (T2DM), with multi-organ morbidity, surely seems to be a good candidate for a multi-organ chip model. An analysis of rodent models mimicking human T2DM reveals significant interspecies differences at every level of glucose regulation. This seems to explain why animal studies have poor translations to understand and improve glucose metabolism in humans [12]. Bauer et al. interconnected liver spheroids, which are packages of cells with many features of the human hepatocyte and human pancreatic islet microtissue. In this chip-based model, glucose tolerance tests (GTT) showed a functioning

feedback loop for insulin and glucose. Moreover, when they performed the second and third GTT, they noticed that the islet microtissue had a reduced ability to release insulin. This indicates that prolonged hyperglycemia impairs islet function and therefore the multi-organ chip model mimics basic T2DM pathophysiology. Still, the model in this study is far from representing what happens *in vivo*, because the model only consists out of two organs. Additional organs could be incorporated such as kidneys, a gut, the heart or fat tissue, to further unravel the disease progression of T2DM. Also, a chip-based pathophysiological model could potentially provide a helpful tool to identify new drug targets [13].

Human-on-a-chip and other future ambitions

Thus far we reviewed the potential of an organ-on-a-chip and even a multi-organ system to help resolve problems of animal models. But what about a body-on-a-chip? Could such a system actually reflect *in vivo* parameters of the human body accurately?

A body-on-a-chip would consist of linked together human organ-on-a-chip models (Figure 2). This model would basically resemble a human, scaled down roughly 100,000 times. However, when the human body is scaled down to a micro-device model, imbalance seems inevitable [13]. Organ models would need to have the same relative volume as they have in the human body. In addition, a cell culture medium would be required that mimics blood and with the correct blood flow rate, because both factors influence diffusion across the endothelial membrane. However, there is lots of variety in the culture media used for differently established organ-on-a-chip models, because they use different cell lines. Therefore, making a body-on-a-chip is not as easy as just connecting existing organ-on-a-chip models. Regarding this compatibility issue, tissue engineers are still looking for the "Swiss Army knife" among cell sources [11]. Another consideration is the bioavailability of drugs, which is a phenomenon of drug disposition in the gut and liver or skin whereby the concentration of a drug is reduced before it reaches the systemic circulation [11,13]. Several companies, such as TissUse in Germany, are already trying to perfect this new technique. It is hard to tell when the human-on-a-chip will be common practice in laboratories, but experts estimate this to happen within the next 20-30 years.

There are numerous future ambitions for both human-on-a-chip as well as organ-specific chips. Preclinical testing of pharmacodynamics and kinetics, as well as body toxicology, would be exiting applications for the organ-on-a-chip models. Animals have a different genetic background and therefore often translate poorly to the human clinic [11]. Still, researchers are obligated to test their new medicines on at least two species of rodents and two bigger mammalian species. Altered legislation and involvement of regulatory agencies would be needed to implement the organ-on-a-chip models more in preclinical testing. This could eventually help bridge the gap between preclinical predictions based on animals and outcomes in clinical trials [13]. Furthermore, if newer stem cell technology is integrated into organ-on-a-chip models, personalised models

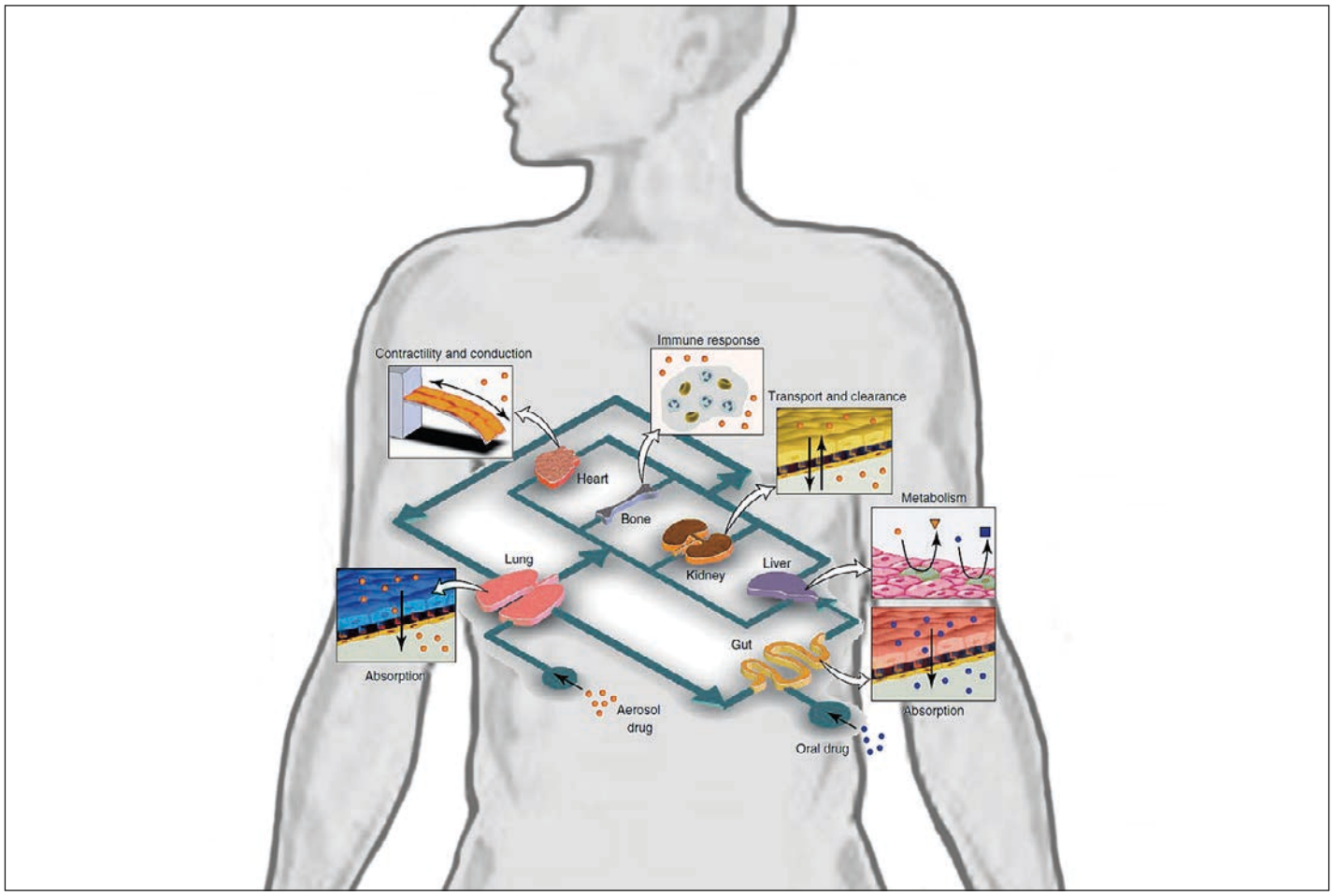

Figure 2: *Human-on-a-chip. Designing a whole body biomimetic device will potentially correct one of the most significant limitations of organ-on-a-chip models: the isolation of organs. Image from https://commons.wikimedia.org/wiki/File:Conceptual_Schematic_of_a_Human-on-a-Chip.jpg, user Timothy Ruban, reuse under CC BY-SA 3.0 licence.*

could make patient-specific predictions concerning toxicology and drug efficacy [13]. Moreover, organ-on-a-chip models could be used in research where actual clinical trials are hard to carry out (e.g. in paediatric diseases and rare diseases).

Conclusion

Organ-on-a-chip models have great potential in their various forms. Kidneys-on-a-chip already prove useful in pharmacokinetics. Multi-organ systems and the body-on-a-chip are likely to let us learn more about (patho)physiology and could allow for preclinical testing of new drugs. Hopefully, organ-on-a-chip models will eventually be able to replace animal experiments or reduce them to an absolute minimum, without the need for compromises; both ethical and scientific. In the meantime, the organ-on-a-chip can reduce the number of animals needed for research, by playing a role in studies like toxicology, with the great advantage of the genetic resemblance of the human.

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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 the bachelor!

We challenge you!

Question 1

A patient with an acute asthma attack is treated with intravenously administered prednisolone, in addition to inhalers. Prednisolone acts on the glucocorticoid receptor, a so-called nuclear receptor. When is the effect of prednisolone likely to occur?

A. Within seconds.
B. Within minutes.
C. Within hours.

(Topic: Farmatoxocology, Module Q6 Movement and Flow 2017)

Question 2

Gastric acid secretion can be inhibited by negative feedback from the intestine. Which of the following hormones from the duodenum inhibits the release of gastrin from the pyloric antrum?

A. Acetylcholine.
B. Bombesin.
C. Histamine.
D. Secretin.

(Topic: Digestion, Module Q6 Movement and Flow 2017)

The answers to these questions can be found on page 16 in this journal.

ZEBRAS OF MEDICINE

OESOPHAGEAL DYSPHAGIA: HOW TO DIFFERENTIATE BETWEEN ACHALASIA AND GASTRO-OESOPHAGEAL REFLUX DISEASE

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Abstract

Review

BACKGROUND: Gastric acid reflux is a normal physiologic process. It involves into gastro-oesophageal reflux disease (GERD) when the patient has unpleasant symptoms or mucosal damage of the oesophagus. Achalasia is a rare motor disorder of the distal oesophagus and lower oesophageal sphincter (LES). Achalasia is often not recognised by clinicians or confused with peptic strictures, a complication of GERD.
OBJECTIVE: This article aims to provide insight into the different forms of dysphagia, in particular, to differentiate between achalasia and GERD.
RESULTS: Achalasia and GERD are both causes of oesophageal dysphagia. Patients suffering from GERD as well as patients suffering from achalasia, show symptoms of dysphagia, heartburn and regurgitation. However, patients with GERD experience dysphagia only for solid foods. Patients with achalasia experience progressive dysphagia for solids and liquids. GERD can best be diagnosed by clinical symptoms, though an endoscopy is helpful if peptic strictures are suspected. The best diagnostic tools for achalasia are a barium swallow test and oesophageal manometry.
CONCLUSION: Differentiation between achalasia and GERD can be done by anamnesis and diagnostic testing. Patients with GERD typically complain about postprandial and nocturnal regurgitation. Patients with achalasia complain about regurgitation of undigested food, and dysphagia for solids and liquids. Diagnostic testing includes endoscopy, barium swallow tests and oesophageal manometry.

KEYWORDS: oesophageal dysphagia, GERD, achalasia

Introduction

Dysphagia is the medical term for difficulty in swallowing. It can be classified into oropharyngeal and oesophageal dysphagia. Patients with oropharyngeal dysphagia have difficulty initiating a swallow, and this could lead to regurgitation and aspiration. Patients with oesophageal dysphagia have the unpleasant sensation of food and or liquids being obstructed between mouth and stomach resulting in pain at the suprasternal notch or behind the sternum [1]. Gastro-oesophageal reflux disease (GERD) is common in the Western population. It causes heartburn and regurgitation. Heartburn is a burning sensation in the retrosternal area. Achalasia is a rare disorder in which there is a loss of normal peristalsis in the distal oesophagus and disfunction of the lower oesophageal sphincter (LES). The LES cannot relax properly, causing food particles to remain in the oesophagus. Often achalasia is initially not recognised by healthcare professionals, as the symptoms can be similar to other disorders of the digestive tract, such as gastro-oesophageal reflux disease (GERD) [2]. Achalasia and GERD are both forms of oesophageal dysphagia. Differentiation between achalasia and GERD might be a clinical challenge [1,3]. The aim of this article is to give insight into the different forms of dysphagia, in particular, to differentiate between achalasia and GERD.

Oropharyngeal and oesophageal dysphagia

To differentiate between achalasia and GERD, we must first differentiate between oropharyngeal and oesophageal dysphagia. Taking an accurate clinical history is key in differentiating between both types of dysphagia. Oropharyngeal dysphagia is also called transfer dysphagia and arises from the oral cavity, pharynx, upper oesophagus or upper oesophageal sphincter (UES). Patients with oropharyngeal dysphagia complain about repetitive swallowing, nasal regurgitation, coughing, nasal speech, drooling, choking, halitosis and/or recurrent pneumonias [1]. Oropharyngeal dysphagia is often present in neurological patients. Therefore, it is helpful to include neurological symptoms and family history in your anamnesis.

Patients with oesophageal dysphagia have different symptoms. They experience discomfort a few seconds after initiating a swallow, and often locate their pain distal to the suprasternal notch. This type of dysphagia can be caused by both liquids and solids. Patients may have a history of heartburn, scleroderma, congenital oesophageal webs and rings or radiation therapy [1]. Once again, an accurate clinical history can give direction to oropharyngeal or oesophageal dysphagia, but diagnostic tests are needed most of the time. Different causes of oropharyngeal and oesophageal dysphagia can be found in Table 1 and 2 of the appendix on *www.ramsresearch.nl*.

Gastro-oesophageal reflux disease and peptic strictures

Gastric acid reflux into the oesophagus is a normal physiologic process. Gastro-oesophageal reflux becomes GERD when it causes unpleasant symptoms or mucosal injury to the oesophagus [4]. GERD is common in the overall population with a prevalence of 10-20% in western countries, and 5% in Asia [5]. GERD is frequently diagnosed in adults from Western countries, whereby smoking and obesity seem to increase the risk for developing GERD [6]. Heartburn and regurgitation are characteristic symptoms of GERD. Complications include reflux oesophagitis, ulceration, peptic strictures, Barrett's oesophagus and adenocarcinoma of the oesophagus [4]. Peptic strictures are a result of healed erosive oesophagitis lesions. Collagen is deposited during this healing process, and the collagen fibers may contract and narrow the oesophageal lumen. These peptic strictures lead to progressive dysphagia for solid food only. Peptic strictures occur in up to 10% of patients with GERD, but this number decreases with proton-pump inhibitor (PPI) use [1]. GERD can be diagnosed based on the symptoms described above. To diagnose peptic strictures, an endoscopy is needed and a histological biopsy can be

performed if a malignancy is suspected [1]. Treatment of benign peptic strictures comprises of dilation by a mechanical or balloon dilator. Short-term outcomes are good, longer-term outcomes are best when a luminal diameter greater than 12mm is achieved by dilation [7]. Patients with peptic strictures should be treated with a PPI, to prevent more damage to the oesophagus. Apart from surgical and pharmacotherapeutic management, lifestyle and dietary modification should be recommended [8]. Weight loss is recommended for overweight patients and the use of tobacco and alcohol should be diminished. Selective elimination of dietary triggers, such as fatty or spicy foods, can be useful in some patients. Elevation of the head during the night is useful in patients with nocturnal symptoms [6]. These lifestyle modifications are recommended for all patients with GERD. An overview is given in Table 1.

Achalasia

Achalasia is a primary oesophageal motor disorder caused by a lack of myenteric neurons that coordinate oesophageal peristalsis and LES relaxation. However, it remains unclear what causes this lack. It is a rare disorder, with a prevalence of 11 per 100.000 adults. Incidence increases with age, with a mean age of 53 years at diagnosis [9]. Some patients have symptoms for years before achalasia is confirmed. Symptoms are dysphagia, regurgitation of undigested food, respiratory symptoms such as a nocturnal cough, aspiration and pneumonia, chest pain and weight loss [2,3]. Dysphagia after meals and heartburn can lead to misdiagnosis as GERD. However, achalasia leads to progressive dysphagia for both solids and liquids. Moreover, regurgitation caused by achalasia is unresponsive to adequate use of PPI, as the problem is located in the oesophagus instead of the stomach [3]. Achalasia can be treated with pneumatic dilation or laparoscopic surgical myotomy [10]. These are preferred options for initial treatment. Botulinum toxin therapy is recommended only for patients who cannot or do not want to undergo surgery [11]. Oral pharmacologic therapy with calcium channel blockers and long-acting nitrates is an option when other treatment options fail. Short-term efficacy is excellent, but the effectiveness of mentioned treatments decreases with time. The risk of oesophageal squamous cell carcinoma and adenocarcinoma is higher in patients with achalasia, although recent guidelines do not recommend routine endoscopic screening for these patients [2]. An overview is given in Table 1.

Diagnostic tests

GERD can be diagnosed by clinical symptoms only. However, an endoscopy is helpful to exclude peptic strictures or malignancies. A barium swallow test can be done when a motility problem is suspected [12,13]. During a barium swallow test a film is made, while the patient swallows a liquid containing barium sulfate. Barium sulfate lights up on X-ray, so anatomical or motility abnormalities can be seen clearly (Figure 1). A barium swallow test can be used to support, but not confirm, the diagnosis of achalasia [14].

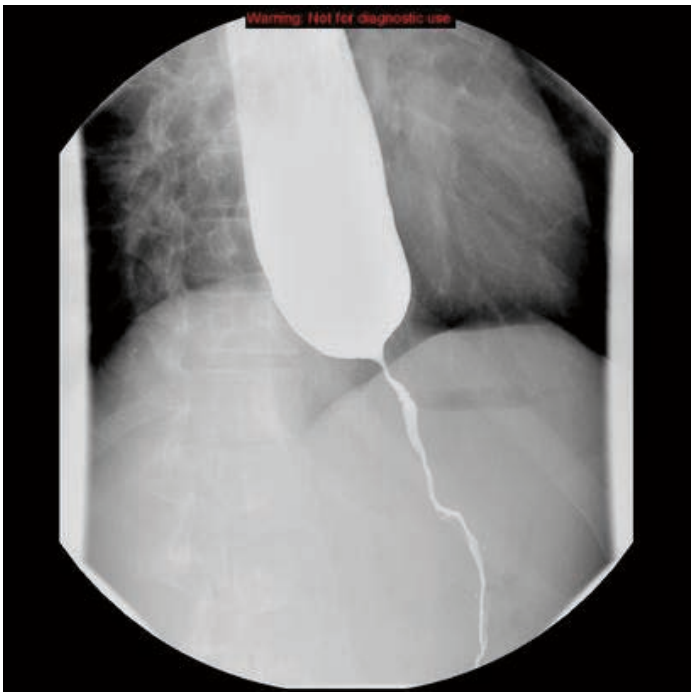

Figure 1: *X-ray of a patient with achalasia during a barium swallow test. A wide, atone oesophagus and a thin gastro-oesophageal junction (Bird's beak) is seen. Image from https://pediatricimaging.wikispaces.com/Achalasia, reuse under CC BY-NC-ND 3.0.*

The best test for confirmation of achalasia is an oesophageal manometry [15]. During manometry, a thin pressure-sensitive tube is inserted in the nose of the patient through the oesophagus and into the stomach. The patient is asked to swallow repeatedly a small fixed amount of water. This test evaluates the pressure in the oesophagus. Findings of aperistalsis and incomplete LES relaxation without a mechanical obstruction present, confirm the diagnosis of achalasia [2]. However, even manometry does not have a sensitivity of 100% [15].

Conclusion

Differentiation between achalasia and GERD can be done by anamnesis and diagnostic testing. Both groups of patients complain about heartburn and regurgitation. Patients with GERD typically complain about postprandial and nocturnal regurgitation. Patients with achalasia complain about regurgitation of undigested food, and dysphagia for solids and liquids. Endoscopy is helpful if GERD with peptic strictures is suspected. A barium swallow test and oesophageal manometry can be used to

Table 1: *Overview of gastro-oesophageal reflux disease versus achalasia.*

	GERD	Achalasia
Pathofysiology	Gastric acid reflux into the stomach, sometimes complicated by peptic strictures	Primary oesophageal motor disorder, lack of myenteric neurons
Prevalence	10-20% in the Western population, 5% in Asia	0,01% of adults in the world population
Dysphagia	For solid food when peptic strictures are present	Progressive for solids and liquids
Other clinical symptoms	Heartburn, postprandial and nocturnal regurgitation	Heartburn, regurgitation of undigested food, nocturnal cough, aspiration
Diagnostic tests	Diagnosis based on clinical symptoms Endoscopy if peptic strictures are suspected	Barium swallow test Oesophageal manometry

confirm the diagnosis of achalasia. On top of that, symptoms of GERD will diminish with the use of a PPI, while symptoms of achalasia do not.

Acknowledgements

I would like to thank dr. T.M. Bisseling, Radboud university medical center, from the Departement of Gastroenterology, Hepatology and Intestinal failure, Nijmegen, the Netherlands for proofreading this article.

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CORRECT ANSWERS TO THE EXAM QUESTIONS

Answer question 1:
C. Within hours.

A patient with an acute asthma attack is treated with inhalers to deliver medication straight into the lungs. This medication is aimed at providing immediate relief by reducing inflammation and/or opening up the airways. In addition to this, prednisolone is administered intravenously. Prednisolone is a corticosteroid drug that can reduce the inflammatory response for a longer period of time. It does so by acting on the glucocorticoid receptor. This is a nuclear receptor, which means that prednisolone alters gene transcription. So, even though prednisolone has a lipophilic structure that allows for easy and rapid passage through the cell membrane, it takes a while for the effects of prednisolone to occur.

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

Answer question 2:
D. Secretin.

Secretin secretion from the duodenum can inhibit the release of gastrin from the pyloric antrum. Gastrin is a peptide hormone that stimulates the secretion of gastric acid by the parietal cells of the stomach and is able to increase antral muscle mobility.
Acetylcholine is a neurotransmitter in the autonomic nervous system and has a muscle-activating function. Bombesin stimulates gastrin release and thereby stimulates the secretion of gastric acid. Histamine is involved in local immune responses but is also located in the enterochromaffin-like cells within the gastric glands. Histamine release is halted when the pH of the stomach starts to decrease.

During the exam, 55% of the participants answered this question correctly.

The exam questions can be found back on page 13 in this journal.

RAMS AT THE NVMO CONGRESS

Every year, the Nederlandse Vereniging voor Medisch Onderwijs (NVMO) organises a congress to gather everyone involved in the development and research of education in the (bio)medical field in the Netherlands. Students, researchers, doctors and others involved in (bio)medical education share and discuss the latest experiences and developments in education during these two days.

This year, RAMS had the honour to share its perspective on medical education by hosting a roundtable session together with the Erasmus Journal of Medicine (EJM). Mirjam Schaap (Chair Editorial Board of RAMS h.t.), Ferhat Beyaz (Vice-Chair of RAMS e.t.) and Linda Al-Hassany (Student Editor of EJM) gave the participants a brief overview of the ins and outs of a student scientific medical journal. After this overview, both scientific education and the differences between EJM and RAMS were discussed. Participants from various faculties in the country gave their opinion on different statements. This inspiring session provided us with many great ideas to improve RAMS and scientific education for students. Some ideas included the organisation of a symposium to present the articles published in RAMS and providing students from other universities the opportunity to participate in the learning process provided by RAMS. In the upcoming period, RAMS will explore these ideas. RAMS would like to thank those who participated and contributed to this successful roundtable session, in particular the Erasmus Journal of Medicine, but also the NVMO for organising this wonderful congress.

Picture 1: *From left to right: Mirjam Schaap (Chair Editorial Board of RAMS h.t.), Linda Al-Hassany (Student Editor of EJM) and Ferhat Beyaz (Vice-Chair of RAMS e.t.) and hosting the NVMO congress.*

Picture 2: *Roundtable discussion during the NVMO congress.*

A META-ANALYSIS : THE EFFECT OF MOBILE PHONE RADIATION ON THE INCIDENCE OF MALIGNANT TUMOURS IN ANIMALS

Tanja Reutelingsperger*[1], Carlijn Litjens[2], Jan Bookelaar[2], Else van Gerresheim[2], Joost Kools*[1]

This meta-analysis was conducted in 2013 by second-year Biomedical Sciences students.
Two authors recently translated and revised the textual part of the report to make it eligible for publication.*

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Abstract

Meta-Analysis

BACKGROUND: In the last few years, the use of mobile phones has increased exponentially. We are warned by media stations for unhealthy consequences of the mobile phone radiation. Among those is a Belgian thesis from 2008 that stated that mobile phone radiation is harmful to our health. Furthermore, the International Agency for research on cancer of the World Health Organisation reported a possible risk of cancer caused by exposure to electromagnetic radiation. However, no meta-analysis has been performed on this subject yet. Most of the studies on this subject are animal studies, because in those studies we can completely control the amount of radiation received while keeping the possible confounders to a minimum.
OBJECTIVE: To clarify whether mobile phone radiation leads to a higher incidence of malignant tumours in animals.
METHODS: An extensive search was performed in Pubmed and Embase using a search filter created by SYRCLE (the SYstematic Review Center for Laboratory animal Experimentation) to include all test animals. After the critical appraisal, we deducted the incidence of malignant tumours in the animal population for each study and used these to compute odds ratios according to the Mantzel Haenszal method. We looked at whole body tumour incidence, brain tumours, breast tumours and lymphomas.
RESULTS: The search resulted in 15 relevant articles for our research question. The computed odds ratio (OR) of whole body tumour incidence was 1.01; 95% CI [0.86-1.20]. The computed OR of brain tumour incidence was 0.94; 95% CI [0.75-1.17], the breast tumour incidence was 1.11; 95% CI [0.83-1.47] and the lymphoma incidence was 0.77; 95% CI [0.46-1.29].
CONCLUSION: The evidence found shows that there is no etiological connection between mobile phone radiation and tumour growth in rats and mice. More research is needed to clarify whether this also holds true for humans.

WHAT'S KNOWN: It is known that other types of radiation can increase the risk of cancer. Controlled clinical trials on humans regarding mobile phone radiation are hard to conduct because almost everybody is exposed to mobile phone radiation. Therefore, a lot of animal studies are conducted regarding this subject, while the amount of radiation can be controlled and the confounders can be kept to a minimum in animal studies.

WHAT'S NEW: Although systematic reviews have been published about the effect of mobile phone radiation on tumour incidence in animals, a meta-analysis wherein all the existing data is combined has not yet been conducted. It is of great importance that this meta-analysis is performed to achieve the highest level of evidence on this topic.

KEYWORDS: mobile phone radiation, cancer, tumour, animals

Introduction

The last twenty years, the use of mobile phones has increased significantly. More people than ever before own mobile phones and phones are used more hours per day. To keep connected with satellites, mobile phones use electromagnetic radiation between 450-3800 MHz. Amongst the population, it is thought that this electromagnetic radiation used, could increase the risk of getting cancer. The idea that tumours might arise due to this radiation is partly fed by messages originating from the media. For example, in 2007, a Belgian thesis was published which concluded that mobile phone radiation is harmful to our health [1]. This conclusion led to turmoil amongst the population and also in the House of Representatives of the Netherlands. The Health Council of the Netherlands asked the Commission of Electromagnetic Fields (CEF) to critically review the thesis to identify the risk of the population [2]. After an extensive research, the CEF concluded that the thesis contained invalidations and imperfections. First of all, the report seemed written by only three persons, instead of a full commission with several independent specialists. One of those persons did not have a scientific background, which was noticeable in the incomplete and selective search strategy. Lastly, the aim of the report was "to document the reasons why current public exposure standards for non-ionizing electromagnetic radiation are no longer good enough to protect public health" [1], meaning they never aimed to make an objective analysis.

In 2011, the International Agency for Research on Cancer (IARC) of the World Health Organisation (WHO) evaluated the available literature on the possible carcinogenic effects of electromagnetic fields [3]. They reported a possible risk of (brain)cancer caused by exposure to electromagnetic fields, based on epidemiological and (animal) experimental data. However, they also reported that the evidence was limited for brain tumours and inadequate for other types of cancer. They concluded that more scientific research is needed to clarify the possible risk.

Table 1: *Our search strategy was made up of three parts: mobile phone radiation, tumours and an animal search filter made in SYRCLE [22,23]. It resulted in 337 studies in PubMed and 265 in Embase.*

Database	Search term	Results
PubMed	(GSM[Title/Abstract] OR cell phone[Title/Abstract] OR mobile phone[Title/Abstract] OR mobile phones[Title/Abstract] OR cellular phone[Title/Abstract] OR cellular phones[Title/Abstract] OR cellular telephone[Title/Abstract] OR cellular telephones[Title/Abstract] OR Radio Wave[Title/Abstract]) OR radio frequency[Title/Abstract]) OR radio frequencies[Title/Abstract]) OR radio-wave[Title/Abstract]) OR radio-frequency[Title/Abstract]) OR radio-frequencies[Title/Abstract]) OR radio-waves[Title/Abstract] OR global system mobile[Title/Abstract] OR radio waves[Title/Abstract] OR radio wave[Title/Abstract] OR "Cellular Phone"[Mesh] OR "Radio Waves"[Mesh]) AND (neoplasm[Title/Abstract] OR neoplasms[Title/Abstract] OR neoplasia[Title/Abstract] OR cancer[Title/Abstract] OR brain tumour[Title/Abstract] OR brain tumours[Title/Abstract] OR malignancy[Title/Abstract] OR malignancies[Title/Abstract] OR brain tumor[Title/Abstract] OR brain tumors[Title/Abstract] OR malignant tumor[Title/Abstract] OR malignant[Title/Abstract] OR tumor[Title/Abstract] OR tumour[Title/Abstract] OR tumors[Title/Abstract] OR tumours[Title/Abstract] OR carcinogenic[Title/Abstract]) OR carcinogenesis[Title/Abstract]) OR cancers[Title/Abstract] OR "Neoplasms"[Mesh]) AND animal search filter [2]	337
Embase	(GSM OR cell phone OR mobile phone OR mobile phones OR cellular phone OR cellular phones OR cellular telephone OR cellular telephones OR Radio Wave OR radio frequency OR radio frequencies OR radio-wave OR radio-frequency OR radio-frequencies OR radio-waves OR global system mobile OR radio waves OR radio wave).ti,ab. OR exp mobile phone/ OR exp radiofrequency radiation/ AND (neoplasm OR neoplasms OR neoplasia OR cancer OR brain tumour OR brain tumours OR malignancy OR malignancies OR brain tumor OR brain tumors OR malignant tumor OR malignant OR tumor OR tumour OR tumors OR tumours OR carcinogenic OR carcinogenesis OR cancers).ti,ab. OR exp neoplasm/ AND animal search filter [3]	265

Although the IARC made an analysis of the available literature and several systematic reviews have been published, no meta-analysis on this topic has been conducted yet. Most of the trials conducted regarding mobile phone radiation are animal studies. This can be easily explained while in these type of studies we are able to control the environment, expose the population to a preferred amount of radiation and keep possible confounders to a minimum. Therefore, in this report we provide an independent overview of the evidence concerning mobile phone radiation as a possible cause of the development of tumours based on animal experimental data and combined it in several meta-analyses.

Methods

Search strategy and study selection

Many synonyms for the determinant "cell phone radiation" and the outcome measure "tumour incidence" were used to conduct our search strategy. The animal filter of SYRCLE (the SYstematic Review Center for Laboratory animal Experimentation) was used for the research population to include all types of animals. Since the interest in the mobile phone radiation topic has only arisen recently, we chose to search for studies published in the last ten years (2003- February 2013). Title and abstracts were screened based on the following exclusion criteria: systematic reviews, studies in humans, *in-vitro* studies, studies using radiofrequency as a therapy, studies without abstract or full-text version, studies not written in English or Dutch and studies with another primary outcome measure than tumours. The remaining studies were screened on full-text. If eligible for our research question, a critical appraisal based on the Cochrane Risk of bias tool was performed. Both screening and appraising of the studies were done independently by two researchers and compared afterwards. A discussion was started until consensus was reached, when differences between the two researchers in screening or appraising were encountered.

Critical appraisal

The articles were scored on their validity by scoring different domains using the Cochrane risk of bias tool [4]. For each type of bias we assessed if the authors did or did not take any measurements to reduce the risk of bias, or did not report measurements taken to reduce the risk of bias. The risk of selection bias was assessed by scoring the type of sequence generation and allocation concealment. The subgroup "baseline characteristics" was supplemented to the original Cochrane tool to assess the risk of selection bias in studies that were lacking a clear explanation of the

used techniques for sequence generation and allocation concealment. In these cases, the population characteristics of the different groups were checked on comparability at baseline.

The risk of performance bias was assessed depending on blinding of the researcher and/or caregiver. The risk of detection bias was assessed by identifying which measures were used to blind outcome assessors from knowing of which animal received which intervention. Attrition bias was assessed by reviewing if the outcome data was complete.

Outcome measures

The primary outcome was whole body tumour incidence in the animals exposed to cell phone radiation compared to sham radiation, last-mentioned meaning that the animals have been placed in the radiation apparatus without being exposed to actual radiation. The amount of received radiation or absorbed energy per time unit was expressed in specific absorption ratio (SAR) in Watt per kilogram. We also chose to report brain tumours separately, since the IARC reported that electromagnetic radiation could be a possible risk factor for these specific types of tumours. Also breast tumours and lymphomas were frequently reported tumours after radiation in literature and were therefore separately reported besides the whole body tumour incidence. For all these tumour incidences, separate meta-analyses were performed.

The SAR in the studies differed. Therefore, we pooled the amount of exposure into three groups, ≤1.0 W/kg defined as low, 1.1-2.9 W/kg as medium and ≥3 W/kg as high exposure. To see whether the amount of exposure could increase the risk of developing tumours, we compared the incidence of whole body tumours and brain tumours of each different SAR groups to sham radiation.

Statistical analysis

Using ReviewManager 5.0, we performed the meta-analyses computing a Mantzel Haenszel odds ratio for the tumour risk in each exposure group. We also calculated I^2 of Higgins et al., to assess whether the data used for the meta-analyses were heterogeneous, with cut-off points <25% as low heterogeneity, 25-50% medium heterogeneity, >50% high heterogeneity [5].

Results

Studies

The search strategy resulted in 337 articles on Pubmed and 265 articles in Embase (Figure 1). After removal of the duplicates, 456 articles remained. Screening on title and abstract resulted in 20 studies eligible for full text screening. Five studies were excluded after full text screening, because one appeared to be a review and four articles did not have the right outcome measures for the meta-analysis. Critical appraisal of the 15 remaining studies showed a lack in the reporting of used measures to decrease the risk of bias (Figure 2a, 2b) [6-20]. Saran et al. and Sommer et al. did not even report one of the domains used in the critical appraisal tool [13,17]. Furthermore, Tillman et al. did not take any measurements to randomise the outcome assessment, resulting in a high risk of bias regarding the outcome measurements [18].

An overview of the baseline characteristics of the research population and the radiation exposure method of each study can be found in the appendix. Ten studies used rats [6-10,14-16,19,20], the other five used mice [11-13,17,18]. Five studies used only female animals [6,8, 9,17,19] and the remaining ten studies used both males and females [7,10-16,18,20]. Six studies exposed only the head to radiation [7,10,12,14,15,20] and the other nine studies exposed the whole body [6,8,9,11,13,16-19].

Whole body tumour incidence

Three studies reported whole body tumour incidence as an outcome measure [12,16,18]. None of the studies showed a statistically significant increase in tumour incidence after radiation.

All the data combined resulted in an OR of 1.01; 95% CI [0.86-1.20] for whole body tumour incidence in exposed groups compared to the control groups (Figure 3), with an I^2 of 32%.

When pooled in the different SAR groups, the combined OR, compared to sham radiation, for low exposure was 1.04; 95% CI [0.71-1.53], for medium exposure the OR was 1.11; 95% CI [0.73-1.70] and for high exposure the OR was 0.94; 95% CI [0.69-1.29].

Table 2: *Risk of bias per item for each article. Each study was scored on (the reporting of) measurements taken for various items that could lead to a risk of bias.*

	Sequence generation	Allocation concealment	Blinding researcher	Blinding care- giver	Blinding outcome	Randomisation outcome assessment	Incomplete outcome data
Anane [6]	o	o	o	o	o	o	o
Anderson [7]	o	o	o	o	o	o	o
Heikkinen [8]	o	o	o	o	o	o	o
Hruby [9]	o	o	o	o	o	o	o
La Regina [10]	o	o	o	o	o	o	o
Lee [11]	o	o	o	o	o	o	o
Oberto [12]	o	o	o	o	o	o	o
Saran [13]	o	o	o	o	o	o	o
Shirai2005 [14]	o	o	o	o	o	o	o
Shirai2007 [15]	o	o	o	o	o	o	o
Smith [16]	o	o	o	o	o	o	o
Sommer [17]	o	o	o	o	o	o	o
Tillmann [18]	o	o	o	o	o	o	o
Yu [19]	o	o	o	o	o	o	o
Zook [20]	o	o	o	o	o	o	o

o: Yes (low risk of bias)
o: Unclear risk of bias
o: No (high risk of bias)

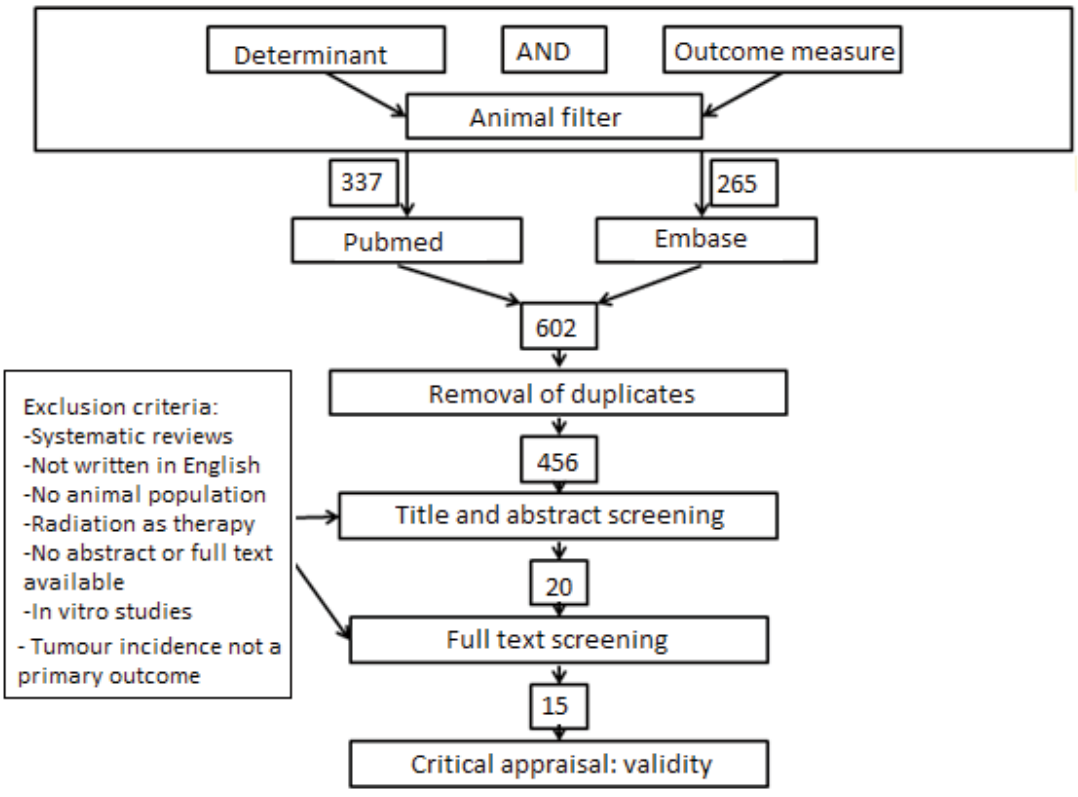

Figure 1: *Flowchart of search strategy and critical appraisal. We found 602 studies using our search strategy, of which 146 were duplicates. The 456 remaining studies were screened on title and abstract resulting in 20 eligible studies. After full text screening 15 studies were included in the meta-analyses.*

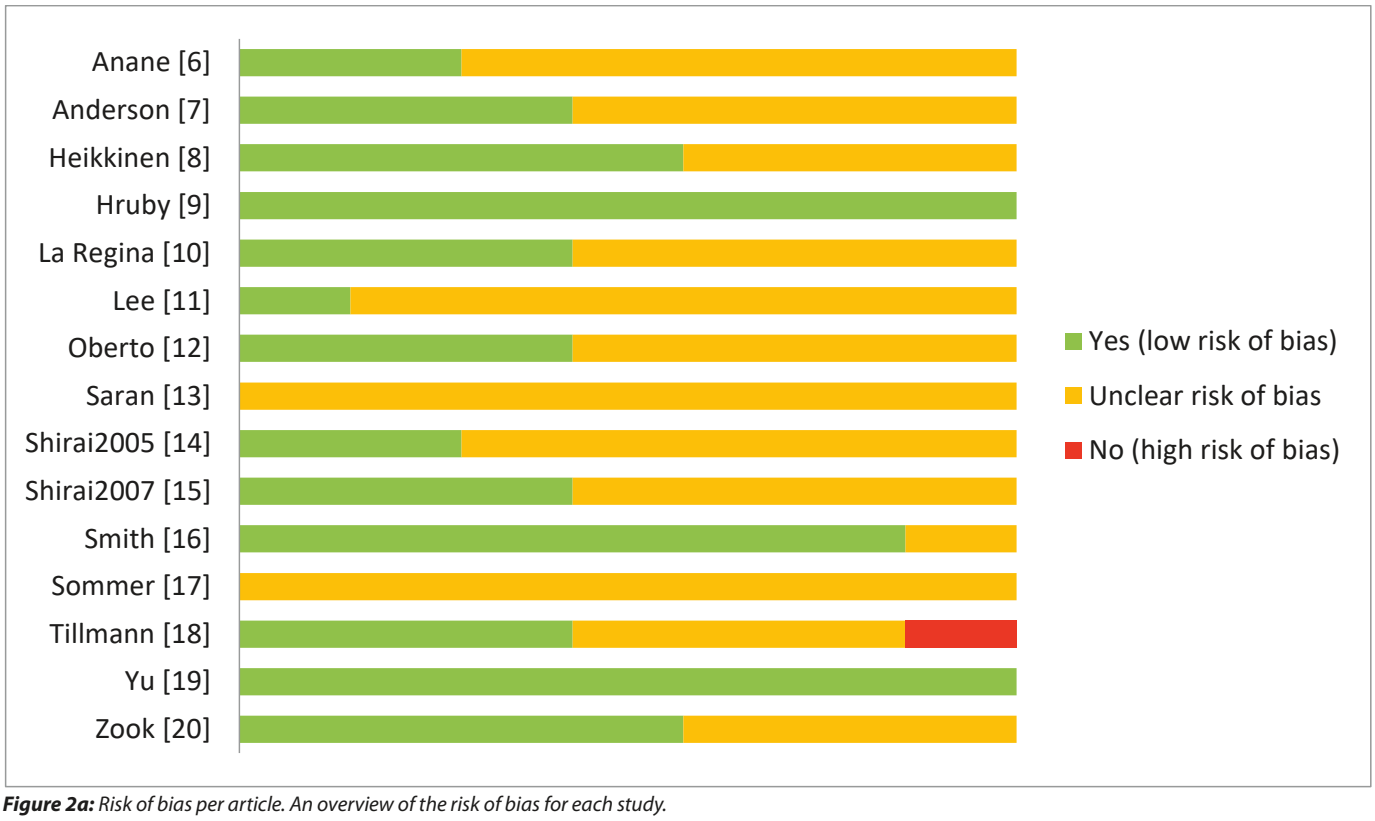

Figure 2a: *Risk of bias per article. An overview of the risk of bias for each study.*

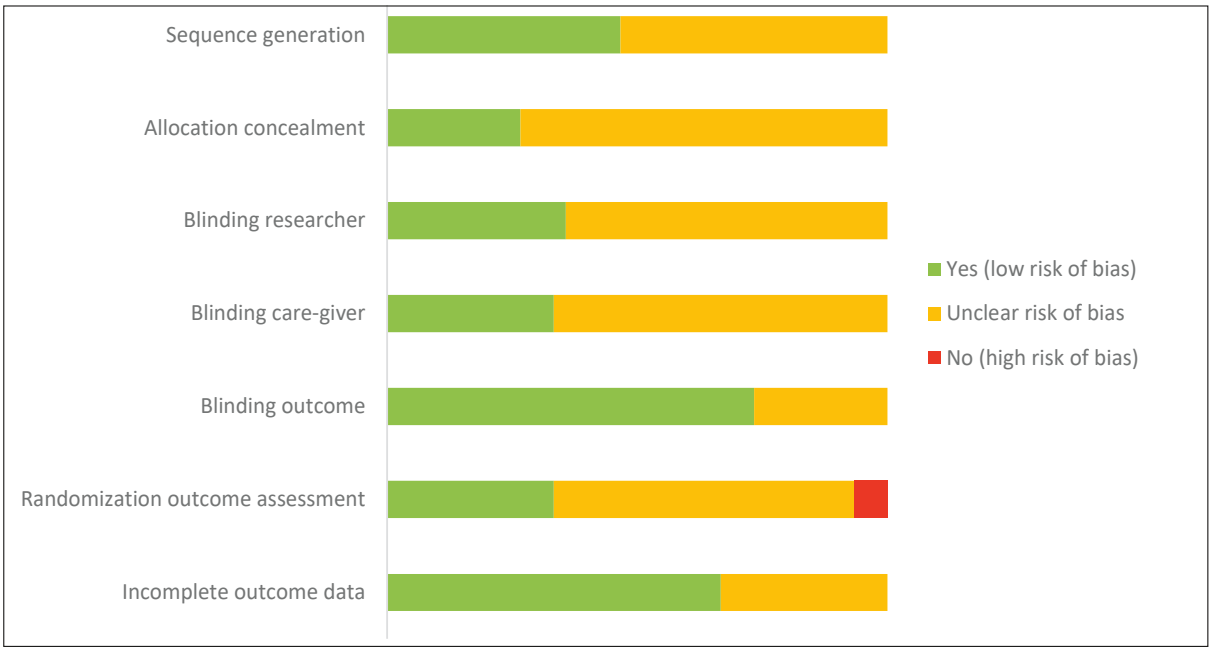

Figure 2b: *Risk of bias per item. An overview of how often measurements were taken for the different items that could lead to risk of bias.*

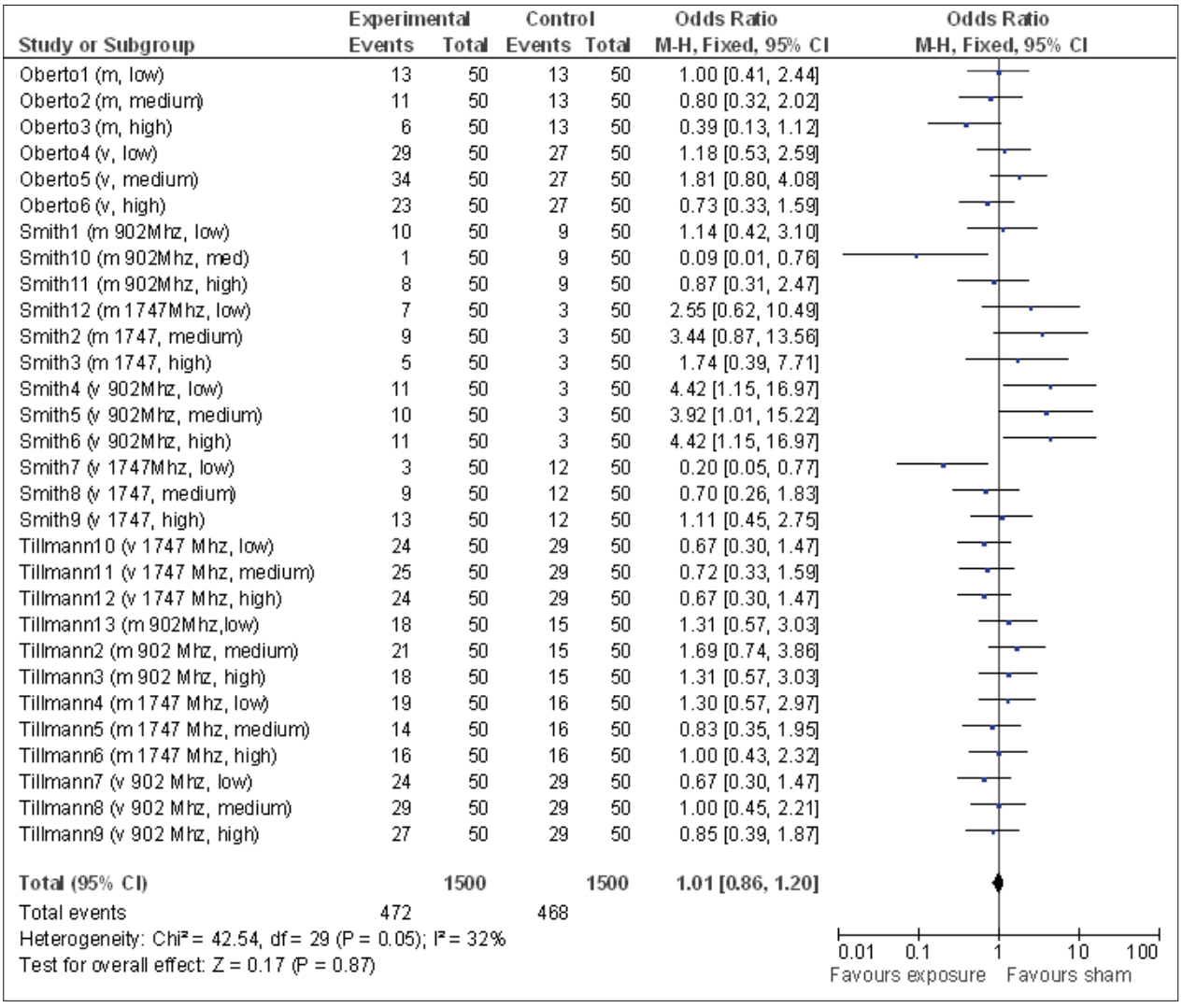

Figure 3: *Meta-analysis of whole body tumour incidence. A forest plot including all studies found that researched whole body tumour incidence [12,16,18].We separated each study population in female (v) and male (m). Furthermore, we separated the populations according to the amount of exposure they received: low was defined as ≤1.0 W/kg, medium as 1.1-2.9 W/kg and high as ≥3 W/kg. Where possible we reported the frequency (in Megahertz) used.*

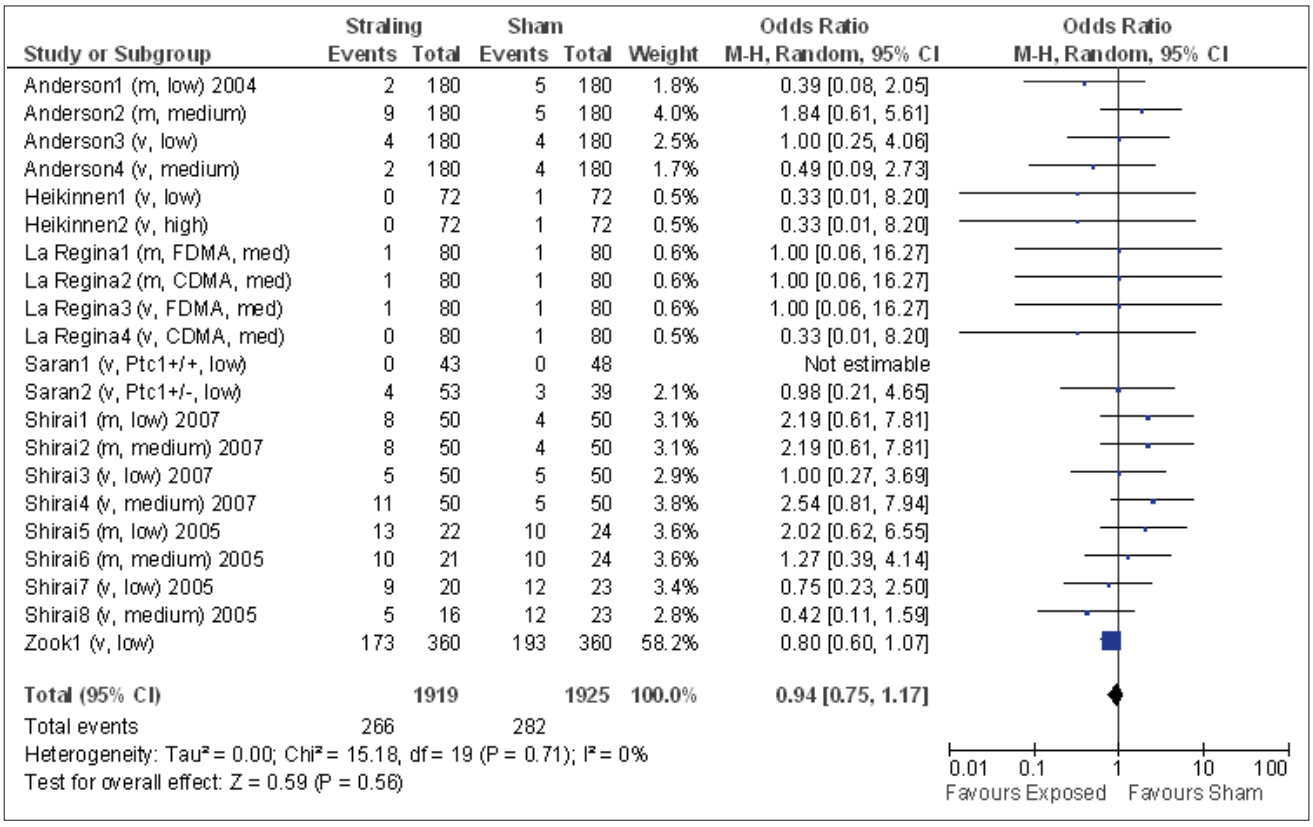

Figure 4: *Meta-analysis of brain tumour incidence. A forest plot including all studies found that researched brain tumour incidence [7,8,10,13,14,15,20]. We separated each study population in female (v) and male (m). Furthermore, we separated the populations according to the amount of exposure they received: low was defined as ≤1.0 W/kg, medium as 1.1-2.9 W/kg and high as ≥3 W/kg. Where possible we reported the type of model used in the study (FDMA, CDMA, Ptc1+/+, Ptc1+/-).*

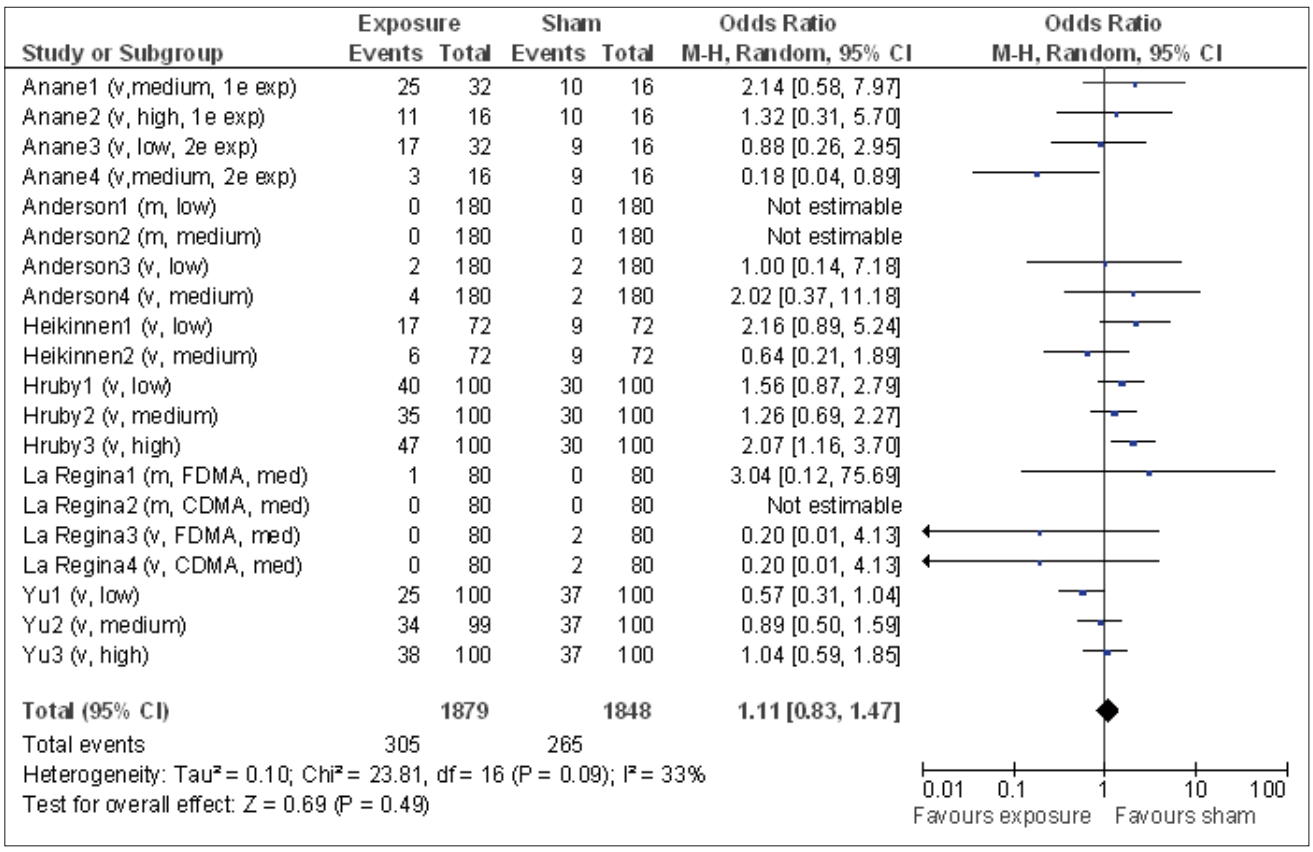

Figure 5: *Meta-analysis of breast tumour incidence. A forest plot including all studies found that researched breast tumour incidence [6-10,19]. We separated each study population in female (v) and male (m). Furthermore, we separated the populations according to the amount of exposure they received: low was defined as ≤1.0 W/kg, medium as 1.1-2.9 W/kg and high as ≥3 W/kg. Where possible we reported the type of model used in the study (FDMA, CDMA).*

Brain tumour
Six studies reported brain tumours as an outcome measure [7,8,10,13-15,20]. None of the studies showed a statistically significant increase in brain tumour incidence after radiation. The largest of these studies (Zook et al.) reported that 193 of the 360 exposed rats developed at least one brain tumour compared to 173 of the 360 rats in the sham group (OR 0.80; 95% CI [0.60-1.07]) [20].

All the data combined resulted in an OR of 0.94; 95% CI [0.75-1.17] for the incidence of brain tumours in exposed groups versus control groups (Figure 4), with an I^2 of 0%.

When pooled in the different SAR groups, the combined OR, compared to sham radiation, for low exposure was 0.87; 95% CI [0.67-1.11], for medium exposure the OR was 1.27; 95% CI [0.78-2.06] and for high exposure the OR was 0.33; 95% CI [0.01-8.20].

Breast tumour
Six studies reported breast tumours as an outcome measure [6-10,19]. Hruby et al. found a statistically significant increase in the incidence of breast tumours in the high exposure group (OR 2.07; 95% CI [1.16-3.70]) [9]. They conducted a big trial with 400 rats and found a statistically significant increase in the amount of tumours in other organs after exposure to radiation. However, they discussed that, based on literature, their results might be accidental since the used rat model leads to a great variety in results.

Anane et al. performed two almost identical trials [6]. The first trial showed a higher incidence of breast tumours in the medium exposure group. However, when performed for the second time, this result could not be replicated. Due to the inconsistency of the results they concluded that no valid evidence on the possible co-promoting effect of mobile phone radiation on breast tumour incidence in rats could be deduced from these results alone. The other studies did not find a statistically significant increase in breast tumour incidence after exposure to radiation.

All the data combined resulted in an OR of 1.11; 95% CI [0.83-1.47] for the incidence of breast tumours in exposed groups versus control groups (Figure 5), with an I^2 of 33%.

Lymphomas
Three studies reported lymphoma incidences as an outcome measure [8,11,17]. None of the studies reported a statistically significant increase in lymphoma incidence after exposure to radiation. All the data combined resulted in an OR of 0.77, 95% CI [0.46-1.29] for the incidence of lymphomas in exposed groups versus control groups (Figure 6), with an I^2 of 0%.

Discussion

This is the first meta-analysis on the existing data of the effect of mobile phone radiation on tumour incidence in rats and mice. Although some studies did find a statistically significant increase in tumour development after exposure to radiation, when combined with other data in several meta-analyses, no statistically significant increase was found for any of the tumour types. Furthermore, the amount of exposure to radiation did not statistically significant influence the development of tumours.

We consider our data to be of high validity, because of the clear and systematic method used. The inclusion of the relevant articles was done independently by two researchers to decreases the risk of excluding any relevant article. As with the inclusion, the appraisal of the relevant studies was done independently by two researchers, reducing the risk of observer bias. Lastly, all our included studies used mice or rats, which makes the data more comparable than it would be when different types of animal were used. This is also confirmed by the low to slightly medium percentages of I^2.

However, for a more valid extrapolation of the results to humans, studies on bigger animals exposed to mobile phone radiation are needed. We expect that bigger animals would resemble us humans more in the body content exposed to radiation while the content in relation to the surface area is more comparable. Furthermore, the methods used in the studies were not always comparable. Firstly, there was a variety of mouse and rat models used. Some studies used genetically modified animals while others used chemical substances to induce tumour growth. Therefore it can be discussed whether the data of these studies can be combined in one meta-analysis. Secondly, the studies used different amounts of SAR. It is imaginable that a higher amount radiation dose will lead to more

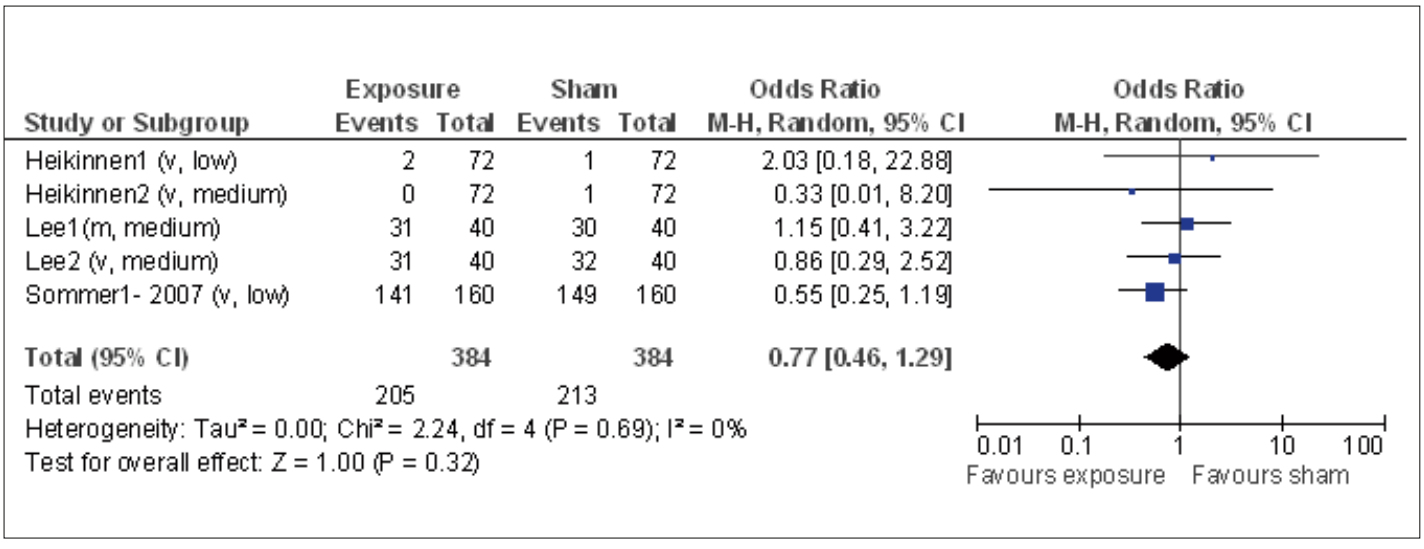

Figure 6: *Meta-analysis of lymphoma incidence. A forest plot including all studies found that researched lymphoma incidence [8,11,17]. We separated each study population in female (v) and male (m). Furthermore, we separated the populations according to the amount of exposure they received: low was defined as ≤1.0 W/kg, medium as 1.1-2.9 W/kg and high as ≥3 W/kg.*

DNA damage and therefore more tumour growth. However, we aimed to correct for this by pooling the different SAR amounts in three levels (low, medium, high). Thirdly, the duration of exposure differed between the studies, varying from 45 minutes to 24 hours per day. A longer exposure to radiation will possibly lead to more tumour growth. By combining the data of these studies with different exposure lengths, the effect of radiation may be underestimated.

In September 2014, the CEF released a systematic analysis regarding mobile phones and cancer based on animal studies [21]. They concluded that it is highly unlikely that exposure to electromagnetic radiation may have initiating or promoting effects on the development of cancer.

Unfortunately, we cannot report anything on the long-term effect of exposure to mobile phone radiation, since rats and mice have a short lifespan. Studies on long-term effect should therefore use animals with a longer lifespan. Furthermore, it is difficult to extrapolate data from studies with small animals, like rats and mice, to humans. Controlled clinical trials in primates would be helpful, since primates have more resembles with humans and have a longer life-span than rats and mice. Moreover, more data on the consequences of mobile phone radiation for humans is needed. However, clinical trials in humans are very hard to conduct, but epidemiological data could support the consequences for humans.

Conclusion

Based on the evidence found by the extended literature search, we conclude that mobile phone radiation is not a risk factor for the development of tumour growth in rats and mice, regardless of the amount of exposure. Further research should be performed to investigate whether this also holds true for humans.

Acknowledgements

We would like to thank dr. Carlijn Hooijmans, member of SYRCLE and Assistant Professor at the Department of Health Evidence and Anesthesiology of the Radboud university medical centre (Nijmegen, the Netherlands) for supervising this project in 2013, before it was revised and translated.

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RECENT HIGH-IMPACT PAPERS FROM RADBOUDUMC RESEARCHERS

Janneke Elzinga[1]

Summary

With over 3000 publications per year, scientific research is a cornerstone of the Radboud university medical centre [1]. In this section, recent high-impact papers – published by researchers from the Radboudumc – will be discussed.

[1]Master Student Molecular Mechanisms of Disease, Radboud university medical center, Nijmegen, the Netherlands

How emotional memories are stored

While everyday memories slowly wear down with time, emotional events are often longer and more vividly remembered. It is known that noradrenergic activation of the amygdala, involved in emotional processing, enhances the initial formation of memory in the hippocampus. In the long term, memory traces are reorganised and partly transferred to neocortical networks, called systems consolidation. This is accompanied by the transformation of memory quality and time- and brain-specific epigenetic modifications. However, whether this process can be actively influenced by emotional arousal status is unknown. In a study by researchers from the Donders Institute, rats were subjected to a so-called inhibitory avoidance discrimination task training (footshock versus no footshock), after which they were treated with either norepinephrine (NE) or saline [2]. It was demonstrated that NE treatment supports accurate memory of the shock-context association and thus affects systems consolidation dynamics. This was shown to be dependent on hippocampal activity and accompanied by time-regulated epigenetically driven changes in transcription of memory-related genes in hippocampus and neocortex. The study contributes to further understanding the neuronal pathways involved in long-term accuracy of memory.

Non-haploinsufficiency in neurodevelopmental disorders

Intellectual disability (ID) and other developmental disorders (DDs) are often caused by de novo mutations in protein-coding genes. Previously, haploinsufficiency (i.e. the loss of one copy of a gene) was thought to be the main mechanism by which dominant mutations exert their disease-causing effect. Opposed to this is non-haploinsufficiency (NHI), of which gain-of-function and dominant-negative mechanisms are examples. In the case of NHI, mutations are often spatially clustered, affecting only particular regions of a gene. Researchers of the Department of Human Genetics exploited this phenomenon to identify genes with significant spatial clustering patterns of de novo mutations in large cohorts of people with ID and DDs [3]. From the 15 genes with clustering mutations identified, 12 had already been associated with neurodevelopmental disorders, of which 11 indeed had been associated with NHI mutation mechanisms. The three newly-identified genes opened new diagnostic possibilities. The results were complemented with 3D modelling of the affected proteins, which showed that the majority of the clustered mutations probably does not affect the overall structural integrity and may possibly act through another mechanism than haploinsufficiency. Furthermore, it was found that NHI-associated genes are less tolerant to normal genetic variation. The study shifts the focus of study to a mutation mechanism that may contribute to a larger extent to ID/DD than previously thought.

From mRNA modifications to an autism spectrum disorder

The field of epitranscriptomics investigates the biochemical modification of RNA and its effect on RNA metabolism, as in line with epigenetics (e.g. splicing, translation and degradation). The most common and well-understood mRNA modification is the addition or removal of m6A. This is a mRNA nucleotide modification commonly found in mammalian cells and has been linked to (patho)biological processes including cancer, obesity and fertilisation. The mechanism by which m6A influences RNA homeostasis is not exactly known. The so-called YTH-domain has previously been identified as a potential "m6A-reader" domain, but the existence of other interacting proteins, either attracted or repelled by m6A, remains elusive. Researchers from the Department of Molecular Biology, in collaboration with international partners, screened for m6A readers in various cell types and mRNA sequence contexts [4]. They demonstrated the conservation of YTH-domain-containing proteins across cell-types. In addition, sequence-context-dependent m6A readers were identified, including FMR1, of which loss is known to lead to fragile X-linked mental retardation. On the opposite, other proteins were shown to be repelled by m6A modified mRNA. This study demonstrates the effect of m6A modifications on mRNA homeostasis by regulating, for instance, mRNA stability or translation rates. Moreover, this study is the first to report a link between a mRNA modification and the fragile X-linked mental retardation syndrome.

Linking magnesium homeostasis to metabolic disorders

Magnesium (Mg^{2+}) homeostasis is tightly regulated by renal reabsorption. Disturbed levels of this cation have been associated with metabolic disorders, but the main genes regulating renal Mg^{2+} handling, however, remain to be identified. In a multidisciplinary, international collaboration, researchers from the Department of Physiology performed a genome-wide meta-analysis of Mg^{2+} homeostasis to identify genetic components [5]. They combed existing data on genetic and biological (e.g. plasma and urine) parameters from over 9,000 individuals. This resulted in the identification of two loci associated with urinary magnesium: one was located near a gene coding for a Mg^{2+}-channel (TRPM6), the other was located on a gene (ARL15), which previously had been linked to obesity and insulin biology, respectively. Next, ARL15 was demonstrated to regulate TRPM6-mediated currents in human kidney cells. This *in vitro* data was complemented with *in vivo* data from zebrafish. The expression of ARL15 zebrafish orthologue was regulated by dietary Mg^{2+} and its knockdown resulted in Mg^{2+} wasting and metabolic disturbances. Finally, in the population-based studies, the association between urinary Mg^{2+} and metabolic phenotypes were modified by a genetic variant of ARL15. This study increases insight in Mg^{2+} homeostasis in relation to metabolic disorders and identifies ARL15 as a novel key player in these processes.

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A Word from the Board of RAMS

Dear reader,

In the spirit of this anniversary edition, I will dwell upon the number 10. In the scientific field, 10 is the atomic number of neon and it mathematically serves as a triangular number. Moreover, the Snellen chart, an eye chart that can be used to measure visual acuity, uses 10 different letters. Lastly, the blood value of C-reactive protein, often abbreviated to CRP, should not be over 10 mg/L; if it is, it might be an indication of a state of acute inflammation in the human body.

Fortunately, the tenth edition will not be the last RAMS-edition; and that is why this edition is the first edition that is not a single digit, but a number. Therefore, I feel highly honoured to have the final word in this anniversary edition of RAMS. In my opinion, this anniversary edition demonstrates the development and the success of RAMS has in the past few years; it started in 2013 and now, RAMS already published its 10th edition.

Hopefully, RAMS will have the opportunity to celebrate some more anniversary editions and be able to give more students the possibility to publish their (first) articles. So, do not hesitate and take your chance in one of our next editions!

On behalf of the Board of RAMS,

Bart de Vries
Vice-Chair RAMS 2017-2018

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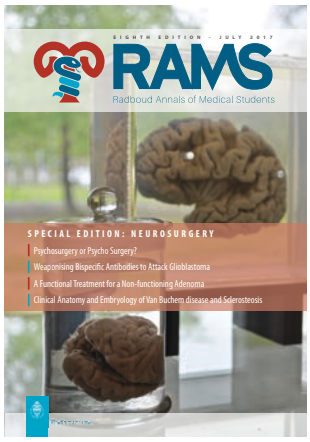

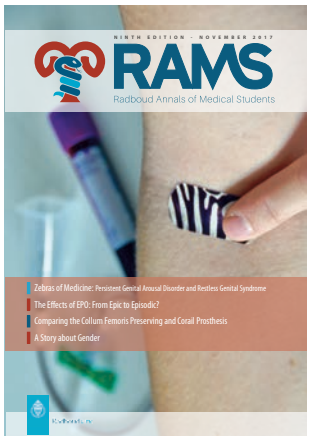

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