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# FROM THE EDITORIAL BOARD

Dear reader,

I am very delighted you picked up our 22nd edition! Once again, we have put a lot of time and effort into this edition, and I think reading it will definitely be worth your time.

Spring is finally coming our way again, meaning the dark and gloomy days are almost over! I expect you are just as excited as I am for better weather and some lovely sunny days. To connect this transition of seasons to science, one of our editors has written an article that discusses spring fever. Did you ever wonder whether spring fever is a myth or an actual phenomenon? And what would be the mechanism behind it if it actually exists? This will be discussed in our Myth or Science article. We also had a fascinating interview with Anne Sophie Kruit about the first bilateral hand transplant in the Netherlands performed in the Radboudumc. You can read all about it in this edition. Furthermore, we discuss the drug development pipeline needed to treat diseases medicinally.

We also have a new Zebras of Medicine article, which will dive into how you can deviate between Wernicke encephalopathy and delirium as both can present with the same symptoms. In addition, we showcase a number of articles that have been published recently by researchers from the Radboudumc in high impact journals. Guus, our columnist, has also written an enlightening column about hospital series with a response of Károly Illy, MSc., on the topic. Other topics include a look into the future of neurodiversity and the relevance of the gut microbiome in health and disease. I hope you will learn something new while reading through these interesting articles!

I want to thank all our editors and reviewers for their hard work and the specialists in the field who reviewed every single piece to check its contents to ensure the best quality.

Enjoy the read!

On behalf of the eighth board of RAMS,

**Sofie Knipping**
Editorial Editor-in-Chief of the VIIIth Editorial Board

# INDEX

# SPRING FEVER:
## LOVE IS IN THE AIR – OR NOT?

**Anna-Lena Boller**[1]

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***Insight***

"If we had no winter, the spring would not be so pleasant" (Anne Bradstreet). After a long winter, people appreciate the first sun rays in spring. Flowers are blooming, the temperature increases, people seem to be happier, and "Love is in the air". Poets have called this phenomenon 'spring fever'. But is that actually a thing? Are people happier, and do they show increased amorousness? And if so, why is that the case?

### The definition of spring fever

When the days start to become longer and the temperature increases, many people are more restless, energetic, and romantic. This phenomenon is called spring fever and it is, as the name implies, associated with the onset of spring [1]. Since these episodes of good mood are associated with a season, it seems evident that they are bound to seasonal patterns, but is that really true? Is the mood seasonally changing? Spring is accompanied by more sunlight, warmer temperatures, and blooming plants all over, compared to the winter months that precede it; everything appears friendlier. It seems logical that the changes in the landscape can enlighten someone's mood. However, is there merit to believe that there might also be an underlying biological process that influences the mood and could lead to this so-called spring fever?

### Circadian rhythm

The most significant seasonal change is the amount of daylight someone experiences. In spring, people usually experience higher exposure to sunlight on average due to increased amount of daylight. What does this have to do with the seasonality of mood? Humans, as well as other organisms, need to adapt to these environmental cues, which are key regulators of organisms' biological processes, including quality of sleep or hormone production [2]. The circadian rhythm is the response to environmental changes involving sunrise and sunset [3]. By gene expression, the underlying circadian system can regulate the activity of brain regions involved in mood control and, therefore, indirectly affect one's temper [2]. Amongst other things, the circadian rhythm manages the production of melatonin, the so-called "darkness hormone", which suppresses neuronal activity during the night [4]. Altered melatonin secretion has been shown to be associated with changes in mood in major depressive disorder or seasonal affective disorder (SAD) [5]. Seasonal variations can affect melatonin production in the sense of longer days delaying the onset of its production in the evening [4]. Overall, longer days and, therefore, higher exposure to daylight positively affects one's circadian rhythm and the resulting inhibition of melatonin production. This results in increased mood and (neuronal) activity compared to shorter days: the spring fever.

### Sunlight and Vitamin D

The increased sunlight in spring and summer affects not only the circadian rhythm but also the production of Vitamin D. The penetration of sunlight, containing Ultraviolet B (UVB) radiation, activates the Vitamin D production in the human body. It has been shown that depression, which is partly characterised by episodes of a bad mood, is linked to the level of Vitamin D [6]. Although there is no global cut off for Vitamin D levels, an insufficiency or deficiency is expected to provide a risk for the development of depression [7]. An optimal range in blood is assumed to be between 30-60 ng/ml, while insufficiency is defined at a level of 21-29 ng/ml and deficiency below a level of 20 ng/ml [7]. Knowing the link between low Vitamin D levels and depression leads to the assumption that sufficient Vitamin D levels, on the contrary, lightens up one's mood, meaning there could be an indirect link between sunlight and happiness [7, 8]. Apart from the effect on Vitamin D production, serotonin production is also affected by sunlight. Serotonin belongs to the class of neurotransmitters and functions as a neuromodulator. Imbalances in the serotonin levels have been associated with depression and compulsive disorders [9]. Lambert *et al.* showed that the serotonin turnover in the brain is the lowest in winter compared to the other seasons; however, there is also daily fluctuation based on the changes in luminosity [10]. This means that the longer the day and the higher the light intensity, the greater the serotonin turnover. Due to the natural upregulation that usually comes along with spring patterns, serotonin levels could be one of the factors, next to Vitamin D production, that leads to increased energy and enlightened mood during spring - the spring fever [8].

### Summer – in favour

As discussed above, the increased exposure to sunlight in spring can have positive effects on one's serotonin and Vitamin D levels and, thus, indirectly on the mood and energy level. However, what does this intend for the darkest season in the year, winter? Murray *et al.* showed that mood was significantly lowered in winter compared to preceding summer in a study conducted over three years [9]. It is necessary to mention here that "positivity bias" exists in reports regarding happiness and well-being. People tend to judge experiences or feelings more favourably than they actually are, meaning in this study, the summer might be a generally positive baseline [11, 12]. Therefore, the investigated winter might

be experienced worse than it actually is, compared to the previous summer, but not below subjective norms [11].

## Winter blues

Although there might be a positivity bias, there are people who experience a severely lowered mood in winter, having episodes of depression. This phenomenon is called SAD, also known as winter blues [13, 14]. SAD is considered a subtype of affective disorders that is accompanied by seasonal major depression and bipolar disorders, whereby the onset is often associated with the winter months. In the cooler months, these patients experience hypersomnia, increased appetite, and depressed mood, while spring and summer can be characterised by elevated mood, social activity, and energy as well as increased libido - patients experience full remission [9, 13, 14]. SAD is usually treated with phototherapy to provide artificial sunlight during the winter months mimicking the positive effects of sunlight on biological processes such as the aforementioned circadian rhythm, Vitamin D production and serotonin levels [9].

## Spring fever: just the absence of winter blues?

Both winter blues and spring fever appear to be connected to sunlight and, thus, the seasons seem to affect one's mood by altering biological processes. However, not all people experience spring fever or winter blues. Actually, only a small part of the population is affected by severe mood shifts due to the seasons. People that are affected are usually known to be highly neurotic, which makes them more vulnerable to environmental factors [15]. This leads to the conclusion that there are, in fact, seasonal biological patterns that can be described in a poetic way as spring fever. However, the effect of spring fever is very different per individual and mainly associated with the mood during the winter season; as Anne Bradstreet said: "If we had no winter, the spring would not be so pleasant". If people suffer from winter blues or SAD, they experience a bigger mood shift due to biological changes caused by the environment. People who do not mind the winter season do not feel a strong shift in mood due to the start of the spring. Therefore, for some people, it seems that spring fever is just the absence of the winter blues or the other way around. However, it needs to be stressed that humans and their mood are affected by many factors such as the seasons, some more and some less. So, keep in mind, if somebody is having a 'rainy' day, try to 'lighten' them up. And do not forget the sunscreen over prioritising your mental health.

## Acknowledgments

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## References

1. Nicholson, C. Does "spring fever" exist? *Sci Am* **298**, 116 (2008).
2. Cho, C.-H. & Lee, H.-J. Why Do Mania and Suicide Occur Most Often in the Spring? *Psychiatry Investig* **15**, 232-234 (2018).
3. Kim, J., *et al.* Implications of Circadian Rhythm in Dopamine and Mood Regulation. *Mol Cells* **40**, 450-456 (2017).
4. Porcu, A., *et al.* Photoperiod-Induced Neuroplasticity in the Circadian System. *Neural Plast* **2018**, 5147585-5147585 (2018).
5. Srinivasan, V., *et al.* Role of melatonin in mood disorders and the antidepressant effects of agomelatine. *Expert Opin Investig Drugs* **21**, 1503-1522 (2012).
6. Geng, C., *et al.* Vitamin D and depression: mechanisms, determination and application. *Asia Pac J Clin Nutr* **28**, 689-694 (2019).
7. Parker, G.B., *et al.* Vitamin D and depression. *J Affect Disord* **208**, 56-61 (2017).
8. Penckofer, S., *et al.* Vitamin D and depression: where is all the sunshine? *Issues Ment Health Nurs* **31**, 385-393 (2010).
9. Gupta, A., *et al.* Role of serotonin in seasonal affective disorder. *Eur Rev Med Pharmacol Sci* **17**, 49-55 (2013).
10. Lambert, G.W., *et al.* Effect of sunlight and season on serotonin turnover in the brain. *Lancet* **360**, 1840-1842 (2002).
11. Murray, G., *et al.* A longitudinal investigation of seasonal variation in mood. *Chronobiol Int* **18**, 875-891 (2001).
12. Sears, D.O. The person-positivity bias. *Journal of Personality and Social Psychology* **44**, 233-250 (1983).
13. Leahy, L.G. Overcoming Seasonal Affective Disorder. *J Psychosoc Nurs Ment Health Serv* **55**, 10-14 (2017).
14. Sher, L. The role of brain thyroid hormones in the mechanisms of seasonal changes in mood and behavior. *Med Hypotheses* **55**, 56-59 (2000).
15. Winthorst, W.H., *et al.* Seasonality of mood and affect in a large general population sample. *PLoS One* **15**, e0239033 (2020).

# TRUST YOUR GUT:
# THE RELEVANCE OF THE GUT MICROBIOME IN HEALTH AND DISEASE

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***Abstract***

The gut microbiome contains 100 trillion microorganisms inhabiting the gastrointestinal tract. Research into the gut microbiome began in the 16th century, with some accounts that date even earlier. Increasing interest in the gut microbiome has led to the founding of multiple international consortiums like the Human Microbiome Project and the American Gut Project. The gut microbiome confers multiple benefits to the host due to the synthesis of essential vitamins and regulation of immunity, gut health, as well as modulation of behaviour and mood through the gut-brain axis, which is a network that connects the nervous system to the gastrointestinal tract. Dysbiosis of the microbiome is associated with several diseases such as gastric and non-gastric cancers, inflammatory diseases, neurodegenerative, and neuropsychiatric disorders. Current therapies, mainly for gut-based diseases, include faecal microbiota transplantation and the use of prebiotics and probiotics either through diet or as supplements. Future lines of research include studying the molecular mechanisms underlying dysbiosis and screening the microbiome for diagnostic purposes, as well as establishing therapies for gut-brain linked diseases.

## Introduction

The gut microbiome, which inhabits the gastrointestinal tract, is a collection of archaea, eukarya, and bacteria [1]. It is estimated that the gut microbiome has 100 trillion microorganisms, consisting of 150-400 species, depending on ethnicity [1, 2]. It is also estimated that the microbiome encompasses 2-20 million genes compared to the 20,000 genes in the human genome, hence being called the last organ of the human body [3, 4].

Dysregulation of the gut microbiome is associated with many diseases such as Alzheimer's disease, depression, cardiovascular disease, diabetes, obesity, and autoimmune disorders [5]. Undoubtedly, the interest in the human microbiome has increased rapidly over the years, with almost US $1.7 billion being spent on medical research for the human microbiome [4]. This has led to swift advancements not only in terms of laboratory screening techniques - for identification and labelling every microbe in the gut but also therapeutically - with poop pills, faecal transplantation, probiotics, and prebiotics for certain diseases [5].

This review aims to elucidate the role of the gut microbiome in health and disease: the history behind gut microbiome research; its association with various diseases such as cancer, neurological disorders, neurodegenerative disorders, and gut disorders; the current therapies ranging from faecal microbiota transplants (FMTs) to poop pills leading on to what the future holds for gut microbiome research.

## A brief history of gut microbiome research

In scientific research, one of the early descriptions of the human gut microbiome was documented by a Dutch scientist, Antonie van Leeuwenhoek in 1683. He examined microbes under the microscope, from his own mouth and stool, and concluded the presence of more than 1000 'animalcules' which differed based on their origin [3]. In the 16th century, Li Shizhen, a pharmacologist, successfully treated patients suffering from an abdominal disease with a soup containing fresh, dry, or fermented stool [6]. Escherich and Metchnikoff, doctor and zoologist respectively, laid the foundations for understanding host-microbe interactions. Escherich proposed the potential role of endogenous microbes in the physiopathology of intestinal disease. He was later known for discovering the bacteria *Escherichia coli* [3]. In the 18th and 19th centuries, scientists from Britain and Germany documented the presence of microorganisms in the stomach and intestines of men [3]. The German botanist Uffelman also documented the presence of a large number of microorganisms in normal faeces of breast-fed babies [3].

Henry Tissier, a paediatrician, used a pure culture of *Bacillus acidiparalactici,* known to have strong fermentative power, to treat children with gastrointestinal disease. He noticed the restoration of the normal flora of his patients when they recovered [3].

Hermann Senator, a doctor of internal medicine, postulated that poisons generated in the gastrointestinal tract could enter the vascular system and cause a systemic effect [3]. The theory of autointoxication proposed by Charles Bouchard states that toxic metabolites produced by microorganisms, such as from the intestinal waste within the body, can lead to poisoning and is a major contributor to diseases [7, 8]. Furthermore, he suggested that one of the common ways to prevent autointoxication was through colon resection. However, Metchnikov suggested the introduction of harmless or beneficial microorganisms to replace harmful flora. He demonstrated its efficacy by popularising the age-old remedy of fermented milk to treat diarrhoea and claimed to have found a cure for ageing [3].

## Global research initiatives

Increasing revelations connect the gut flora to many vital physiological functions. However, the entire repertoire of gut microbes remains undefined. DNA sequencing techniques such as the 16S rRNA gene-based sequence analysis and metagenomic analysis are being used to identify new species [9]. Hence, countries have come up with multiple global projects like the Human Microbiome Project, MyNewGut funded by the EU, the British Gut Project, and the

American Gut Project to study, understand, and characterise gut microbial diversity as well as the human microbiome [10].

## Factors that influence the gut microbiome

The origin of the development of the gut microbiome is a controversial debate [10]. Some studies suggest that the placenta and the womb are sterile, and the GI tract of the infant is colonised at birth. However, recent studies report the presence of microorganisms in the placenta, umbilical cord, amniotic fluid and foetal membranes [11]. The mode of delivery also influences the gut and faecal microbiota composition in infants. An infant delivered through C-section has lower Bacteroides composition and higher levels of *Clostridium* species, resembling only 41% of the mother's faecal microbiome [1]. Infants delivered through vaginal birth contain a high abundance of *Lactobacilli* (due to the *Lactobacillus* present in the vagina), and their microbiota resembles 72% of the mother's faecal microbiome [1].

The composition of the microbiome evolves depending on the infant's diet and environmental exposure. After birth, two phyla temporarily dominate the microbiome, namely, *Actinobacteria* and *Proteobacteria*. Thereafter, lactic acid bacteria and *Bifidobacterium* are present in the gut till the infant is introduced to solid food. From then on, adult-type microbes such as *Bacteroides*, *Clostridium*, *Ruminococcus*, *Veilonella* and *Prevotella* dominate the infant's intestine [11]. An adult-like gut microbiota is established by about three years of age [11]. The diversity of the microbiome alters due to multiple factors such as illness, antibiotics exposure, diet, and other environmental factors such as smoking, geographical location, and living arrangements (urban or rural) [1].

For instance, in the case of antibiotic treatment, a study performed in humans found that β-lactam antibiotics (like ampicillin, cefazolin etc.) affect the microbiome by hampering the production of important cellular metabolites like acetyl CoA and acetyl phosphate that regulate cell metabolism [1]. Investigations in mice whose microbiome was depleted by antibiotic treatment showed that not only secondary bile acid and serotonin metabolism were affected in the colon but also that the mice were more susceptible to pathogenic infection [1].

Diet also plays an important role in determining microbial composition in the gut [12]. A study performed in 2018 involving immigrants originating from Thailand who migrated to the United States showed that the immigrants displayed a 'westernised' gut flora compared to their original microbiome [13]. Differences in populations that lead agricultural or early hunter-gatherer lifestyles versus urban populations are mostly in the number of fibre-digesting bacteria, with the former communities having 60% of their microbiome composed of these bacteria. In another study, mice that were fed a low-fibre diet were shown to have a loss in microbial diversity as opposed to mice fed with a high-fibre diet [12].

## The gut microbiome in health and disease

The gut microbiome offers multiple benefits to the host by supporting many physiological functions such as regulation of immunity, protection against pathogens, and maintaining the intestinal epithelium [14]. It also produces vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and cobalamin (vitamin B12), which are not produced by the host and are supplied through diet. [14]. The microbiota performs these functions by fermenting non-digestible substances like dietary fibres, thereby supporting the growth of microbes that produce short-chain fatty acids (SCFAs) like acetate, butyrate, and propionate [1, 14].

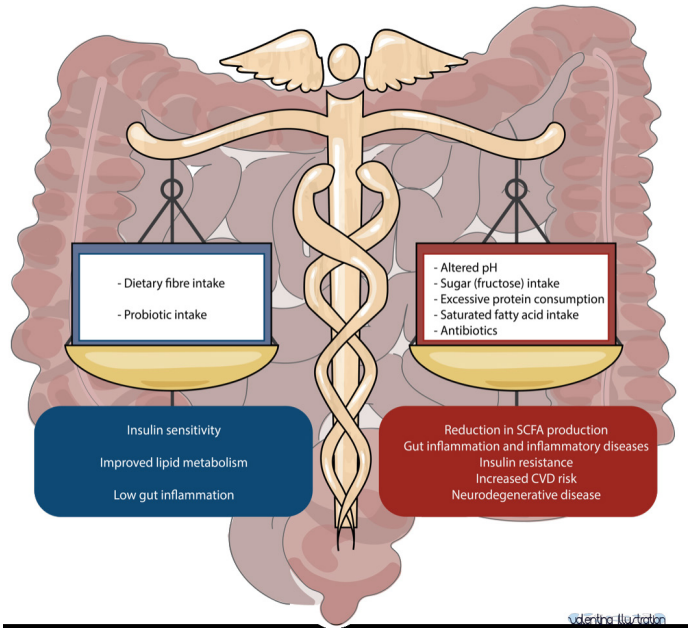


***Figure 1:*** *Illustration displaying the factors that influence the gut microbiome and the consequences during dysbiosis. The balance depicts the health effects when there is adequate fibre and probiotic intake (blue box) vs when there is an intake of foods with high sugar, protein and saturated fatty acid content, which alters the gut microbiome and increases the risk of various diseases (red box)[14].*

The most abundant short-chain fatty acid, acetate, regulates appetite and is important for lipogenesis and cholesterol metabolism [14]. Butyrate plays an important role for colon cells by not only being their main energy source but also being anti-inflammatory and anti-cancer by inducing apoptosis in colon cancer cells [14]. It also regulates glucose metabolism and is responsible for maintaining a state of hypoxia in the intestine to prevent dysbiosis. Propionate also plays a role in glucose metabolism and in satiety signalling.

Disturbance in the microbial composition causes dysbiosis which leads to conditions such as altered gut hormone production and immune dysregulation [15]. Dysbiosis of the gut can occur due to increased sugar, fat, and protein intake and low dietary fibre intake, which alters pH levels. Other factors include lack of sleep, frequency of exercise, and exposure to antibiotics [14]. Dysbiosis is linked to multiple diseases and disorders such as obesity, inflammatory bowel disease, cancer, metabolic disorders, neurological disorders, and neurodegenerative diseases (figure 1) [15].

## Gut microbiota and cancer

Dysbiosis of the gut microbiome has been reported to contribute to the development and progression of cancer as well as influencing the efficacy of anti-cancer therapies [16]. For instance, bowel cancer is the world's third most commonly occurring cancer [17]. *Bacteroides fragilis*, a strain of bacteria found in the gut, produces a toxin- Bacteroides fragilis toxin- which is implicated in the tumorigenesis of colon cancer as it triggers inflammation through common molecular pathways involving NF-κB and Stat3 in colon epithelial cells [18]. Similarly, a strain of *E. coli* (*E. coli* NC101) was found to promote colorectal cancer due to the production of a DNA-damaging toxin that leads to an inflammatory and tumorigenic environment [19]. Besides certain strains of bacteria directly causing tumorigenesis, even just the presence of certain strains indicates trouble. Similarly, the presence of *Fusobacterium nucleatum* in patients with bowel tumours is linked to worse survival outcomes as it directly interacts with

cancer signalling and immune pathways and promotes resistance to chemotherapy [20]. Besides intestine-related cancers, the gut microbiome also influences the prognosis and treatment response in other cancers such as liver, breast, and pancreatic cancers [14]. Animal experiments have shown that the presence of specific gut microbes (*Blautia producta*, *Clostridium saccharogumia*) influences the protective effect of phytoestrogens in breast cancer [14].

The gut microbiome may also improve the efficacy of anti-cancer drugs such as chemotherapy and immune checkpoint inhibitors [21]. Germ-free mice that do not have a gut microbiome are resistant to a common chemotherapy drug, cyclophosphamide (CTX). Similarly, these mice resist CTLA-4 blockade, a common immune checkpoint treatment, until introducing a certain *Bacteroides* strain that restores the response to CTLA-4 treatment by inducing and promoting the maturation of dendritic cells [21].

## Gut microbiota and immune-related disorders

Multiple studies have documented altered gut microbiome composition in inflammatory diseases such as Crohn's disease, rheumatoid arthritis, ulcerative colitis, asthma, diabetes mellitus, and obesity [22]. Short-chain fatty acids such as butyrate play an important role in the suppression of the NF-kB pathway and IFN-γ, regulating inflammatory and innate immune responses. SCFA's bind to G-protein coupled receptors present on immune cells such as macrophages, thereby conferring their anti-inflammatory effect [22]. Taking the example of inflammatory bowel disease, certain species of bacteria are reduced, causing a reduction in the production of SCFAs. This further causes an imbalance in the number of Th17 and T-reg cells which are vital for anti-inflammatory processes and, as a consequence, increase proinflammatory cytokine production [23].

## Gut microbiota and neurological and neurodegenerative diseases

Alteration in gut microbiota is associated with having consequences for the central nervous system [24]. The gastrointestinal tract and the nervous system communicate via the gut-brain axis, a bidirectional network that connects the autonomic nervous system, enteric nervous system, central nervous system and the hypothalamic-pituitary-adrenal axis, to the gastrointestinal tract [25]. The gut-brain axis regulates behaviour, cognition, and even brain development [26].

Recently, it was found that 90% of serotonin, a neurotransmitter responsible for the regulation of behaviour, sleep, and mood, is produced in the gut [24]. Neuronal physiology can be influenced by microbes such as *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Bifidobacteria* that produce neurotransmitters [24]. Patients suffering from neurodegenerative diseases like Alzheimer's, Parkinson's, multiple sclerosis, and neuropsychiatric disorders had altered microbial compositions when compared to healthy individuals [24]. A change in microbe composition can lead to neuroinflammation and changes in permeability in the blood-brain barrier. A similar observation was made in patients suffering from neuropsychiatric disorders such as depression. In another study concerning Alzheimer's disease, animal models that were germ-free, transgenic and susceptible to Alzheimer's showed lower levels of β-amyloid precursor protein when compared to non-germ free, transgenic, Alzheimer's susceptible animal models, which suggested the role of the gut microbiome, with over-representation of certain bacteria, in Alzheimer's disease [24].

Therefore, the gut microbiome plays a role in many neuropsychiatric disorders and neurodevelopmental disorders [26]. Germ-free mice present depression-like and anxiety-like behaviour, exhibit social avoidance, and show fatigue. These symptoms were reduced by supplementing them with prebiotics and fermented foods, indicating potential targeted therapies in this field [12, 26].

## Current therapies and future perspectives

Since the dysbiosis of the gut microbiome is linked to multiple diseases, various therapies have been developed that target certain microbial species linked to specific diseases, thereby restoring healthy microbial composition [27]. These therapies are more established for gut diseases rather than gut-brain linked disorders. One of the first therapies developed is faecal microbial transplantation (FMT). Even though this treatment was documented to be first used in Ancient Chinese medicine, in 1958, Ben Eiseman's team used faecal microbial transplantation as a method to treat patients suffering from pseudomembranous colitis. FMT is performed by transferring the gut microbiota from a healthy donor individual to the patient who has a dysbiotic microbiome via capsules, either orally or via a surgical colonoscopy. FMT was performed successfully for patients who suffered from recurrent *Clostridoides difficile* infection when antibiotic treatments failed. The cure rate was reported to be 80-90% [28, 29, 30].

FMT has not been restricted as a treatment only for *C. difficile* infection and shows promise in treating inflammatory bowel disease, gastric and non-gastric cancers, obesity, and neurodegenerative disorders [23, 26, 31]. Due to potential safety and regulatory issues, FMT is now also being tested in the form of capsules commonly known as poop pills [32].

Moreover, prebiotics and probiotics are well-established strategies for modulating the gut microbiome [33]. Prebiotics are non-digestible carbohydrates that serve as substrates for fermentation in the gut and hence lead to an increase in beneficial bacteria. Probiotics are live bacteria and yeasts that, when ingested, are beneficial to gut health [14]. Multiple animal studies have been performed that demonstrate the efficacy of these treatments for various diseases. However, there are limited numbers of clinical trials [33].

Future research involves targeting the gut microbiome in a specific manner, understanding the molecular interactions that occur due to dysbiosis, and advancement in technology for screening and diagnostics to prevent diseases.

## Conclusion

This review elaborated on multiple roles of the gut microbiome in the human body in health and disease pathophysiology. Targeting the gut microbiome as a form of therapy is a promising approach and is already being implemented in clinical trials. Future research involving the use of the microbiome for diagnostic purposes is therefore warranted.

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## References


1. Thursby, E. and N. Juge, *Introduction to the human gut microbiota.* The Biochemical journal, 2017. **474**(11): p. 1823-1836.
2. Davenport, E.R., et al., *The human microbiome in evolution.* BMC Biology, 2017. **15**(1): p. 127.
3. Farré-Maduell, E. and C. Casals-Pascual, *The origins of gut microbiome research in Europe: From Escherich to Nissle.* Human Microbiome Journal, 2019. **14**: p. 100065.
4. Berg, G., et al., *Microbiome definition re-visited: old concepts and new challenges.* Microbiome, 2020. **8**(1): p. 103.
5. *Hype or hope?* Nature Reviews Microbiology, 2019. **17**(12): p. 717-717.
6. Du, H., et al., *Fecal medicines used in traditional medical system of China: a systematic review of their names, original species, traditional uses, and modern investigations.* Chinese Medicine, 2019. **14**(1): p. 31.
7. Mathias, M., *Autointoxication and historical precursors of the microbiome-gut-brain axis.* Microbial ecology in health and disease, 2018. **29**(2): p. 1548249-1548249.
8. Ernst, E., *Colonic irrigation and the theory of autointoxication: a triumph of ignorance over science.* J Clin Gastroenterol, 1997. **24**(4): p. 196-8.
9. Almeida, A., et al., *A new genomic blueprint of the human gut microbiota.* Nature, 2019. **568**(7753): p. 499-504.
10. Cryan, J.F., et al., *The Microbiota-Gut-Brain Axis.* Physiological Reviews, 2019. **99**(4): p. 1877-2013.
11. Tanaka, M. and J. Nakayama, *Development of the gut microbiota in infancy and its impact on health in later life.* Allergology International, 2017. **66**(4): p. 515-522.
12. Zmora, N., J. Suez, and E. Elinav, *You are what you eat: diet, health and the gut microbiota.* Nature Reviews Gastroenterology & Hepatology, 2019. **16**(1): p. 35-56.
13. Vangay, P., et al., *US Immigration Westernizes the Human Gut Microbiome.* Cell, 2018. **175**(4): p. 962-972.e10.
14. Valdes, A.M., et al., *Role of the gut microbiota in nutrition and health.* BMJ, 2018. **361**: p. k2179.
15. Shreiner, A.B., J.Y. Kao, and V.B. Young, *The gut microbiome in health and in disease.* Current opinion in gastroenterology, 2015. **31**(1): p. 69-75.
16. Zitvogel, L., et al., *Cancer and the gut microbiota: an unexpected link.* Science translational medicine, 2015. **7**(271): p. 271ps1-271ps1.
17. Rawla, P., T. Sunkara, and A. Barsouk, *Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors.* Przeglad gastroenterologiczny, 2019. **14**(2): p. 89-103.
18. Chung, L., et al., *Bacteroides fragilis Toxin Coordinates a Pro-carcinogenic Inflammatory Cascade via Targeting of Colonic Epithelial Cells.* Cell host & microbe, 2018. **23**(2): p. 203-214.e5.
19. Arthur, J.C., et al., *Intestinal inflammation targets cancer-inducing activity of the microbiota.* Science (New York, N.Y.), 2012. **338**(6103): p. 120-123.
20. Castellarin, M., et al., *Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma.* Genome research, 2012. **22**(2): p. 299-306.
21. Cheng, W.Y., C.-Y. Wu, and J. Yu, *The role of gut microbiota in cancer treatment: friend or foe?* Gut, 2020. **69**(10): p. 1867.
22. Wang, J., W.-D. Chen, and Y.-D. Wang, *The Relationship Between Gut Microbiota and Inflammatory Diseases: The Role of Macrophages.* Frontiers in Microbiology, 2020. **11**(1065).
23. Khan, I., et al., *Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome.* Pathogens (Basel, Switzerland), 2019. **8**(3): p. 126.
24. Rutsch, A., J.B. Kantsjö, and F. Ronchi, *The Gut-Brain Axis: How Microbiota and Host Inflammasome Influence Brain Physiology and Pathology.* Frontiers in Immunology, 2020. **11**(3237).
25. Vivarelli, S., et al., *Gut Microbiota and Cancer: From Pathogenesis to Therapy.* Cancers, 2019. **11**(1): p. 38.
26. Liang, S., X. Wu, and F. Jin, *Gut-Brain Psychology: Rethinking Psychology From the Microbiota–Gut–Brain Axis.* Frontiers in Integrative Neuroscience, 2018. **12**(33).
27. Wong, A.C. and M. Levy, *New Approaches to Microbiome-Based Therapies.* mSystems, 2019. **4**(3): p. e00122-19.
28. Gupta, A., S. Saha, and S. Khanna, *Therapies to modulate gut microbiota: Past, present and future.* World journal of gastroenterology, 2020. **26**(8): p. 777-788.
29. Grinspan, A., *FMT for Severe C. difficile Infection: If at First You Do No Harm, the Second Time's a Charm.* Digestive Diseases and Sciences, 2021. **66**(1): p. 5-6.
30. Kho, Z.Y. and S.K. Lal, *The Human Gut Microbiome – A Potential Controller of Wellness and Disease.* Frontiers in Microbiology, 2018. **9**(1835).
31. Chen, D., et al., *Fecal microbiota transplantation in cancer management: Current status and perspectives.* International journal of cancer, 2019. **145**(8): p. 2021-2031.
32. Kao, D., et al., *Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial.* Jama, 2017. **318**(20): p. 1985-1993.
33. Marchesi, J.R., et al., *The gut microbiota and host health: a new clinical frontier.* Gut, 2016. **65**(2): p. 330.

# A LOOK INTO THE FUTURE: NEURODIVERSITY

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***Insight***

For a lot of psychiatric disorders, our perspective has changed over the past century. As an example, homosexuality was removed from the Diagnostic and Statistical Manual (DSM) only in 1973 [1]. Until 1980, it was possible for women to be diagnosed with 'hysteria', a condition for women showing attention-seeking and labile behaviour [2]. At the same time, many new disorders have been defined or redefined, such as hoarding disorder and binge eating disorder in the most recent version of the DSM in 2013 [3]. How do we decide if something is a disorder? Recently, the neurodiversity movement stated that mental disorders are just variations on normal brains and should be treated as such, respecting their unique challenges and capabilities. In this article, I will explore what a neurodiverse future of psychiatry would look like.

## Introduction

The neurodiversity paradigm is the concept that there is no normal or healthy brain, but there is a lot of variation between brains. There is no better kind of brain, just like there is a lot of variation in, for example, height or hair colour, but no better or worse hair colour or height. Besides the term neurodiversity, there is also the term neurodivergence, which refers to brains that differ from what we, as a society, see as the standard. For instance, people with autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), or dyslexia are seen as neurodivergent. The opposite of neurodivergent is neurotypical. Neurodiversity does not diminish the struggles neurodivergent people experience as a consequence of their neurodiversity.

In present times, neurodivergent people often struggle with issues like stigma, low self-esteem, and lower quality of life [4–6]. The neurodiversity movement advocates for better outcomes for neurodivergent people. What would a society where we value neurodiversity look like? Let us explore this, using ADHD as an example.

## A brief look into the past

Before speculating what the neurodiverse society would look like, it is important to know where we come from. The first description of ADHD comes from Sir Alexander Crichton (1763-1856), who described a case that resembled ADHD but only mentioned inattentive symptoms [7]. He reported ADHD to be purely physiological. Sir George Frederic Still (1868-1941), on the other hand, described ADHD as abnormal moral control of the child [7]. Later, Franz Kramer (1878-1967) and Hans Pollnow (1902-1943) put the focus on hyperactivity, coining the term "Hyperkinetic disease of infancy", but observed other symptoms as well [7]. The next postulated cause for ADHD was minimal brain damage because differences in brain function were visible [7]. This term was later replaced by minimal brain dysfunction [7]. In the second edition of the DSM, ADHD was named "hyperkinetic reaction of childhood" [7]. Inattention was mentioned in the description, but hyperactivity and impulsivity were seen as more important [7]. In the third edition, inattention returned as a core symptom, and the name changed to attention deficit disorder (with or without hyperactivity) [7]. In later versions of the DSM, subtle changes, mainly about the three presentations, were made [8].

From this history, we can learn several things. First of all, we learn that definitions and names of disorders can change over time and that this is related to what is thought to be the core problem. Secondly, the perceived cause of the disorder has varied over time, from entirely nature to entirely nurture and in between. Lastly, the focus has always been on the deficit and the problems a disorder causes for a person and their environment.

## Always look on the bright side

As mentioned before, throughout history, the focus for many psychiatric disorders was on the deficits. This in itself is not surprising, as this is what people and their environment experience problems with, and seek help for. Lately, some researchers are slowly shifting away from this deficit-oriented view and are exploring positive aspects of psychiatric disorders. Sticking with the example of ADHD, one of the positive aspects associated with ADHD is creativity [9]. In two qualitative studies, several other positive aspects were brought to light, such as being energetic, having a great sense of humour, or having great social intelligence [10, 11]. The research into positive aspects related to psychiatric disorders is still in its infancy.

In the future, we should know what strengths are commonly associated with different disorders and how they correlate with symptom severity and different symptom domains. For instance, we might discover that sociability and ADHD are positively correlated and, more specifically, that it is correlated with hyperactive symptoms. In that case, a person with ADHD can discover their strength and use this in their daily life. Knowing and using your strengths can boost self-esteem, general well-being, and quality of life.

## Adapting the environment

Another important point from the neurodiversity movement is that problems an individual experiences related to their disorder are not inherently caused by the person but by a mismatch with the environment. In the same way, not being able to enter a building because of stairs for a wheelchair user is not inherently caused by the wheelchair but by a lack of a wheelchair ramp. In the same way, we could say that not being able to sit still in a classroom for someone with ADHD is not inherently a problem caused by their ADHD but by a mismatch between our societal expectations and their brain. By adapting the way we teach, we can meet their needs instead of them meeting our expectations.

Currently, this is already done for the working environment on a small scale with, for example, special companies for neurodivergent people, such as Authentict, an ICT company for people with ASD. They focus on the strengths of their employees and provide an optimal work environment with, for instance, little stressors, low sensory stimulation, and extra coaching for areas they struggle with [12].

Although these initiatives are great, it would be even better if, in the future, all companies seek to be neurodiverse and inclusive. This would mean that neurodivergent people are valued for their strengths and different perspectives, and the workplace is adapted to their unique needs.

Moreover, the society of the future should not only strive to be inclusive for neurodivergent people at their workplace but also in all sects of society. This includes schools and universities, but also supermarkets, sports clubs, and cinemas.

## Implications for the clinic

Adopting the neurodiversity paradigm in clinics has several implications. In the first place, clinicians have the possibility to change the story of a diagnosis from the beginning. In their article, Brown *et al.* give useful tips on how clinicians can adopt a neurodiversity perspective in diagnosing psychiatric disorders [13]. The article focuses on the use of positive language (e.g. not "deficit" or "co-morbid"), the tone used, the needs and emotions of the parents, how to present treatment, and intersectionality [13]. In the future, this should be adopted by all diagnosing clinicians. Moreover, attention should be given to recognizing strengths and not only difficulties a person experiences. If this is included from the start of the diagnosis, this could prevent a decline in self-esteem.

Not only psychiatrists and other clinicians that diagnose individuals with psychiatric disorders should adopt this strategy. The whole health care system should be more inclusive of neurodivergent people, providing an environment where they can feel safe and understood. This could, for example, mean providing online consults with patients who feel anxious in the hospital itself or dimming the lights for patients with sensory sensitivities.

## Implications for research

The neurodiversity paradigm should not only change our society or the clinic but also the way we do research. In their article, Edmund Sonuga-Barke and Anita Thapar argue that the shift away from deficit-oriented research will radically change the way we perform research [14]. We should not be looking for a cure anymore. Instead, we should put the experiences of neurodivergent people first and listen to what they think is worth researching, as neurodivergent people often see their neurodiversity as an important part of their identity. Moreover, the primary outcome of trials should change from merely a reduction of symptoms to improvement in quality of life, self-esteem, and other positive indicators.

Additionally, we should pay attention to how the environment can be (in)accessible for neurodivergent people, the stigma, and attitude towards them. Researchers should take the experiences of neurodivergent people as a starting point for research, focusing on positive outcome measures.

## Conclusion

This article discussed what a neurodiverse society in the future would look like. This includes approaching neurodivergence from a positive angle and focusing on their strengths. At the same time, we should adapt society to make it more accessible to neurodivergent people. This should be done at, for instance, the workplace and in the clinic. Clinicians should provide a more nuanced story when diagnosing an individual, listening to their needs and experiences. Finally, our research should focus on positive outcomes for neurodivergent people. The future should value neurodiversity and will be better because of it.

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## References

1. J. Drescher, 'Out of DSM: Depathologizing Homosexuality', *Behav. Sci.*, vol. 5, no. 4, pp. 565–575, Dec. 2015, doi: 10.3390/bs5040565.
2. R. E. Kendell, 'Hysteria', in *International Encyclopedia of the Social & Behavioral Sciences*, N. J. Smelser and P. B. Baltes, Eds. Oxford: Pergamon, 2001, pp. 7133–7138. doi: 10.1016/B0-08-043076-7/03725-6.
3. *Diagnostic and statistical manual of mental disorders : DSM-5*. Arlington, VA: American Psychiatric Association, 2013.
4. T. V. Masuch, M. Bea, B. Alm, P. Deibler, and E. Sobanski, 'Internalized stigma, anticipated discrimination and perceived public stigma in adults with ADHD', *Atten. Deficit Hyperact. Disord.*, vol. 11, no. 2, pp. 211–220, Jun. 2019, doi: 10.1007/s12402-018-0274-9.
5. P. E. Newark, M. Elsässer, and R.-D. Stieglitz, 'Self-Esteem, Self-Efficacy, and Resources in Adults With ADHD', *J. Atten. Disord.*, vol. 20, no. 3, pp. 279–290, Mar. 2016, doi: 10.1177/1087054712459561.
6. R. Agarwal, M. Goldenberg, R. Perry, and W. W. Ishak, 'The Quality of Life of Adults with Attention Deficit Hyperactivity Disorder', *Innov. Clin. Neurosci.*, vol. 9, no. 5–6, pp. 10–21, 2012.
7. K. W. Lange, S. Reichl, K. M. Lange, L. Tucha, and O. Tucha, 'The history of attention deficit hyperactivity disorder', *Atten. Deficit Hyperact. Disord.*, vol. 2, no. 4, pp. 241–255, 2010, doi: 10.1007/s12402-010-0045-8.
8. J. N. Epstein and R. E. A. Loren, 'Changes in the Definition of ADHD in DSM-5: Subtle but Important', *Neuropsychiatry*, vol. 3, no. 5, pp. 455–458, Oct. 2013, doi: 10.2217/npy.13.59.
9. M. Hoogman, M. Stolte, M. Baas, and E. Kroesbergen, 'Creativity and ADHD: A review of behavioral studies, the effect of psychostimulants and neural underpinnings', *Neurosci. Biobehav. Rev.*, vol. 119, pp. 66–85, Dec. 2020, doi: 10.1016/j.neubiorev.2020.09.029.
10. S. Mahdi *et al.*, 'An international qualitative study of ability and disability in ADHD using the WHO-ICF framework', *Eur. Child Adolesc. Psychiatry*, vol. 26, no. 10, pp. 1219–1231, Oct. 2017, doi: 10.1007/s00787-017-0983-1.
11. J. A. Sedgwick, A. Merwood, and P. Asherson, 'The positive aspects of attention deficit hyperactivity disorder: a qualitative investigation of successful adults with ADHD', *Atten. Deficit Hyperact. Disord.*, vol. 11, no. 3, pp. 241–253, Sep. 2019, doi: 10.1007/s12402-018-0277-6.
12. 'AUTHENTICT - ICT mét autisme :: Waarom ICT'ers met autisme?' https://authentict.nl/waarom-ict-ers-met-autisme- (accessed

Dec. 12, 2021).
13. H. M. Brown, A. C. Stahmer, P. Dwyer, and S. Rivera, 'Changing the story: How diagnosticians can support a neurodiversity perspective from the start', *Autism*, vol. 25, no. 5, pp. 1171–1174, Jul. 2021, doi: 10.1177/13623613211001012.
14. E. Sonuga-Barke and A. Thapar, 'The neurodiversity concept: is it helpful for clinicians and scientists?', *Lancet Psychiatry*, vol. 8, no. 7, pp. 559–561, Jul. 2021, doi: 10.1016/S2215-0366(21)00167-X.

## EXAM QUESTION

**Question 1**
The so-called 'tricyclic antidepressants' inhibit the reuptake of two specific neurotransmitters. One of these is norepinephrine. Which one is the other neurotransmitter?

A. 5-hydroxytryptamine
B. Dopamine
C. Acetylcholine

*(Topic from Q8 MGZ Psychology, 2020)*

**Question 2**
Which cells can be grown ex vivo and applied therapeutically to reduce rejection?

A. Donor regulatory T cells
B. Donor tolerogenic dendritic cells
C. Both donor tolerogenic cells and regulatory T cells

*(Topic from Q5 MGZ Immune system, 2021)*

**The answer to these questions can be found on page 24 in this journal.**

# FINDING THE DRUGS OF TOMORROW:
## THE DRUG DEVELOPMENT PIPELINE

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***Abstract***

Developing novel drugs and therapies is a labour-intensive, costly, and tightly regulated process spanning multiple years. As students of the medical faculty will likely interact with this process during or after their studies, this article is aimed at giving a general overview of the drug development pipeline and explains the individual phases in more detail. In the drug development pipeline, researchers examine their disease of interest to find a druggable target. Following this, massive compound libraries are screened for hits that are able to influence the target. These drug candidates are often tested *in vitro* and *in vivo* to establish pharmacokinetics, off-target effects, and proof-of-concept efficacy. Subsequently, the drug candidates are studied in more detail, optimised, and prepared for the clinical phases. In the first couple of clinical phases, the emphasis is on safety. In phase I, extensive safety testing is performed in a small group of healthy participants. In phase II, the efficacy of the drug is tested in a small group of patients with the disease of interest while further examining safety. In phase III, the efficacy is assessed in a larger group of patients with the disease of interest, and more rare side effects are studied. Following a successful, positive phase III trial, the novel drug can be submitted for regulatory approval. If the drug is approved, phase IV focuses on all data gathered from the drug prescriptions to all patients. These data may indicate rare side effects not found in earlier trials or indicate a different efficacy compared to what was found in the controlled clinical trial settings. The regulatory approval may be rescinded if the drug proves to have too little efficacy or too many severe side effects outside of the study setting.

## Introduction

As the quality of global health increases, so does the need for novel, efficacious drugs and therapies. Older drugs can be improved, current diseases may develop treatment resistance, or new diseases can surface at any time. When the need for a new drug arises, a multi-step, multi-year, and multi-million-dollar cascade must be traversed before a novel drug can enter the market. From time to time, older ideas can be refined or expanded upon, shortening the process. However, from start to finish, the development of a new drug can take over 15 years, and the mean estimate of costs is around US$2.6 billion per approved drug, although newer studies report a decreasing mean estimate of costs of around US$1 billion per approved drug [1, 2].

The drug development pipeline consists of a preclinical development phase, in which a target is defined and a possible drug candidate is selected, and a clinical development phase, in which a promising drug candidate is tested in humans [1]. Both phases are subdivided into individual, more detailed phases [1]. Due to the high sunk costs into potential drug candidates, failure in a later phase of development proves to be extremely costly. As such, drug development companies will put in great effort to only select the most favourable candidates for the development of a new drug. Potential candidates are optimised as much as possible before moving into human trials.

## The drug development pipeline

### Preclinical development

During the preclinical development of a novel therapy, the goal is to find a suitable target which is causally involved in the disease of interest and to subsequently find a way to influence this target to combat the disease (Table 1). As the name "preclinical development"

***Table 1:*** *The simplified goals of preclinical phases.*

| **Preclinical phase** | **Goal of the phase** |
|---|---|
| Target identification | To obtain a list of targets that may be causally related to the disease of interest. |
| Target validation | To verify that the target is causally related to the disease of interest. |
| Lead discovery | To test many compounds or methods to attempt to influence the target. |
| Lead optimisation | To gather all information on the lead, improve the safety and efficacy of the lead, and prepare it for clinical phases. |

suggests, this is all performed without applying the therapy in humans. *In vitro* assays on cell lines, disease models, or patient material combined with *in vivo* animal disease models and *in silico* tools are used to find a suitable target and subsequently find a therapy that can influence the target. At the end of the preclinical development, information on safety, toxicology, and efficacy has to be collected from previously mentioned models, and if proven to be favourable enough, the development will continue into the clinical phase. The therapy is finalised and ready to be applied in a human setting. The preclinical development is the shorter of the two, taking roughly five years and 30% of all costs associated with developing a new drug [3].

## Target identification

The first step in the drug development pipeline consists of defining a health problem or disease. The goal of the target identification is to identify key molecules or processes which are associated with the disease. However, not all diseases may have molecules or processes which are known to directly influence the disease burden, or these cannot be influenced through external intervention (yet). Target identification requires intricate knowledge of the disease aetiology and may require additional research before a suitable target can be found.

Omics techniques, such as genomics and proteomics, can offer valuable insight into a disease phenotype if used on patient material. Applying several omics techniques on patient materials and combining the results will lead to a large list of factors that are altered, such as upregulation or downregulation of genes or unexpected metabolites. These "changes" can be relevant to the disease aetiology. However, not all of these factors may be related to the disease of interest. Narrowing the list down to a few promising, disease-causing factors is key. By constructing the disease aetiology using the literature and experimental results, key molecules or processes can be pinpointed. These can be cross-referenced with the results from the disease phenotype analysis to yield interesting targets. Once a shortlist of possible targets is constructed, these can move to the target validation phase.

## Target validation

During the target validation, the goal is to establish proof that the target truly is a disease-causing factor. Causal relations can be proven if 1) the target is present in the diseased state and 2) influencing the target influences the disease condition. For example, the function of the enzyme angiotensin-converting enzyme (ACE) is to convert angiotensin I into the vasoconstrictive angiotensin II [4]. The activity of this enzyme has been linked to high blood pressure and heart failure [4]. A method to prevent or at least inhibit this conversion would lead to a decrease of angiotensin II, and as a result, a decrease in blood pressure. On the other hand, increasing ACE activity will increase the prevalence of angiotensin II, increasing blood pressure and worsening the disease state. In this case, ACE activity is linked to the diseased state; increasing its activity directly increases the disease burden, and preventing its activity relieves disease burden, making ACE activity a causative factor and thus a suitable target to combat high blood pressure and heart failure [4]. The target validation makes use of patient material and is partially performed *in vitro*, but currently often requires *in vivo* animal studies to confirm whether the selected target is truly disease-causing.

## Lead discovery

Once the target is defined, therapy can be designed to influence the target. Note that, depending on the type of target (e.g., genetic, receptor, enzyme, etc.), different types of therapies can be considered (Table 2).

### Genetic defects

In the case of genetic defects, numerous approaches can be taken to influence the impact of the genetic defect, such as trying to repair the gene using genomic editing techniques or by influencing the genetic product if no suitable approach can be taken to repair the gene. Before designing a gene-repairing therapy, it is important to thoroughly investigate the genetic defect itself. How often does it occur? What kind of mutation is it (e.g., missense, nonsense, or frameshift)? What is the canonical function of the gene? Can this defect be amended later in life, or is there a specific window of opportunity? Most of these questions can be answered by research performed in the target identification and target validation phases, but knowing the inner workings of the genetic defect at hand is key. Knowledge of the genetic defect can render one approach more suitable in comparison to another (e.g., supplementation of the correct genetic product may be a more suitable approach than attempting to restore the "correct" gene in case of a gene whose function is relatively unknown). Depending on which approach is taken, the process of lead discovery is different.

***Table 2:*** *A few examples of approaches that can be taken to find a hit, depending on the type of target.*

| **Type of target** | **Examples of therapy methods** |
|---|---|
| Genetic | Gene repair techniques;<br>Supplementation of correct genetic product (in case of loss-of-function of genetic product);<br>Inhibition of the diseased genetic product (in case of gain-of-function genetic product) |
| Enzymatic | Exogenous enzyme supplementation (in case of loss-of-function);<br>Enzyme inhibition (in case of gain-of-function); |
| Receptor | Receptor agonists (if receptor must be activated);<br>Receptor antagonists (if receptor must be inactivated) |

If gene repair appears to be the most promising route, several types of delivery vectors can be used. A delivery vector is a method to get the correct genetic material to the correct place. Viruses such as adenoviruses or herpesviruses are versatile vectors as they can deliver a relatively large genetic load but cannot integrate this into the host DNA, causing only temporary expression [5]. However, other viruses, such as retroviruses and lentiviruses, can insert a smaller genetic load into the host DNA using reverse transcriptase and integrase enzymes [5]. Viruses are unique in terms of which cells they can infect, how effective they are at delivering the genetic load, and how they must be prepared. Newer techniques of gene editing, such as the CRISPR-Cas9 complex, can also be used. However, as the potential effectivity and preparation for each technique are different, it is necessary to choose a specific method of gene-editing early in the process.

The lead discovery for gene-editing of genetic defects consists mostly of testing different genetic codes of the vector. In the case of CRISPR-Cas9-mediated gene editing, this consists of testing multiple sequences of guide RNA.

If gene repair is not an option, downstream elements can be targeted. For example, the transcribed mRNA can be intercepted and degraded, the formed product can be inhibited, or a functional version of the genetic product can be supplemented.

### Non-genetic defects

In the case of non-genetic defects, therapies are focused on affecting the target directly. This is often in the form of small molecules or biologicals. Long lists of known compounds, called compound libraries, are screened to see whether any of the existing compounds interact with the target in a process called high throughput screening (HTS). Thousands of compounds are screened for interactions with the target. Any compounds that are found to interact with the target in the initial HTS are tested again to confirm their activity using a positive selection and a negative selection. Once a compound is confirmed as active against the target, its selectivity for the target is tested, and a dose-response curve for the target is constructed. If the compound passes this test, it is considered a "hit".

Finally, after a promising hit is selected, it is further tested in *in vitro* systems. Data on cellular toxicity and genotoxicity is gathered, off-targets are tested, and a primitive absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile is generated. If these appear favourable, the hit is considered a "lead" and can be tested in *in vivo* systems. Testing the lead *in vivo* gives valuable information on the characteristics of the compound in a systemic setting. New off-targets can be found, unexpected metabolites can be analysed, toxicities can be discovered, and an *in vivo* ADMET profile can be generated. Additionally, this will also serve as the first proof-of-concept that the drug or therapy can achieve the desired goal.

## Lead optimisation

The lead optimisation focuses on improving the safety and toxicology of the lead. If the lead is to ever become a successful therapy for humans, the benefit versus risk ratio must be favourable. In the lead optimisation phase, *in vitro* assays, cellular disease models, and *in vivo* disease models are used to ascertain the efficacy and the safety of the lead. The lead optimisation is aimed at finding out everything there is to know about the lead, amplifying the positives, mitigating the negatives, and creating the optimal ADMET profile. For example, the compound may be coated or slightly altered to improve the oral bioavailability, or the route of administration may be changed to reduce off-target exposure. At the end of the lead optimisation, the therapy is ready to be tested in humans. During the lead optimisation, the lead is tested in similar doses and routes of administration as planned in humans. Generally, after the lead optimisation, no more changes are made to the molecular contents of the lead.

The dose administered to the animal test subjects in the *in vivo* disease models at which no adverse effects were observed is used as a basis for human dosing in the clinical phases [6]. Interspecies and intraspecies safety factors are applied to reduce the dose to a level that is thought to be safe for humans. Generally, the intraspecies factor is 10, while the interspecies factor depends on which animal model was used in the *in vivo* safety testing [6].

## Clinical development

The clinical phases of drug development are aimed at finding out whether a treatment is safe to use and if it has the intended effect in humans (Table 3). Next to this, the dosing regimen is also established and confirmed. The clinical development of a therapy is generally the most time consuming and costly part of the drug development pipeline, taking up to 10 years and 70% of all costs [3]. The following sections describe the clinical development phases of a "classic" drug candidate, a drug candidate to be used for relatively common diseases. The clinical phases can be different in the clinical development of "orphan" drugs or therapies, drugs that would not be profitable to develop without government funding due to the rarity

***Table 3:*** *The simplified goals of the clinical phases.*

| **Clinical phase** | **Goal of the phase** |
|---|---|
| Phase I | To establish whether the therapy is safe to use in humans in a small group. |
| Phase II | To establish whether there is an effect of the therapy on the disease of interest in a small group. Includes further safety assessment. |
| Phase III | To assess the effectiveness of the therapy and possibly compare it to other therapies in a large study group. Includes further safety assessment. |
| Phase IV/post-marketing surveillance | To continually check for the safety of using the therapy in all patients using the therapy. |

of the treated condition. Specific immunotherapy for a rare cancer or gene repair therapy for a rare, severe hereditary disease falls under the orphan drug development. For example, phases I and II can sometimes be performed simultaneously, and market approval may be easier to attain in these cases.

### Phase I

Once the green light is given for human testing, phase I can begin. Phase I is focused on finding information regarding the safety of systemic exposure to the drug or therapy in humans. The subjects in phase I are usually healthy subjects without the disease of interest unless the therapy is known to actively harm healthy subjects, such as new chemotherapeutic drugs. In that case, a small patient group is selected. Phase I usually uses between 20-50 test subjects.

The primary outcomes of phase I studies are focused on parameters regarding the ADMET profile of the compound in humans. Next to this, phase I studies often include a dose-escalation. Dose-escalation means that a fraction of the study subjects is exposed to increasing doses of the drug to assess the safety of higher than intended exposures. For example, while overexposure to antibiotic Vancomycin will almost certainly kill off a bacterial infection, it may also cause significant nephrotoxicity. The dose-escalation is divided into phase Ia (single dose escalation) and phase Ib (multi-dose escalation). The dose-escalation allows for dose-related toxicities to be found and an estimate of the optimal dose to be selected. This optimal dose is the dose at which the positive effects of the treatment maximally outweigh the negative effects of the treatment. This optimal dose is confirmed in later phases and will become the therapeutic dose if the drug gains market approval.

Phase I lasts around one and a half years, with estimated success rates of up to 65% [7].

### Phase II

If a drug or therapy is proven to be safe in phase I, phase II can commence. For some drug candidates that have a high expected toxicity, phase I and phase II are combined. In phase II, the goal is to demonstrate the efficacy of the drug or therapy. Next to this, additional safety data is gathered. The results of a phase II trial will determine the sample size, expected effect size, time schedule, and other parts of the following phase III trial. Phase II uses a hundred to several hundred participants with the disease of interest. Phase II is also used to establish the final, optimal dosing regimen, based on the safety data gathered from the phase I dose escalation.

Even though the phase may not seem that complicated, only 33% of compounds pass from phase II to phase III, with phase II lasting around three years [7]. The failure to pass phase II can often be attributed to interspecies and intraspecies differences, rendering the novel drug or therapy not effective for human subjects.

### Phase III

Phase III is the most critical phase of the clinical phase. Phase III trials, sometimes referred to as "pivotal trials", include hundreds to thousands of patients to confirm the dosing regimen, the safety, and the efficacy of the novel therapy or drug [8]. Phase III trials are incredibly expensive due to the large number of participants and the monitoring involved. Costs may even be driven up if the novel therapy or drug is compared to the current golden standard, as this requires additional study participants. If the novel therapy or drug is proven to be effective at treating the disease and is considered safe (enough) to use in patients, the trial can be considered a success. Following a successful phase III trial, the researchers can submit a request for regulatory approval in the tested population to the regulatory agencies. The European Medicines Agency (EMA, for approval in the European Union) and the Food and Drug Administration (FDA, for approval in the United States of America) carefully review all the evidence gained from the preclinical and clinical phases. If the novel therapy or drug is deemed to be efficacious and safe, approval for marketing will be granted. The novel therapy or drug can now be prescribed for the indicated disease in the indicated population.

Phase III trials take the longest, taking a median of 3.8 years to completion, with a success rate varying between 50% and 60% [7].

The applicability of the drug or therapy can be extended to other diseases or other populations (e.g., some antivirals that were approved for treatment against HIV also display efficacy for treating hepatitis B), but these require new trials to prove the safety and efficacy profile in the novel population.

### Phase IV/Post-marketing surveillance

After a new drug or therapy is approved, more patients gain access to the product. This can be seen as the final "trial". As more patients are exposed to the drug or therapy, much more data will become available regarding the safety and efficacy of the product. It is possible for new effects – positive and negative – to only show up after the product has already gained regulatory approval. The manufacturer must keep collecting data regarding the safety of their product and periodically submit this data to the regulatory authorities, who will act accordingly. If severe health effects are found, or a drug with severe side effects is found to not be as efficacious as thought after the regulatory approval, the regulatory authorities can withdraw marketing approval.

The most well-known cases are likely the recall of approval for diethylstilbestrol (DES) and the recall of thalidomide (Softenon). DES is a synthetic oestrogen approved to prevent miscarriage and other pregnancy-related complications, but the use of DES resulted in clear cell adenocarcinomas and breast cancer not only in the women who took the drug but also in their daughters and possibly their granddaughters [9]. Prescribing DES to pregnant women was finally banned in the USA in 1971 and in 1978 in Europe [9]. Thalidomide was approved for use against anxiety and morning sickness [10]. However, following a series of cases in which severe birth defects were observed, thalidomide was withdrawn from the European market in 1961 [10]. Both incidents had a large impact on the way clinical trials and drug approval are handled today. Pregnant women are now a special population for which a new drug or therapy must gain separate regulatory approval. The post-marketing surveillance system is a direct result of the thalidomide effects.

## Discussion

The drug development pipeline is a long and meticulous process. Each individual section of the pipeline is important and plays its own part in bringing a new drug to the market. However, the pipeline is changing. With novel SARS-CoV-2 vaccines achieving emergency approval for clinical testing and bringing a working vaccine from an idea to the public in as little as 11 months, it is clear to some that the pipeline can be altered [11]. The massive gain in time-to-product in the development of COVID-19 vaccines came mostly from running clinical trials in multiple phases back-to-back. Additionally, the preclinical development was also significantly reduced, as most vaccines were developed using prior research performed for SARS-CoV or other viral infections [12]. However,

the vaccine manufacturers must continue to submit data to the regulatory authorities to prove long time safety and efficacy. This will continue until the manufacturers have fully completed the regular clinical phase studies.

Parts of the preclinical development are also up for debate, as the voices of animal rights activists grow louder every year. Currently, animals are used in multiple steps of the preclinical development, such as disease models to confirm the target in the target validation phase or in tests for the systemic exposures in the lead discovery phase. *In vitro* systems such as organs- or humans-on-a-chip models or *in silico* modelling could serve as a suitable replacement for animal testing [13, 14]. However, it is not clear yet whether these systems can model the real human situation sufficiently. As about only 10% of all leads currently make it through the clinical phases, using new systems must at least be as good as the old systems and preferably provide a benefit in predictive value for the human situation. Only time will tell how the drug development pipeline will develop in the future and what this will mean for research and researchers alike.

## Acknowledgements

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## References

1. Mohs, R.C. & Greig, N.H. Drug discovery and development: Role of basic biological research. *Alzheimers Dement (N Y)* **3**, 651-657 (2017).
2. Wouters, O.J.*, et al.* Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018. *JAMA* **323**, 844-853 (2020).
3. Harrer, S.*, et al.* Artificial Intelligence for Clinical Trial Design. *Trends Pharmacol Sci* **40**, 577-591 (2019).
4. Messerli, F.H.*, et al.* Angiotensin-Converting Enzyme Inhibitors in Hypertension: To Use or Not to Use? *J Am Coll Cardiol* **71**, 1474-1482 (2018).
5. Bouard, D.*, et al.* Viral vectors: from virology to transgene expression. *Br J Pharmacol* **157**, 153-165 (2009).
6. Nair, A.B. & Jacob, S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* **7**, 27-31 (2016).
7. Wong, C.H.*, et al.* Estimation of clinical trial success rates and related parameters. *Biostatistics* **20**, 273-286 (2019).
8. Umscheid, C.A.*, et al.* Key concepts of clinical trials: a narrative review. *Postgrad Med* **123**, 194-204 (2011).
9. Giusti, R.M.*, et al.* Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med* **122**, 778-788 (1995).
10. Lenz, W. A short history of thalidomide embryopathy. *Teratology* **38**, 203-215 (1988).
11. Pearson, H. How COVID broke the evidence pipeline. *Nature* **593**, 182-185 (2021).
12. Padron-Regalado, E. Vaccines for SARS-CoV-2: Lessons from Other Coronavirus Strains. *Infect Dis Ther*, 1-20 (2020).
13. Festing, S. & Wilkinson, R. The ethics of animal research. Talking Point on the use of animals in scientific research. *EMBO Rep* **8**, 526-530 (2007).
14. Pinero, J.*, et al.* In silico models in drug development: where we are. *Curr Opin Pharmacol* **42**, 111-121 (2018).

# INTERVIEW:
# THE FIRST BILATERAL HAND TRANSPLANT IN THE NETHERLANDS

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***Insight***

When the first attempt at hand transplantation was performed in 1964 in Ecuador, the recipient experienced acute and irreversible rejection of the donor hand within two weeks [1]. Hand transplantation has come a long way since then, with the development of enhanced immunosuppressive treatment and advances in microsurgery. While hand transplantation is an amazing feat, it remains a complex procedure, limited by difficulties surrounding limb preservation and accompanied by ethical, financial, and psychological complications [1, 2]. If you lost your hands, would you jump at the chance to get a hand transplant?

Worldwide, over 130 hand-arm transplantations have been performed. However, none of these hand-arm transplantations had taken place in the Netherlands [3]; until recently, that is. After years of intense preparation, the first bilateral hand-arm transplantation was successfully performed in the Radboudumc in July of 2019, led by Prof. Dr. Hovius and Prof. Dr. Ulrich. Maartje Bijl was the first patient to undergo hand transplantation in the Netherlands. In order to discover the ins and outs of this complicated procedure, RAMS sits down with Anne Sophie Kruit, a plastic surgeon in training who was closely involved in this surgery. Anne Sophie also recently successfully obtained her PhD in extracorporeal perfusion and limb preservation.

## Limb preservation: current state and research

A crucial part of any transplantation is to ensure the transplant is not damaged. When tissue is no longer attached to the body, damage occurs due to ischemia. This is a lack of oxygen and nutrients, which are usually supplied by blood flow. Considering that ischemia can develop quickly after tissue removal, the current standard is to store the tissue on ice, increasing the time until ischemia occurs. 'The common practice for a limb is to flush it with preservation fluid to prevent clotting inside the vascular system, to cool it, and then store it in bags on melting ice,' Anne Sophie explains. This can be done up to approximately six hours before the tissue is damaged irreversibly [2, 4]. 'Clinically, this type of damage will give fibrosis, which means that the muscle contraction will be less powerful or the nerve will function a bit less.'

In comparison, a kidney can be stored on ice for up to 24 hours before the tissue starts to deteriorate [5]. This difference results from limbs containing a lot of muscle and nerves, which are both tissues that are highly susceptible to ischemia and cell death [2, 4]. Transplanting a hand or arm is also exceedingly more difficult than transplanting an organ, as you have to connect nerves, tendons and bones instead of 'just' blood vessels. 'You are battling against time to keep the surgery as short as possible before replanting [the limb], and you hope the muscle is still vital.'

Due to the more complicated nature of hand-arm transplantations, research is necessary to find ways to improve the procedures surrounding limb preservation. One of those researchers is Anne Sophie: 'Most of the time I start explaining [my research] by referring to a heart-lung machine, which most people know from organ donation and cardiac surgery. My main goal was to change this machine to also be able to use it for limbs. There has been some research in the field, but a lot of it lacks methodology or has contradicting outcomes, and the quality of all these studies was quite low.'

'You have to see the heart-lung machine as the replacement of the body', Anne Sophie explains, 'You can attach the arm to it, and there is a fluid running through the machine. This can be blood, which is the most body-like, or you can use a cold over-the-counter available fluid, and this is what we have done to keep it simple. A pump, similar to the heart, has to pump [the fluid] back and forth through the tissue. There is also a heater-cooler machine, so you can set the temperature either at body temperature or cool [the limb], and there is an oxygenator. So there is a fluid, which is cooled, and there is oxygen and nutrients in it.' Anne Sophie and colleagues then compared this updated heart-lung machine to the current practice. 'Cold storage is able to preserve tissues from four to six hours, and then it starts to degrade. With the machine, we were now able to at least store it [tissues] for 18 hours. We ended up with my final model, which was to amputate a pig's forelimb, put it on the machine for 18 hours, and then replant it back to the pig. We did that, compared it to four hours on ice and replanted it back. The outcomes were actually quite equal, so that was encouraging.'

Finding the right setting for this machine was not as easy as Anne Sophie makes it sound.

'The tricky part was to find the right balance and the right setting. The second thing, which was really important, was to find an outcome that can reliably measure the amount of muscle damage. This is a holy grail in this research, to find one measure and one cut-off value which tells you if the muscle is dead or alive, and that is something we are still searching for'.

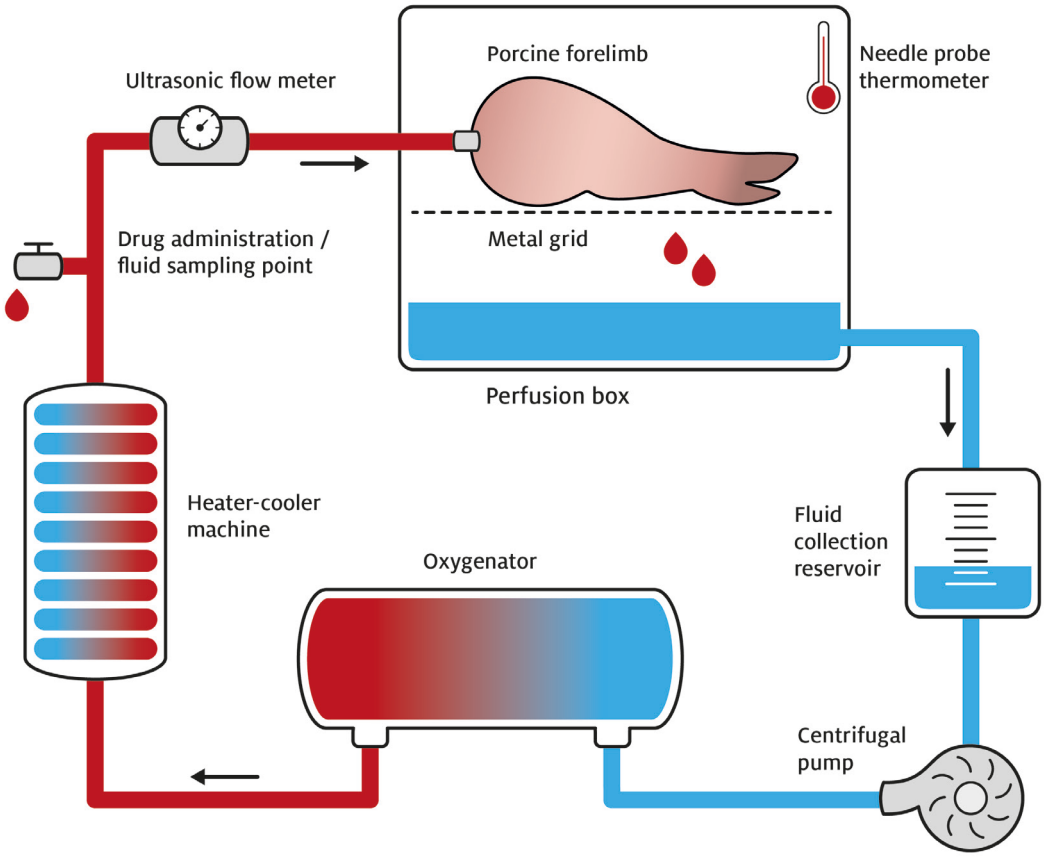


***Figure 1:*** *A systematic illustration of the extracorporeal perfusion set-up, as optimized by Anne Sophie and her colleagues.*

## First double hand transplantation

One example that illustrates the importance of this research is the first double hand transplantation recently performed in the Netherlands on Maartje Bijl. She lost both arms and legs due to sepsis. This surgery was performed according to the current standards but could benefit greatly from Anne Sophie's research: 'With the research I did, we proved that 18 hours of extracorporeal perfusion is feasible, and my successor is researching 24 hours. So, this would mean a prolonged time period for patient stabilisation and surgery'.

The preparation for the surgery took nearly three years, including ethical and legislative approval, writing protocols, and practising in the anatomy lab. However, one of the most important and equally tricky parts was finding a donor. 'First of all, it has to match with the patient. You cannot just put a really large manly hand on a petite woman's wrist, so it had to match.' Besides the hands needing to match cosmetically, the Human Leukocyte Antigen (HLA) type also had to match. HLA molecules are expressed on most cells, and they help the immune system distinguish between the body's own 'self' cells and non-'self' cells [6]. 'This was the tricky part because our patient had some antigens against HLA-specific-types because she had had blood transfusions previously. There was a 5% chance of getting a match.'

Next to finding an appropriate donor, the patient needed to be prepared, although Anne-Sophie wonders if you can really prepare a person for such a life-changing procedure. This specific preparation for the patient included psychological testing as well as a moral and ethical debate where all the complications and possible side effects were addressed. This is not without its reasons. 'Because donation is a procedure that involves immune medication, which has large side-effects when you use them for a lifetime – for instance, the risk of developing diabetes and increased risk of skin malignancies. Furthermore, the patient had to be mentally very strong. You have to accept there is a chance that you end up with nothing if the procedure fails or if you get a rejection. She [Maartje] was that kind of person.'

After two mismatches, a match was found, and the 24-hour surgery could begin. 'It felt surreal', Anne Sophie says, 'it was an adrenaline rush just to start.' Anne Sophie was involved in the flushing of the transplant and was one of the two people who overviewed the surgery and made sure everything happened at the right time in the right order. 'All the plastic surgeons and trainees were involved in this procedure, and we had one surgical room, and the OR next to it was a dormitory. I felt tiny because I was just one wheel in the entire machine. On the other hand, it was an extremely unique experience because we already had two times of no match, and I was in the state where I thought, 'well, probably this time it won't be a match again, but then it suddenly was a right match'. We were waiting for that moment, and then we really had to do everything that we had prepared so long for.'

The double hand transplantation gave Maartje Bijl a lot of freedom and independence back. Small tasks as preparing a sandwich, but also things most people would probably not think about. 'Now she can go on an electric stair. She never dared to go on it because you can imagine that with prosthetic legs and no hands to hold the sidebar, it is very dangerous. Now she dares to go on it because she

can at least hold herself.' Having visible transplanted arms could sound uncomfortable to some people, but the patient adapted very fast and well to her transplants. From day one, she said, 'they're mine, they feel like my own, they really match me well' and from day one, 'she was able to move them because the tendons were connected to her own.' The quality of her life has been improved drastically by the transplants giving her the freedom to perform everyday tasks on her own [7].

As with every intervention, this surgery came with a risk of dangerous complications; especially rejections are a risk. However, the patient is doing very well so far. 'She is really well-set with her immune medication regime, so the chances for her are good that nothing of that [rejection] will happen.' Also, there is still room for improvement in controlling the transplants and tasks she can do independently. After full nerve regeneration, further improvements are expected, for instance sensation in her fingers, but also finding ways and tricks on how to solve daily problems.

For the future, Anne Sophie hopes to improve the processes surrounding limb preservation even more. For example, she would like to miniaturise the machine: 'I really envision that it is a pocket-sized machine that you can put in an ambulance and take to the donor hospital or have it at the emergency department and use it in the case of a limb amputation.' Next to the materialistic part, she dreams about improving international collaborations with research groups with different fields of expertise to 'bundle knowledge'. While talking about the future of limb preservation, Anne-Sophie also gave some insight into how she envisions her own future: 'I want to keep being involved in this research. I am still involved and helping my colleagues and doing what I can. […] of course, in the future, I would like to be the surgeon that actually uses the thing that I helped to develop. That would be perfect.'

*Anne Sophie Kruit*

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## References

1. Errico, M., *et al.* History and ethics of hand transplants. *JRSM Short Rep* **3**, 74 (2012).
2. Ozer, K. Advances in Limb Preservation: From Replantation to Transplantation. *J Hand Surg Am* **45**, 626-637.e625 (2020).
3. Hovius, S.E.R., *et al.* [The first bilateral hand transplantation in the Netherlands: results after 1 year]. *Ned Tijdschr Geneeskd* **165**(2021).
4. Blaisdell, F.W. The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. *Cardiovasc Surg* **10**, 620-630 (2002).
5. Lee, C.Y. & Mangino, M.J. Preservation methods for kidney and liver. *Organogenesis* **5**, 105-112 (2009).
6. Sheldon, S. & Poulton, K. HLA typing and its influence on organ transplantation. *Methods Mol Biol* **333**, 157-174 (2006).
7. Herrington, E.R. & Parker, L.S. Narrative methods for assessing "quality of life" in hand transplantation: five case studies with bioethical commentary. *Med Health Care Philos* **22**, 407-425 (2019).

# ZEBRAS OF MEDICINE:
## RECOGNISING WERNICKE ENCEPHALOPATHY AS THE UNDERLYING CAUSE OF A DELIRIUM

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***Abstract***

Wernicke encephalopathy (WE) is an acute neurological disease characterised by altered consciousness (drowsiness, delirium, coma), oculomotor symptoms, and gait ataxia caused by a thiamine deficiency. Thiamine deficiency is often caused by alcohol abuse, but it is also associated with other diseases, such as gastro-intestinal diseases. A timely diagnosis is of pivotal importance, as WE has a 20% mortality rate and, if left untreated, can lead to Korsakoff's syndrome [1, 2]. Korsakoff's syndrome is an irreversible chronic disease characterised by profound anterograde memory impairment, often accompanied by changes in behaviour [3]. Korsakoff's syndrome can be partially prevented by adequate diagnosis and treatment of WE. However, due to varieties in clinical presentation and the (relative) rarity of WE, it is often diagnosed late, especially in patients without a history of alcohol abuse [4]. One of the reasons WE can be misdiagnosed is that patients often present with confusion or altered mental status as the dominant symptom, *i.e.* the clinical syndrome of a delirium. Delirium is an acute neuropsychiatric disorder characterised by fluctuating changes in attention and mental status, often provoked by an underlying disease or infection in individuals already susceptible to experiencing delirium [5]. WE can be an underlying cause of a delirium and also present itself as such. In order to provide an overview of clues indicating WE to be the underlying cause of a delirium, this article delineates the pathophysiology, diagnostic process, and treatment of WE and delirium.

## Pathophysiology

WE is caused by a thiamine (vitamin B1) deficiency due to a restrictive diet or decreased uptake [1]. Thiamine is present in almost all foods, including grain products, potatoes, meat, vegetables, and milk, which means that it is difficult to develop a thiamine deficiency if one maintains a balanced diet [1, 6]. Thiamine is absorbed in the gut by enterocytes, after which approximately 80% is converted into thiamine diphosphate (TPP), which forms our intracellular storage of thiamine [7]. On average, healthy people have a total of 30-50 mg thiamine stored intracellularly [1, 3]. With an average requirement of 1-2 mg of thiamine per day, the storage in the cells would provide 4-6 weeks' worth of thiamine in case of restrictive intake [1, 3]. This is in line with several studies that show that symptoms begin to emerge a few weeks after the start of a decreased intake of thiamine [1, 6]. TPP is involved in a wide range of functions within the cell, the most important of which is its function as a cofactor for several enzymes involved in the Krebs cycle. A lack of TPP will disrupt the Krebs Cycle, resulting in cell death through decreased ATP production, oxidative stress leading to DNA and RNA instability, and a build-up of toxic metabolites such as lactate [7]. As some thiamine-dependent enzymes also require magnesium as a cofactor, it is important to also determine magnesium levels to see if there is an additional deficiency [1]. Thiamine deficiency can cause two different diseases, namely, beriberi, presenting with peripheral neuropathy (dry beriberi) or cardiac failure (wet beriberi) and WE [8]. WE will be the primary focus of this article.

The patient group of WE mainly consists of patients suffering from alcohol dependence, with around 90% of patients with WE actively abusing alcohol in industrialised countries [1]. However, other diseases affecting intake or absorption of thiamine can also lead to WE. These include, for instance, bariatric surgery, malabsorption syndromes, or hyperemesis [9]. Interestingly, alcohol abuse itself, regardless of nutrition state, increases the chance of developing WE [1]. Patients suffering from alcohol abuse were reported to already have lower levels of serum thiamine. A deficiency in thiamine, in turn, decreases the uptake of thiamine by enterocytes, thereby additionally depriving the patients of thiamine [1]. Ethanol can further damage the intestinal epithelium, which can reduce thiamine absorption up to 90% [1]. Moreover, liver disease such as cirrhosis, often seen in alcoholics, impairs the conversion of thiamine into TPP and decreases the amount of thiamine stored in the cells [1, 3]. The first therapy for ascites, a known complication of liver cirrhosis, is the diuretic furosemide, which decreases the renal tubular reabsorption of thiamine [1]. All in all, patients who are alcohol abusers, especially after developing liver cirrhosis, have a higher chance of developing WE due to thiamine deficiency. However, although WE is more common in patients with active alcohol abuse, the absence of alcohol abuse does not rule out WE.

The pathophysiology for delirium caused by other causes is not entirely elucidated; the range of underlying conditions is sheer endless. The cellular mechanisms underlying the development of delirium remain largely unknown. The current theory surrounding the pathophysiology of delirium focuses on patient vulnerability, implying that patients associated with a large number of risk factors require fewer precipitants to develop delirium than individuals with a lower number of risk factors (Figure 1) [5, 10]. The main risk factors include old age, comorbidities, and neurocognitive deficits, with between 20-30% of elderly admitted with acute disease developing delirium [5, 10]. The precipitants can be a wide range of factors, varying in severity. Examples include infection, surgery, trauma, or other diseases [5, 10]. Correctly identifying and treating delirium is important, as delirium negatively affects health outcomes, including long-term symptoms, such as cognitive decline, and mortality [5].

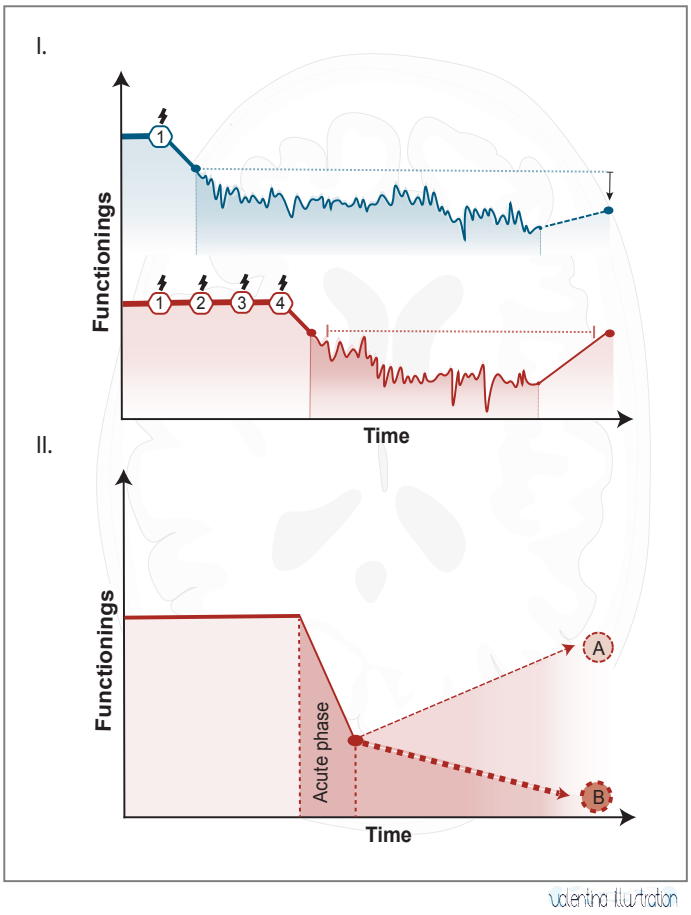


***Figure 1:*** *Wernicke encephalopathy is characterised by an acute decrease in functioning, of which a part will recover fully. However, a significant amount of patients will have residual symptoms or continue to develop Korsakoff's syndrome. A delirium caused by other factors will require fewer precipitants in already susceptible individuals and is characterised by an acute decrease in functioning with a fluctuating course. Development of a delirium can also affect long-term health outcomes and functioning, especially in elderly.*

## Clinical presentation

Carl Wernicke first described WE in 1881, when three patients presented with similar symptoms, namely ataxia, ophthalmoplegia, and changes in consciousness or mental confusion [4]. After this triad of symptoms had been described a few more times in literature, it was referred to as the classic triad. The acute stage of WE, characterised by symptoms from the classic triad, is preceded by a less acute phase with non-specific symptoms, such as gastro-intestinal irritation, fatigue, and irritability (Figure 1) [3]. These non-specific symptoms can emerge six to eight weeks after the start of the thiamine deficiency [1]. During the acute phase, all three symptoms of the classic triad are present in only 16-33% of patients [2-4]. The majority of patients present exclusively with mental changes, e.g. confusion, memory impairment, or problems concentrating [2]. Moreover, patients not suffering from alcohol abuse present less often with the classic triad, making the diagnostic process more difficult [2]. Other symptoms that can occur besides the classic triad are autonomic symptoms, seizures, vestibular dysfunction, and peripheral neuropathy. The European guidelines currently require the presence of two of the following symptoms in order to diagnose WE: I) ocular symptoms, II) cerebellar dysfunction, III) restricted intake, and IV) an altered mental state or mild memory impairment [11].

Patients with delirium due to other causes also present with an altered mental status: an acute and fluctuating decrease in attention, often accompanied by other symptoms. The diagnostic and statistical manual of mental disorders (DSM-V) requires the presence of the following symptoms in order to diagnose delirium: I) an acute onset (hours-days) of symptoms, II) disruption of attention, III) disruption of cognitive function, which can include changes in perceptions, e.g. hallucinations, IV) an underlying physical cause based upon physical examination or laboratory values, and V) no other neurocognitive explanation of the symptoms [12]. Nonetheless, the clinical presentation of delirium differs based on the type of delirium. Hyperactive delirium is often diagnosed more easily, as it is characterised by hyperactive psychomotor activity, which can lead to agitation [12]. Hypoactive delirium, on the other hand, is more often overlooked, as it is characterised by low psychomotor activity and sogginess [12]. However, patients can also present with the mixed subtype, where there is a normal level of psychomotor activity or rapidly fluctuating psychomotor activity [12]. WE should always be considered in the differential diagnosis of a delirium; however, patients with delirium caused by other factors generally do not exhibit the neurological symptoms from the classic triad [12].

## Further testing

WE and delirium are both clinical diagnoses, which means that the diagnosis is primarily based upon the clinical presentation. In the case of WE, it is possible to determine thiamine levels, either by measuring serum thiamine levels or erythrocyte thiamine transketolase activity [13]. However, determination of thiamine levels is not to be awaited in clinical practice. Serum thiamine levels do not necessarily correspond to cerebral thiamine levels and are therefore not used to determine or exclude WE [14]; patients can have WE despite normal serum thiamine levels [14]. Furthermore, treatment should start immediately when the suspicion of WE arises, without awaiting thiamine levels, in order to prevent irreversible damage, including Korsakoff's syndrome [13].

Although typical lesions indicating WE can be seen on neuroimaging, imaging is not used routinely to establish the diagnosis of WE either. Magnetic resonance imaging (MRI) can show lesions typical for WE, which include an increased T2-signal and decreased T1-signal periaqueductally, and within the mamillary bodies, medial thalamus, dorsal medulla, and tectal plates [2]. Atrophy of the mammillary bodies is a specific finding, as is not often seen with other disorders, but that can not be seen yet during the acute stage [15]. However, despite the high specificity of MRI, the sensitivity remains low, which means it can not be used to rule out WE [16]. Nonetheless, MRI can be used to rule out alternative diagnoses, such as rare focal neurological disorders mimicking the symptoms of WE.

Delirium is a clinical diagnosis, with the criteria provided by the DSM-V providing a clear framework [12]. The DSM-V criteria also require further testing in order to identify an underlying physical cause. Therefore, routine laboratory testing is always performed in case of a delirium in order to determine possible underlying causes, e.g. electrolyte disbalance or a urinary tract infection. Imaging is performed according to the patient's (or caregiver's) story and physical examination: if there are indications for neurological causes such as a stroke, a CT-cerebrum can be performed, whereas coughing and a fever are an indication for a chest X-ray. If the cause of the delirium remains unknown, meningitis could be the cause, which is an indication for a lumbar puncture [17].

## Treatment and prognosis

WE is treated with supplementation of thiamine; however, there are discrepancies in terms of the recommended dosage of thiamine in different national guidelines. The EU guidelines suggest administration

of 200 mg intravenously three times a day, whereas the Dutch national guideline advises 500 mg intravenously three times a day [11, 18]. Different clinical trials have been carried out to determine the dosage of thiamine supplementation, but an exact dosage has not been identified hitherto. However, despite differences in the exact dosage of thiamine, the currently existing guidelines do agree that treatment should start immediately when the clinical suspicion of WE arises to prevent complications or death. Furthermore, oral supplementation of thiamine is not recommended, as damage to the gastro-intestinal mucosa by e.g. alcohol abuse causes a decreased uptake of thiamine, hindering its effect [11]. Thiamine must, therefore, always be given intravenously or intramuscularly in the acute phase. Thiamine should be administered before administering glucose as well, since a high glucose load may precipitate WE. After the acute phase, intravenous supplementation of thiamine can be substituted by oral supplementation [11, 18]. In addition, thiamine deficiency is often accompanied by other deficiencies, notably magnesium, sodium, and phosphate deficiencies [11, 18]. Levels there of should be measured at the start of treatment, and these minerals should also be supplemented as needed [11, 18]. Besides supplementation, a crucial component of the treatment of WE is to quit alcohol consumption and prevent relapse.

The prognosis of WE depends on the severity of the disease, time of treatment, and comorbidities of the patient. The symptoms tend to improve in a typical fashion after supplementation of thiamine. The ocular abnormalities will usually improve within hours or days; if the ocular symptoms do not improve after thiamine supplementation, the diagnosis of WE should be reconsidered [19]. The gait ataxia tends to improve, but the recovery is occasionally incomplete, leaving patients with residual symptoms [19]. The confusion will dampen over time, but usually requires weeks to months for a full recovery. Patients can have persistent mental deficits, of which only 20% of patients will recover partially or completely (Figure 1). The other patients will have permanent anterograde memory impairment and will develop Korsakoff's syndrome despite adequate treatment (Figure 1) [13]. It is important to note that thiamine can also be given as a precaution to patients at-risk, e.g. presenting with alcohol withdrawal, to prevent these long-term consequences.

Delirium will not subside until the underlying cause has been treated properly. Therefore, it is crucial to also diagnose and treat the precipitant of the episode [17]. This differs per patient, which means that a patient-specific treatment plan needs to be made – e.g. antibiotics to treat infection or supplementation of electrolytes in case of an electrolyte imbalance [17]. In the meantime, the delirium can be treated symptomatically, using either non-pharmacological or pharmacological interventions. It is advised to start with nonpharmacological interventions: providing a calm and safe environment and reassurance from the staff or family present [17, 20]. If this does not provide enough relief, and the patient remains agitated, scared, or restless, pharmacological treatment can be considered. Antipsychotics, haloperidol specifically, are considered to be the first pharmacological treatment option, but the most suitable medication differs depending on the comorbidities of the patients [17]. In case of (relative) contraindications, newer antipsychotic drugs, often referred to as atypical antipsychotic drugs, can be prescribed. These may or may not be combined with benzodiazepines. However, one must bear in mind that medications such as antipsychotics can also have side effects, including increased risk of cardiovascular events and an increased risk of falls – which can have severe consequences in the elderly [17]. It is therefore advised to prescribe psychotropic medication with caution. There is no psychopharmacological treatment for a hypoactive delirium, as the medication is aimed at reducing agitation or restlessness, which is not present in the case of a hypoactive delirium [20]. Delirium is associated with increased mortality [21]. Furthermore, a recent meta-analysis by Goldberg *et al.* showed that patients with delirium experienced cognitive decline three months after the episode, suggesting that patients can experience chronic long-term cognitive effects of a delirium [22].

## Conclusion

To conclude, the predominant clinical symptom of WE can be a delirium, with confusion and altered mental status as the dominant symptom. In case of WE, patients may also exhibit other symptoms, such as ophthalmoplegia and gait ataxia, whereas patients with delirium caused by other precipitants will show fluctuations throughout time as well as symptoms from an underlying precipitant of the delirium. Hence, it is important to perform a thorough physical examination and history – both with the patient and their caregiver - to determine any symptoms indicating WE as the underlying cause of the delirium or other possible precipitants for a delirium. Correctly identifying WE as the underlying cause of the delirium is crucial, as untreated WE leads to Korsakoff's syndrome, causing permanent cognitive deficits, and an unrecognised and undertreated delirium also affects health outcomes negatively, including mortality and long term cognitive decline. WE should, therefore, always be included in the differential diagnosis of a delirium.

## Acknowledgement

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## References

1. Thomson, AD, Marshall, EJ. The natural history and pathophyiolog of Wernicke's encephalopathy and Korsakoff's psychosis. *Alcohol and Alcoholism* **41(2)**, 151-158 (2005).
2. Ota Y, Capizzano AA, Moritani T, Naganawa S, Kurokawa R, Srinivasan A. Comprehensive review of Wernicke encephalopathy: pathophysiology, clinical symptoms and imaging findings. *Japanese journal of radiology* **38(9)**, 809-820 (2020).
3. Chandrakumar A, Bhardwaj A, Jong GWt. Review of thiamine deficiency disorders: Wernicke encephalopathy and Korsakoff psychosis. *Journal of Basic and Clinical Physiology and Pharmacology* **30(2)**, 152-162 (2019).
4. Yin H, Xu Q, Cao Y, Qi Y, Yu T, Lu W. Nonalcoholic Wernicke's encephalopathy: a retrospective study of 17 cases. T*he Journal of international medical research* **47(10)**, 4886-4894 (2019).
5. Maclullich AMJ, Ferguson KJ, Miller T, de Rooij SEJA, Cunningham C. Unravelling the pathophysiology of delirium: a focus on the role of aberrant stress responses. *J Psychosom Res* **65(3)**, 229-238 (2008).
6. Ott M, Werneke U. Wernicke's encephalopathy - from basic science to clinical practice. Part 1: Understanding the role of thiamine. *Ther Adv Psychopharmacol* (2020).
7. Manzetti S, Zhang J, van der Spoel D. Thiamin Function, Metabolism, Uptake, and Transport. *Biochemistry* **53(5)**, 821-835 (2014).
8. Rolfe M. Beri-beri: "Endemic amongst urban Gambians". *Africa health* **16(3)**, 22-21 (1994).
9. Latt N, Dore G. Thiamine in the treatment of Wernicke encephalopathy in patients with alcohol use disorders. *Internal Medicine Journal* **44(9)**, 911-915 (2014).
10. Young J, Inouye SK. Delirium in older people. *BMJ (Clinical research ed)* **334**, 841-846 (2007).
11. Galvin R, Bråthen G, Ivashynka A, Hillbom M, Tanasescu R, Leone

MA. EFNS guidelines for diagnosis, therapy and prevention of Wernicke encephalopathy. *European Journal of Neurology* **17(12)**, 1408-1418.
12. Assocation. AP. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Washington, DC: APA Press; 2013.
13. Kohnke S, Meek CL. Don't seek, don't find: The diagnostic challenge of Wernicke's encephalopathy. *Annals of clinical biochemistry* **58(1)**, 38-46 (2021).
14. Davies SB, Joshua FF, Zagami AS. Wernicke's encephalopathy in a non-alcoholic patient with a normal blood thiamine level. *Medical Journal of Australia* **194(9)**, 483-484 (2011).
15. Charness ME. Intracranial Voyeurism: Revealing the Mammillary Bodies in Alcoholism. *Alcoholism: Clinical and Experimental Research* **23(12),** 1941-1944 (1999).
16. Antunez E, Estruch R, Cardenal C, Nicolas JM, Fernandez-Sola J, Urbano-Marquez A. Usefulness of CT and MR imaging in the diagnosis of acute Wernicke's encephalopathy. *American Journal of Roentgenology* **171(4),** 1131-1137 (1998).
17. Kukolja J, Kuhn J. SOP: treatment of delirium. *Neurological Research and Practice* **3(1)**, 12 (2021).
18. Specialisten FM. Stoornissen in het gebuik van alcohol2009 [cited 2021 13th of december]. Available from: https://richtlijnendatabase.nl/richtlijn/stoornissen_in_het_gebruik_van_alcohol/alcohol_-_somatische_complicaties/effect_alcoholmisbruik_op_het_zenuwstelsel/indirecte_effecten.html.
19. Isen DR, Kline LB. Neuro-ophthalmic Manifestations of Wernicke Encephalopathy. *Eye Brain* **12**, 49-60 (2020).
20. Hosker C, Ward D. Hypoactive delirium. *BMJ (Clinical research ed)* **357**, j2047 (2017).
21. Aung Thein MZ, Pereira JV, Nitchingham A, Caplan GA. A call to action for delirium research: Meta-analysis and regression of delirium associated mortality. *BMC Geriatrics* **20(1)**, 325 (2020).
22. Goldberg TE, Chen C, Wang Y, Jung E, Swanson A, Ing C, et al. Association of Delirium With Long-term Cognitive Decline: A Meta-analysis. *JAMA neurology* **77(11)**, 1373-1381 (2020).

## CORRECT ANSWERS TO THE EXAM QUESTIONS

**Answer question 1:**
*B. 5-hydroxytryptamine*

The majority of the tricyclic antidepressants act primarily as serotonin (5-hydroxytryptamine) and norepinephrine reuptake inhibitors by blocking both the serotonin and norepinephrine transporter, which results in an elevation of the synaptic concentrations of these neurotransmitters. This enhances neurotransmission. However, they have a weak affinity for the dopamine transporter and, therefore, low efficacy as dopamine reuptake inhibitors. Tricyclic antidepressants block the muscarinic acetylcholine receptors.

For further reading:
Siegel, A., Sapru, H. Chapter 7: Neurotransmitters in Essential Neuroscience, 4th edition (Wolters Kluwer, Philadelphia, 2019).

**Answer question 2:**
*C. Both donor tolerogenic cells and regulatory T cells*

Tolerogenic dendritic cells are heterogenous pools of dendritic cells with immunosuppressive properties, priming the immune system into a tolerogenic state against various antigens. These cells induce immune tolerance by inhibiting the activation of T cells and inducing regulatory T cell proliferation. Regulatory T cells are a specialised subpopulation of T cells that act to suppress immune response, thereby maintaining homeostasis and self-tolerance. It has been shown that they are able to inhibit T cell proliferation and cytokine production. These mechanisms help preventing rejection by antigen specific tolerance.

For further reading:
Li, H., & Shi, B. (2015). Tolerogenic dendritic cells and their applications in transplantation. *Cellular & molecular immunology*, 12(1), 24–30.
Oberholtzer, N., Atkinson, C., & Nadig, S. N. (2021). Adoptive Transfer of Regulatory Immune Cells in Organ Transplantation. *Frontiers in Immunology*, 12.

**The exam questions can be found back on page 12 in this journal.**

# COLUMN: THE GOOD DOCTOR?

**Guus Brand**[1]

[1] Bachelor's student Medicine, Radboud university medical center, Nijmegen, The Netherlands

When I was younger, I grew up with a father working as a paediatrician in the local hospital and a mother who loved hospital soap operas, most especially the show "Grey's Anatomy". Every Friday night was dominated by the daily escapades of Meredith Grey, Miranda Bailey, Richard Webber, and consorts. From 10 p.m. onwards, the living room would be filled with cries like: "I need 3mg of epi stat!"; "His GCS dropped to 5, we need to intubate!"; and "He is not dying on my watch!" These cries were mixed with remarks from my dad, peering over his book or newspaper, about how unrealistic a scene was or that patients with a certain disease do not present themselves with those complaints. Luckily, these were quickly shushed by my mother, who had no patience for realism and facts and just wanted to enjoy her show.

What always struck me as odd were the characters in these television shows. The doctors in medical dramas often show behaviour that is antisocial, labile, aggressive, dishonest, and unprofessional towards patients. Even stranger is that these traits, often associated with bad doctors [1], are the hallmarks of main characters in many medical soap operas. For instance, Dr. Gregory House in *House M.D.* is an antisocial egomaniac with a substance abuse problem who diagnoses extremely rare illnesses on a hunch. Dr. Max Goodwin in *New Amsterdam* is a doctor with cancer who lies about his diagnosis and its effects on his performance. From the same show, Dr. Lauren Bloom has temper issues, a substance abuse problem, and works triple shifts in order to avoid having to go home and face loneliness. The entire hospital staff of *Chicago Med* has slept with each other, the main characters fight over personal reputation—sometimes at the expense of patients— and they sometimes blatantly lie to save their own skin. It would probably make for bad television to make a show about a doctor who duly follows protocol, is cordial to his patients and co-workers, and willingly does all the administration that comes with the job.

However, this dramatisation of the medical profession does come at a risk. For instance, patients may have a distorted view of the medical profession and interventions. This can lead to miscommunication between patients and health care providers but also to the incomprehension of the fallibility of doctors [2]. Another study observed more concrete results. Witzel, Koch, and Kaminski found that patients that watch medical TV shows have more pre-operative fear than patients that do not [3]. Of course, there are more reality-oriented medical TV shows such as *24 Hours in A&E*. However, these shows often focus on the spectacular side of medicine, fast-paced medical interventions in the Emergency Department, helicopter rides, and emergency medicine. To my knowledge, there is no show that follows a doctor on an out-patient clinic of dermatology and shows the drudgingly minutia of medical bureaucracy. That too is medicine, and a show like that might prepare patients and medical students better for the real thing.

From reading this, one might suddenly realise that I know an awful lot about medical television shows, and that is true. I have watched all the television series mentioned above. Would I be inclined to watch a show based around the actual comings and goings of a medical professional? To binge-watch smooth and slow consultations with patients, awkward talks at the coffee machine, and polite communication with other medical professionals? Absolutely not. I prefer to watch the intrigue, action, and drama that fictional medical characters have to deal with. What about you?

## Reaction

I did not grow up in a doctor's family. There was weekly enthusiasm for *M*A*S*H* at our home (in the 1970s). The story is set in 1950 during the Korean War and followed the Mobile Army Surgical Hospital (MASH) 4077. The series mainly followed two surgeons (including the star of the show Alan Alda), a major ("hot lips"), a cleric and a communications officer. It is a series with misery in the operating room, with close friendships and with a lot of (sometimes lame) humor. In my college days there was *St. Elsewhere* (starring Denzel Washington!), *ER* again a little later, and right now I am watching the entire series of *House M.D.* watching again.

So, it cannot be denied: just like Guus, I am a fan of medical shows. However, I am not so afraid of the risk that Guus describes. Take Gregory House for example. In any hospital, this doctor would have been discharged within a week. Immediately. Viewers understand that too. And viewers also know that no doctor can get away with showing up for work under the influence of alcohol or drugs. It is entertainment indeed with intrigue, action, drama, and eroticism. But entertainment with (usually) a sound medical basis. And that is precisely where his strength lies. Viewers have an insight into the complexity of medicine. Viewers are realizing how difficult it can be to diagnose. I therefore expect more understanding and better communication between patients and health professionals.

Finally, these series show the beauty of the profession. Not just from a doctor, but also from a nurse. The shortage of nurses is one of the biggest bottlenecks in healthcare and such a series can give aspiring nurses that extra push. So, I cannot wait for a remake of *Medisch Centrum West*.

Reaction by Károly Illy, MSc., Chair of the Dutch Society of Pediatricians, and a fan of medical TV shows.

*MSc. Károly Illy*

## References

1. Steiner-Hofbauer V, Schrank B, Holzinger A. What is a good doctor? Wiener Medizinische Wochenschrift. 2018;168:398-405
2. Lapostolle F, Montois S, Alhéritière A, De Stefano C, Le Toumelin P, Adnet F. Dr House, TV and Reality… Am J Med 2013;126(2):171-173
3. Witzel K, Koch HJ, Kaminski C. Impact of Medical TV Shows on Preprocedural Fear of Surgical In-House Patients. Eur Surg Res 2017;58:121-127

# RECENT HIGH-IMPACT PAPERS FROM RADBOUDUMC RESEARCHERS

**Richard II Dela Rosa**[1]

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

[1]Master's student Molecular Mechanisms of Disease, Radboud university medical center, Nijmegen, The Netherlands

## How much should you exercise for a healthy heart and a longer life?

Exercise and physical activity have been commonly associated with a healthy heart and a long life. But, how much should you exercise to yield cardiovascular health benefits? In an attempt to answer this question, Bakker et al. performed a cohort study involving ~145,000 individuals from three northern provinces of the Netherlands (PLOS Medicine, impact factor = 11.07) [2]. The participants were divided into three groups: 1) healthy group, 2) group with increased risk of cardiovascular disease due to hypertension and diabetes, and 3) group with a history of heart attack or stroke. The sample population was asked multiple questions regarding topics such as self-reported physical activity and occupation. The results were correlated with mortality and major adverse cardiovascular events within a ~7 year timeframe ($p<0.05$) . The analysis indicated that the ideal amount of exercise differs per group. For healthy and at-risk individuals (group 1 and group 2), a higher amount of exercise correlates to a lower risk of mortality and heart disease. However, the benefits plateau after 10-15 hours of moderate to vigorous physical activity every week. On the other hand, individuals with cardiovascular disease history (group 3) actually showed sustained increases in cardiovascular benefits from physical activity . The more they exercise, the lower their risk of acquiring a new cardiovascular disease. Aside from this, the study also investigated people's daily physical activities, including biking commutes, nature of work, spare time, and household chores. Surprisingly, individuals who perform intensive physical work as part of their jobs do not have a lower risk of heart disease or death. The researchers have no definite explanation for this phenomenon, but they hypothesise that it occurs because this group does not exercise sufficiently outside of their working hours. Additional causes that could have led to this result will be investigated in a future study. The general results of this study support that a physically active lifestyle is crucial in preventing and managing heart diseases. As such, this helps medical practitioners provide evidence-based lifestyle advice, especially to patients that have a history of cardiovascular disease.

## Uncovered: enzyme responsible for hypoxia-induced metastasis in tumours

Hypoxia is a hallmark of cancer that drives metastasis and collective tumour invasion in various cancer types. In environments with normal oxygen levels, different forms of cancer cell masses collectively survey and invade surrounding tissues for further growth. On the contrary, hypoxic environments prompt cancer cells to individually detach from the primary tumour and probe the rest of the body for suitable growth sites. During this process, hypoxia induces epithelial cell transition to mesenchymal cells. Mesenchymal cells, then, can acquire amoeboid cell movement features. This type of movement allows a cell to move alone, mimicking the ancient movement of single-cell amoebas. However, the main drivers of this process remain to be uncovered. A paper published by te Boekhorst et al. in Current Biology (impact factor = 10.83) identifies the enzyme calpain-2 as a key regulator in hypoxia-induced amoeboid cell transition [3]. This enzyme regulates the degradation of integrins, which are adhesive proteins that attach tumour cells together to form a mass. Thus, an increase in calpain-2 detaches a cell from the primary tumour. Additionally, the enzyme also prompts the cell to go into an "energy-saving" mode while travelling to other parts of the body to prevent metabolic burnout. After some time, the cells revert to their previous adhesive state and form new tumours in other tissues. To validate the role of calpain-2 and its pharmacologic targeting potential, the researchers added drugs that inhibit the enzyme in tumour cells in a hypoxic environment. In this condition, the tumour cells were unable to transition, and significantly lower levels of metastasis were observed ($p<0.0001$). These findings demonstrate that calpain-2 is a potential target for therapy, which warrants further drug discovery and translational research studies.

## DeepRank: a novel tool to accelerate protein-protein interaction research

Protein-protein interaction networks are essential for the coordination of complicated cellular processes. Therefore, gathering insight into how these interactions take place in 3D space helps to understand their function and to use this knowledge for drug design, immunotherapy, and other clinically relevant research. Different types of experimental methods, such as X-ray crystallography and cryogenic electron microscopy, have produced numerous atomic-resolution 3D structures of protein complexes. To derive complex interaction patterns from these experimental 3D structures, current machine learning and deep learning methods are used but still need further refinement to increase predictive accuracy. Aiming to circumvent this problem, a novel deep learning framework for data mining 3D protein-protein interfaces called DeepRank has been developed and published in Nature Communications (impact factor = 14.92) by Renaud et al. Using this deep learning approach, scientists can train software to analyse protein structures for specific patterns and predict their interactions [4]. The researchers tested DeepRank's performance based on two challenges in structural biology, being: 1) classification of biologically relevant structures in crystallography products, and 2) ranking of docking models to predict binding between proteins. Results show that DeepRank is comparable and sometimes even outperforms other state-of-the-art methods. The deep learning framework has a variety of applications in medical sciences. For example, in Radboudumc, it is currently used to aid in the development of a novel cancer vaccine [5]. DeepRank is publicly available through GitHub, which makes it accessible for any scientist interested in studying 3D protein interactions. Ultimately, this open-source framework can help accelerate a variety of fundamental and translational research efforts.

## References

1. Radboudumc. "Onze impact in 2020" *Impact 2020* (2020). https://www.radboudumc.nl/over-het-radboudumc/impact-2020
2. Bakker, E.A., et al. Dose–response association between moderate to vigorous physical activity and incident morbidity and mortality for individuals with a different cardiovascular health status: A cohort study among 142,493 adults from the Netherlands. PLOS Medicine 18, e1003845 (2021).
3. Te Boekhorst, V., et al. Calpain-2 regulates hypoxia/HIF-induced plasticity toward amoeboid cancer cell migration and metastasis. Current Biology (2021).
4. Renaud, N., et al. DeepRank: a deep learning framework for data mining 3D protein-protein interfaces. Nature Communications 12(2021).
5. Radboudumc. DeepRank: a deep learning framework for data mining 3D protein-protein interfaces. in, Vol. (ed.^(eds. (2021).

RAMS

## A Word from the Board of RAMS

Dear reader,

What a study year it has been so far. Not being able to go to campus has been a burden for many of us. A lot of activities were cancelled, and everyone felt the impact of the lockdown on either study results, personal life, or both. The board of RAMS wishes everyone who is still dealing with the hardship of COVID-19 well. We are proud that our team of editors and reviewers has written their way through this challenging period and continued delivering their interesting articles.

However, it finally seems we have made it through. As society is increasingly liberated from the shackles that COVID-19 put on her, we can go to campus again to study, experience, and have fun. The second semester of this year has begun, and we hope you, as well as us, will enjoy your studies. We are excited to bring you this new 22nd RAMS edition, and we hope you liked reading it and learned something new! Feel free to always take an edition with you when you see one lying around, as our editions are freely distributed on faculty amongst students.

Don't forget checking in on one of our upcoming activities!

On behalf of the eighth board of RAMS

**Maarten Huigens**
Treasurer of the VIIIth board of RAMS 2021-2022

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

## Editorial Board

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Reviewers have been trained with the help of masterclasses given by professors and teachers at Radboud university medical center. With their knowledge, the reviewers are able to judge the submitted scientific and editorial articles.

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