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Breakfast is the Most Important Meal of the Day: Myth or Science?

MicroRNA Panels show Potential as a Diagnostic Biomarker for Bladder Cancer

Big, Bigger, Big Data

Safety and Effectiveness of the Human Papillomavirus Vaccine

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FROM THE EDITORIAL BOARD

Dear reader,

Admittedly, writing this column as the new chair of the editorial board was not what I was looking forward to the most. Not that I do not like writing, but how do you knit together a single theme from such a diversity of articles? From breakfast to vaccines and from microRNA to big data, I could only find two common denominators: all articles cover medical subjects and all articles have been written by students of our medical faculty. Moreover, discussing the results here and now might spoil the pleasure of reading the articles, and would make the index redundant.

So what is the message I would want to convey instead? While discussing this with some friends, they suggested I could put a *'lorem ipsum'* on this page as metaphorical statement. Unfamiliar with the concept from the press and printing business, I decided to look it up. I discovered that it refers to a latin text that has been used from the 16th century onwards as a filler text to test an old fashioned printing press or, nowadays, evaluate the layout of a page by filling it with meaningless and hence undistracting text. The text, of which the first sentence can be found below, is a scrambled version of a latin piece of writing by Cicero, that has been edited by the inventor to lose meaning while retaining a normal letter frequency.

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Whilst writing this column, it struck me that we live in an era in which much information, but far from all, has become readily accessible. Facts about *lorem ipsum* can be easily retrieved, be it via Google, Wikipedia or nl.lipsum.com, but what about the question whether it is healthier to eat or to skip breakfast? A search on Google will yield varying results, depending on which search you use, but which website do you believe? What to do when UpToDate cannot provide you with an answer? In this edition of RAMS, several systematic reviews, editorial articles, and more, will provide interesting responses to questions with diverging answers. Enjoy reading the sixth edition of RAMS!

Daan Viering
Chair of the Editorial Board

INDEX

From the Editorial Board	2
Breakfast is the Most Important Meal of the Day: Myth or Science?	4
Exam Questions	5
MicroRNA Panels show Potential as a Diagnostic Biomarker for Bladder Cancer	6
Summer School Neurosurgery RAMS 2016 Winning Abstract	12
Safety and Effectiveness of the Human Papillomavirus Vaccine	13
Big, Bigger, Big Data	19
Hydrogen Cyanide Emission by Mono- and Co-cultures of *Pseudomonas aeruginosa* and *Aspergillus fumigatus*	21
Recent High-impact Papers Published by Researchers from Radboudumc	26

BREAKFAST IS THE MOST IMPORTANT MEAL OF THE DAY: MYTH OR SCIENCE?

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Introduction

Not only is this universal advice often presented to children by their parents, it is in fact globally 'known' in every class of society. It often goes hand in hand with the phrase 'eat breakfast like a king, lunch like a prince and dinner like a pauper'. Furthermore, people are reminded constantly that the best start of the day is a healthy breakfast, thanks to all commercials selling 'low- fat diet cereals' with yoghurt, or unsaturated cornflakes. Not to mention the countless health and lifestyle magazines that have carried this for years as their mantra. But is this assertion actually supported by science or is it one of the most popular fables of modern society? Could skipping breakfast even have benefits?

For most people in the Netherlands, breakfast is an essential part of their daily diet and is believed to have many positive effects on our overall health and wellness. For example, several studies have shown that breakfast consumers are less likely to be overweight/obese, and have a lower risk of diabetes and cardiovascular disease [1, 2]. Adversely, it is claimed that skipping breakfast can raise our risk of obesity and such illnesses. This seems a problem, because one third of the Dutch population admitted to skipping breakfast at least once a week! [3]

However, these studies are observational only, and can never confirm a causal relationship. They show that people who eat breakfast are more likely to be healthy, but can not make statements of fact that breakfast itself caused it. What could explain the results that are described in these articles? It is probable that breakfast consumers have other healthy lifestyle habits as well, like a healthier diet that includes more fibers, vitamins and other micronutrients [4]. People who skip breakfast on the other hand, tend to smoke cigarettes, drink more alcohol and be less physically active [5]. These factors could be the reason that breakfast consumers have a better overall health instead of attributing this to breakfast itself.

Several biological mechanisms have been proposed to support the consumption of breakfast as well. One of these mechanisms is the depletion of the glycogen reserves in the liver and muscle tissue during the night, which makes breakfast the first possibility to refill this stock. Therefore, missing breakfast will cause cravings later in the morning and is considered a recipe for pillaging the vending machines at universities and filling up on coffee in the office. This is perceived as the main reason that people who skip the morning meal tend to have a higher body weight than people who do not. For these reasons, even the official Dutch nutrition centre (Het Voedingscentrum) recommends having breakfast. Although this theory does seem very plausible, it is not yet supported by strong scientific evidence.

It might be true that missing breakfast results in more appetite which in turn leads to more consumption of snacks higher in energy and carbohydrates in the afternoon and evening. However, it seems this is not enough to compensate for the energy of the entire meal that was skipped [8]. As a matter of fact, several studies have shown that skipping breakfast may reduce total daily calorie intake by up to 400 kilocalories per day [9].

According to Dr. James A Betts, lead researcher for the Bath Breakfast Project, skipping breakfast and thus remaining in the fasting state, can even make people more sensitive to insulin (protecting against diabetes) and increase the levels of HDL in the blood (reducing the risk of coronary disease). Additionally, due to the restriction in calories it can even promote weight loss [6, 7].

Not long ago, the breakfast dilemma was tested by the American Society for Nutrition in a randomized controlled trial. During four months they compared the relative effectiveness of the recommendation to either eat or skip breakfast in 309 overweight/obese adults. After four months, there was no significant difference in weight whatsoever between the two groups. It did not matter at all whether people had breakfast or skipped it [10]. These results are also supported by various other studies on the effects of breakfast habits on weight loss [4].

According to the Dartmouth-Hitchcock Norris Cotton Cancer Center, however, breakfast is a way to 'initiate' the metabolism and keep this up to speed during the day. They claim breakfast can increase your resting metabolism by up to 10 percent. This theory is derived from the dietary induced thermogenesis (DIT) which is the increase in calories burned after a meal due to the cost of processing food. However, the DIT is determined by the total caloric content of the meals and the macronutrient composition. The meal frequency or time of consumption has little to no effect on the DIT [11]. This is supported by Kobayashi et al., who showed there was no difference in calories burned over 24 hours between people who eat or skip breakfast [12]. Therefore, also this statement can be marked as inaccurate.

One might question whether skipping breakfast would have any drawbacks at all. In support of the people that promote eating breakfast several RCTs have shown that missing the first meal of the day leads to elevated cholesterol in overweight individuals compared to when a daily healthy breakfasts is consumed [13]. Besides that, both average and obese adults reported that they felt less energetic during the morning when remaining in the fasted state than when consuming breakfast [14].

Conclusion

Breakfast is optional. When looking at all the evidence, we can conclude that it simply does not matter whether you eat or skip breakfast. Breakfast does not "start" your metabolism and missing this meal does not automatically make you gain or lose weight. It depends on various other factors, such as your general lifestyle and what you consume the rest of the day. The conceptions regarding the necessity of breakfast were based on observational studies that have never been confirmed by RCTs. From now on you can eat or leave breakfast to your personal preference. If you want to ease your hunger when you wake up, then do eat breakfast, a dietary protein-rich breakfast is the best. [15,16] If you are not hungry, then do not. As a rule of thumb, don't eat unless you are hungry!

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EXAM QUESTIONS

As RAMS aims to enlighten both students and professionals, we would like to present you two exam questions. Find out if you can remember what you learned during the bachelor! The right answers can be found further on in this journal.

We challenge you!

Question 1: The cytosolic enzyme ACAT esterificates cholesterol with unsaturated fatty acids. In what way will consuming unsaturated fatty acids will affect the level of LDL in the plasma? LDL levels will

A. not change
B. increase
C. decrease

Question 2: An increased concentration of the conjugated bilirubin is measured in the blood of a patient with jaundice. The AST and ALT activities in the plasma are normal, while the ALP activity was increased in the plasma. The urine contains bilirubin but no urobilinogen. What is the most likely diagnosis?

A. Intra-hepatic jaundice
B. Post-hepatic jaundice
C. Pre-hepatic jaundice

(Module Metabolism, water and mineral homeostasis 2012-2013)

The answers to these questions can be found on page 11 in this journal.

MicroRNA PANELS SHOW POTENTIAL AS A DIAGNOSTIC BIOMARKER FOR BLADDER CANCER

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ABSTRACT

BACKGROUND: Urothelial carcinoma is the most common form of bladder cancer. Currently, cystoscopy is used for diagnosis of this disease. This invasive method could potentially be avoided by using non-invasive urinary microRNA (miRNA) as biomarkers if these show to be adequate.
OBJECTIVE: The aim of this study was to collect more evidence in order to evaluate the use of miRNA expression as a diagnostic biomarker compared to the diagnostic values of the current golden practice (cytoscopy).
METHODS: PubMed and Embase were searched for relevant articles concerning miRNA and diagnostics for bladder cancer. In duplo, all articles were screened independently for relevance based on title and abstract. QUADAS-2 was used to critically appraise the articles and check for applicability concerns.
RESULTS: All but two of the thirteen relevant articles showed miRNA sensitivity and specificity above 50 percent. Separate miRNAs as well as panels of multiple miRNAs were reported. They all had an area under the curve (AUC) > 0.6, as reported in all but one study. The three panels show a high sensitivity and specificity with a range of 80 to 88 and 87 to 100 percent respectively. For two of the panels the AUC was reported and these were high (0.888 and 0.91).
CONCLUSIONS: Our results show that the miRNA panels outperform most stand-alone RNAs, and some panels also seem to potentially outperform some current diagnostic practices like urine cytology. We therefore recommend more research to be done with miRNA panels compared to cystoscopy, to ultimately validate its potential as a less invasive replacement or precursor for diagnosing bladder cancer.

KEYWORDS: Biomarkers, Bladder Cancer, Diagnostic, MicroRNA

*Supplementary material has been marked with * and can be found online at www.ramsresearch.nl*

Introduction

The most common form of bladder cancer is urothelial carcinoma. This type of cancer starts in the urothelial cells. These transitional cells, which embed the inside of the bladder, cause the bladder to expand when it is full [1]. Worldwide more than 330.000 new cases of bladder cancer are diagnosed and over 130,000 bladder cancer patients die from their disease each year [2]. The development of urothelial cancer is thought to start with the origination of non-muscle invasive bladder cancer (NMIBC). This tumour can potentially grow into the muscle layer of the bladder by which it is called muscle invasive bladder cancer (MIBC). MIBC is associated with poor prognosis [3].

Currently, when a patient is suspected of having bladder cancer (due to symptoms such as haematuria) cystoscopy is used for diagnosis. This method uses a small camera which is brought into the bladder via the urethra. For the patient undergoing cystoscopy, this can be experienced as uncomfortable and painful [4, 5]. Other ways to detect bladder cancer are urine cytology and histology of biopsies, which are labour-intensive and highly invasive respectively. As a new and alternative way to diagnose bladder cancer, the use of biomarkers such as microRNA (miRNA) expression levels could show great potential [6].

MiRNAs are small noncoding RNAs involved in multiple cellular processes, such as cell proliferation and division. It was shown that some specific miRNAs are present in cells during tumour progression but these could also be found in blood and urine [7]. These miRNAs could easily be detected in urine using RT-qPCR (Real-Time quantitative Polymerase Chain Reaction), which makes this experimental method less arduous for the suspected patient [8].

It was the aim of this systematic review to collect more evidence to evaluate the use of miRNA expression as a diagnostic biomarker for the detection of urothelial carcinoma in suspected patients, as compared to the diagnostic values of the current practice.

Methods

Search Strategy

In order to achieve our aim, PubMed and Embase were searched for relevant articles concerning miRNA and diagnostics for bladder cancer. A search query was constructed using the Disease, Determinant, Outcome (DDO) criterion and then translated to match the PubMed and Embase search formats. To find all relevant articles, the following search terms were used: “Urinary Bladder Neoplasms”, “microRNA”, “sensitivity” and “specificity”. Synonyms were added as a search term in title/abstract. Supplementary table 1* contains the full search strategy. The same search was repeated later to ensure no recent articles were missed in the first search. Therefore, in this systematic review all relevant articles up to 16 March 2016 were included.

Inclusion and Exclusion Criteria

In duplo, all articles were screened for relevance based on title and abstract. Articles were included when they contained urinary detection of miRNA, urothelial carcinoma or synonyms, and diagnostic outcomes. Articles were excluded when there was no full text available, the article was not written in english or it involved other systematic reviews. Duplicate articles and subsequent studies were removed using Endnote® (version X7.4).

Quality Assessment

Using QUADAS-2 [19], the risk of bias was determined in duplo by sco-

ring 4 items of a checklist with yes, no or unclear on 4 domains: patient selection, index test, reference test, and flow and timing. The first three domains were also scored on concerns of applicability for this systematic review, where low concerns mean high applicability. Overall risk of bias and applicability were determined using Excel.

Data Extraction
For each of the remaining studies the sensitivity and specificity for diagnosing bladder cancer with miRNA biomarkers were collected. To give an overview of these diagnostic values and the Area Under the Curve (AUC) of all studies, tables were made and graphs were created using Matlab® (version R2012a).

Results

Search Strategy
The queries that were used, which can be seen in supplementary table 1*, resulted in respectively 266 and 647 articles in PubMed and Embase, thus a total of 913 articles for screening. This is depicted in the flowchart (figure 1). A second search two weeks later with an updated outcome syntax in the search criteria (see supplementary table 1) led to 61 extra articles which were also included.

Inclusion and Exclusion Criteria
Based on the inclusion and exclusion criteria mentioned in the methods section, fifteen seemingly relevant articles from the results of the first search were selected, and one relevant article was found in the second search (see figure 1).

Quality Assessment
During the quality assessment using the QUADAS-2 [19], two more articles were found irrelevant and were excluded based on methodology and relevance, giving a total of fourteen relevant articles. Based on poor quality of selection of patients and controls (mixing controls and cases) one more article was excluded, leading to thirteen remaining articles. Of these articles, the risk of bias and the applicability is shown in figure 2A. The summarized risk of bias and applicability for all studies were scored and are shown in figure 2B and figure 2C. Risk of bias in the domain of patient selection is high in all studies, whereas the other three domains mostly had a low risk of bias. The concerns for applicability were low in all three domains.

Data Extraction
For all 13 studies the study characteristics and used miRNAs were compiled and can be seen in supplementary table 2*. The mean ages ranged from 58.4 to 76.5 years among the cases, respectively 34.3 to 69.0 among the controls. The male/female ratio was slightly higher in the cases than in the controls. Nine studies included patients with both NMIBC and MIBC. Zhang X. et al. studied only NMIBC, the remaining three did not mention the invasiveness of the bladder cancer [17].

The following diagnostic values of the 13 studies were collected in table 3: sensitivity, specificity and the AUC. Taking all the studies together, the range of the AUC was 0.629 to 0.91. There were no notable outliers. Concerning the sensitivity, each study found a value between 46.4 and 88 percent. Except for two miRNAs, 21 and 211, from Miah et al., each sensitivity was above 50 percent [13]. The specificity of all studies was above 50 percent as well, with the only exception being miRNA 211 investigated by Miah et al. with a specificity of 41,7 percent. Notable is the specificity of 100 percent found by Snowdon et al. for miRNA 125b and 126, being part of a panel, and Shimizu et al. for miRNA 124-3 [15, 14].

MiRNA 15a
Certain miRNAs were studied in multiple articles. Type 15a has been studied by both De Long et al. and Miah et al. De Long et al. used a panel of multiple miRNAs including 15a with an overall sensitivity of 88 percent. Miah et al. found a sensitivity for miRNA 15a of 51.7 percent. Found by De Long et al. and Miah et al. the specificities were respectively 87 and 72.0 percent and the AUC 0.888 and 0.86 [9, 13].

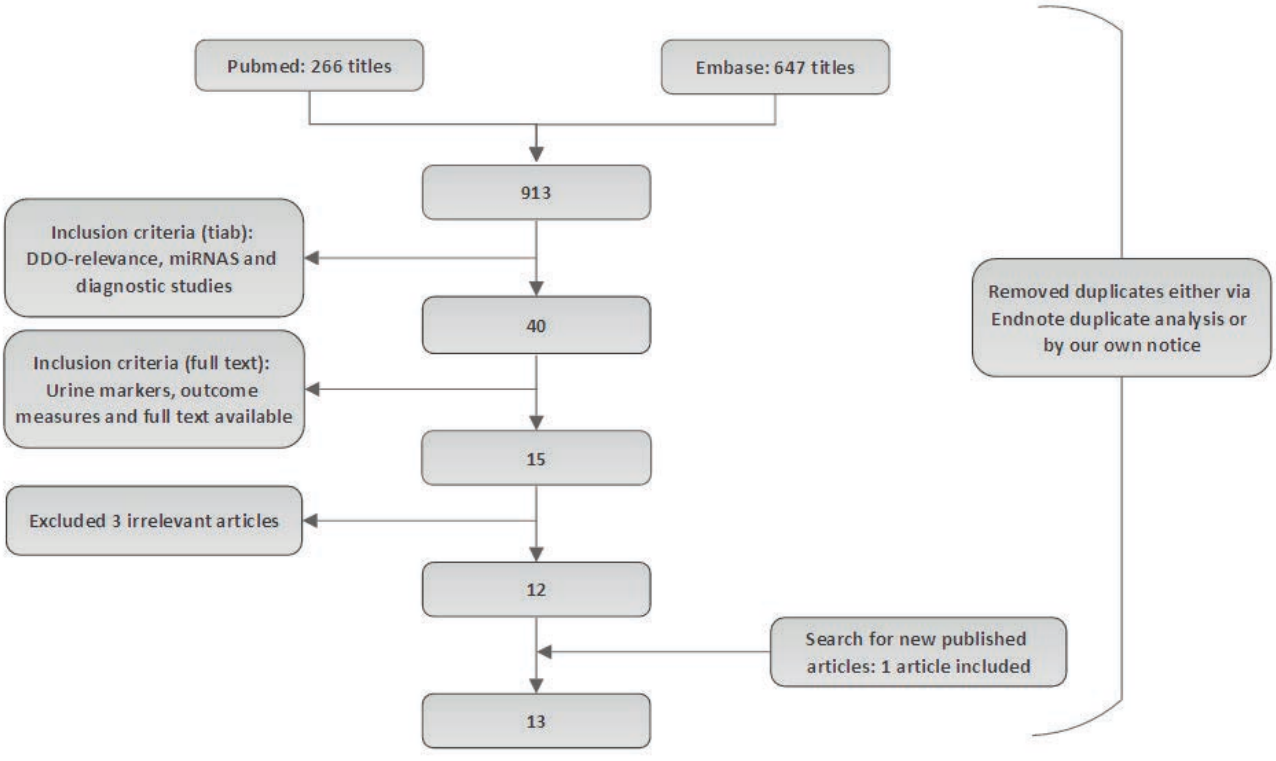

Figure 1: *Flowchart of the search strategy, numbers indicate the amount of articles.*

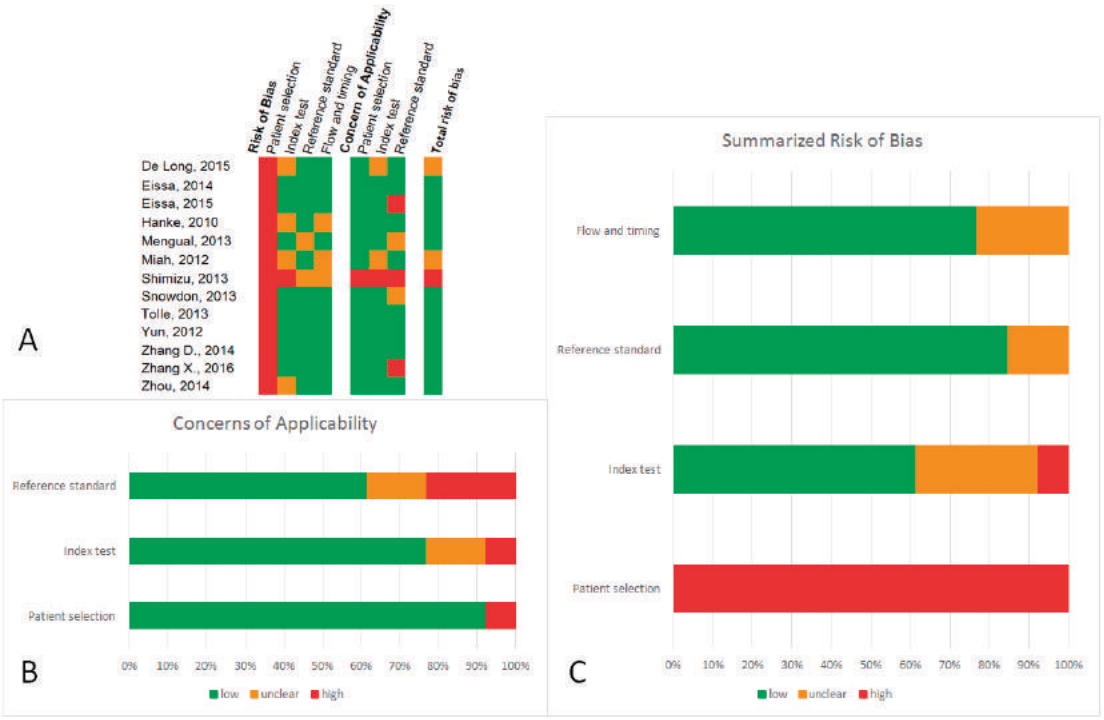

Figure 2: *Results of the critical appraisal using QUADAS-2. Green: low risk of bias or low concern; orange: unclear risk of bias or unclear concern; red: high risk of bias or high concern. A) Risk of bias and concern of applicability per study. B) Summarized concerns of applicability per domain. C) Summarized risk of bias per domain.*

MiRNA 21

Miah et al. and Mengual et al. studied miRNA 21. In the case of Mengual et al., miRNA 21 was used as part of a miRNA panel. The sensitivity and specificity found by Miah et al. were 46.6 and 65.3 percent, Mengual et al. found 83.74 and 87.64 percent respectively for the total panel. The overall AUC reported by Miah et al. is 0.86, Mengual et al. came to an AUC of overall 0.91 [13, 12].

MiRNA 125b

MiRNA 125b was analyzed by Zhang D. et al. and Snowdon et al. In the case of Snowdon et al., this miRNA was part of a panel. Zhang D. et al. analyzed miRNA 125b both individually and in combination with miRNA 99a. A sensitivity of 84.8 and 80 percent respectively was reported by Zhang D. et al. for miRNA 125 individually, with an AUC of 0.813. The combination with miRNA 99a resulted in a sensitivity of 86.7, a specificity of 81.1 percent and an AUC of 0.804. Snowdon et al. found a specificity of 76.2 and 100 percent for miRNA 125b. The AUC was not mentioned [8, 15].

MiRNA 200a

Type 200a was studied by Snowdon et al. and Yun et al. Snowdon found a sensitivity of 80 and a specificity of 100 percent, again for a panel of miRNAs, including 200a. Snowdon et al. did not report the AUC. Yun et al. classified urothelial bladder cancer in two categories; invasive and non-invasive. For the invasive category a sensitivity and specificity of respectively 55.1 and 72.7 percent was reported. The non-invasive category had a sensitivity of 54.4 percent and a specificity of 65.7 percent. The investigated miRNAs for invasive bladder cancers had an AUC of 0.679, where the miRNAs for non-invasive bladder cancers had an AUC of 0.638 [15, 16].

Ratios and combinations

In some cases a combination with urine cytology or a ratio of multiple miRNAs was examined. These combinations sometimes resulted in higher diagnostic values. Hanke et al. used the expression of two miRNAs (182 and 126) relative to the expression of miRNA 152 to distinguish between patients and controls, which resulted in two ratios. The ratio of 182:152, at a threshold of 1.8, gave a sensitivity of 55 percent, a specificity of 82 percent and an AUC of 0.799. The second ratio (126:152) showed a sensitivity of 72 percent, a specificity of 82 percent and an AUC of 0.768 [5].

Zhang X et al. used miRNA 155 and its combination with the results of urine cytology. As a stand-alone, miRNA 155 had a sensitivity of 80.2 percent and a specificity of 84.6 percent. The AUC was 0.804. In the combination the sensitivity increased to 85.8 percent. The specificity and AUC, however, were not reported [17].

Data overview

A complete overview of the sensitivity and specificity of the miRNAs is given in figure 3. Here, each data point has a label that refers to a miRNA/study combination that is given in table 3. The blue diagonal in figure 3 represents a 50 percent chance of correct diagnosis.

A relatively homogeneous cluster can be seen in the right upper corner, which represents an overall good performance. However, some miRNAs or panels performed poorly (i.e. low specificity and sensitivity) . Notable examples of this are data points 17 and 22, which refer to miRNA 211 and 24-1 respectively, both from Miah et al. [13]. Examples that performed well (i.e. high low specificity and sensitivity) are the data points indicated by the numbers 1, 4, 8, 25 and 28. These refer to the panel from De Long et al., miRNA 10b from Eissa et al., the panel from Mengual et al., miRNA 124-2 from Shimizu et al. and the panel from Snowdon et al. respectively [9, 10, 12, 14, 15]. It is worthy to note that all three of the used panels were within this group of five.

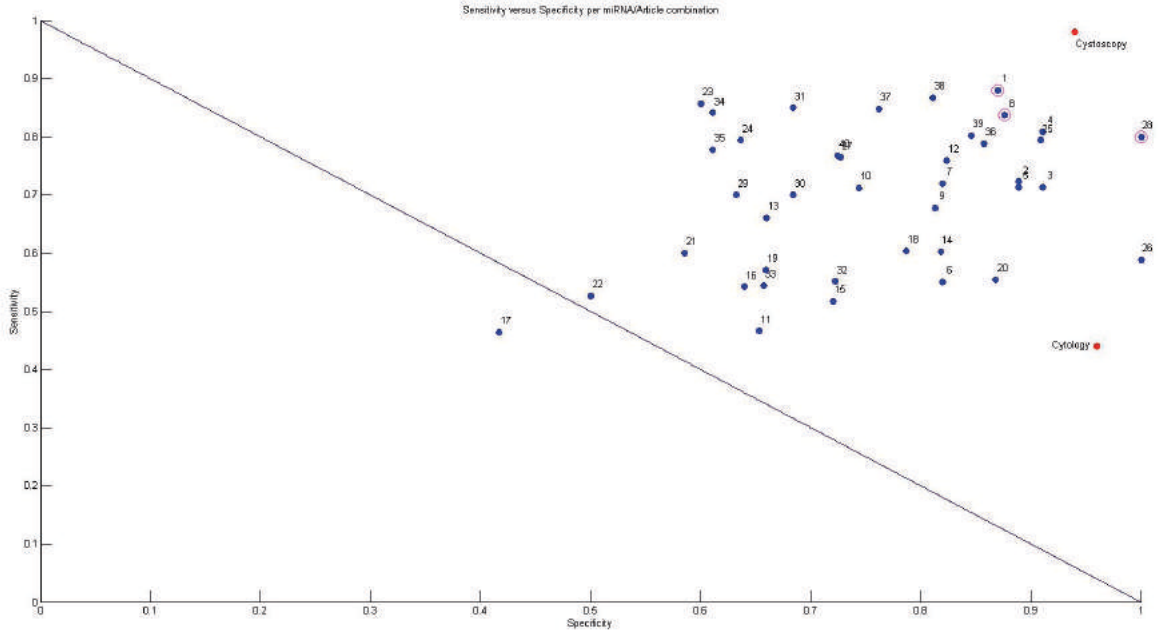

Figure 3: *Scatterplot of all sensitivities and specificities. Each data point has a label that represents a miRNA or miRNA panel which are documented in table 3. Studies using a panel of miRNAs are indicated with magenta circles. The blue diagonal represents the performance at 50/50 chance. Also indicated, in red, are the diagnostic values of cytology and cystoscopy.*

Discussion

The current tools used for diagnosing bladder cancer in suspected patients are cystoscopy, histology and urine cytology. Cytology scores low on sensitivity but high on specificity: a sensitivity of 44 percent and a specificity of 96 percent[20]. Cystoscopy on the other hand has both a high sensitivity (98 percent) and a high specificity (94 percent), but is relatively expensive and invasive[21]. Our results (supplementary table 2*) showed that miRNA panels can to some degree outperform cytology in the sense of sensitivity, but not in specificity. However, in all panels, both specificity and sensitivity were above 80% with an AUC above 0.85.

However, during literature search, articles of which the full text was not readily available were excluded. These excluded articles could have contained some relevant information relevant for our research. The 13 articles included for the critical appraisal were appraised with the QUADAS-2 criteria, which were specially designed for diagnostic studies. However, it can be argued that these criteria are subjective on indexing the risk of bias and the area of applicability.

In figure 2A, it can be seen that Shimizu et al. had a high risk of bias and there were high concerns of applicability according to the QUADAS-2 criteria because they used cell lines instead of patients. However, the article is useful for this systematic review because relevant miRNAs can be identified in cell lines for applicability on urothelial carcinoma, so that only promising miRNAs will be further studied on patient samples. As this article focused on relevant miRNAs for diagnosing bladder cancer, the outcome of such cell line studies can be included.

The results of Snowdon et al. showed a high specificity of 100 percent. The study population contained eight cases and five controls, which means the outcome would probably have had a large confidence interval. In the article of Snowdon et al., a confidence interval has not been mentioned. We included this study because of the applicability to our research question.

As notified in our results, miRNA 15a, 21, 125b, and 200a were all miRNAs that were examined in two separate studies. Although, for each case, the miRNA was studied separately as well as within a panel. Comparison of the sensitivity, specificity and AUC would be optimal but cannot be accomplished because of the different ways of studying these miRNAs, which made it difficult to conclude which miRNA generally performed better.

Recommendations

Further research should follow a different study design (blinded and randomized) in order to avoid a high risk of bias concerning patient selection. An example of a better design would be the following: each suspected patient should be diagnosed independently twice; once by urinary miRNAs and once by cystoscopy. A cross table can be made to analyze the results.

Because the sensitivity is higher than that of single miRNAs, research with miRNA panels is promising. New research should focus on constructing the ultimate panel of miRNAs and implementation in the current practice. In the end, it should show that a miRNA-based diagnosis could be a direct replacement of, or precursor to, the more invasive, yet more decisive methods of diagnosis. MiRNA implementation should therefore definitely deserve scientific attention.

Conclusion

Panels of multiple miRNAs generally scored better on sensitivity and specificity than separately studied miRNAs. The sensitivity and specificity were also higher than those of cytology, one of the current diagnostic methods. Cystoscopy remains the golden standard with a sensitivity of 98 and a specificity of 94 percent [21]. However, using a miRNA would be less invasive for the potential patients and relatively cheaper.

Table 3: *Summarization of studies' results, per study and MiRNA.*

Study	Label fig. 3	MiRNAs	Sensitivity (%)	Specificity (%)	AUC (95%-CI where available)
De Long, 2015	1	Panel[1]	88	87	0.888
Eissa, 2014	2	96	72.3	88.9	0.822 (0.758-0.887)
Eissa, 2015	3	210	71.3	91.1	0.836 (0.769-0.903)
	4	10b	80.9	91.1	0.877 (0.822-0.933)
	5	29c	71.3	88.9	0.845 (0.781-0.908)
Hanke, 2010		Ratios:			
	6	182:152	55	82	0.799
	7	126:152	72	82	0.768
Mengual, 2013	8	Panel[2]	83.74	87.64	0.91
Miah, 2012	9	15b	67.8	81.3	All 15 MiRNAs: 0.83
	10	135b	71.2	74.4	
	11	21	46.6	65.3	8 MiRNAs* 0.86:
	12	1224-3p	75.9	82.4	
	13	203	66.1	66.0	3 MiRNAs**: 0.86
	14	27b	60.3	81.8	
	15	15a	51.7	72.0	
	16	212	54.2	64.0	*MiRNAs:
	17	211	46.4	41.7	15a/15b/27b/100
	18	100	60.4	78.7	/135b/203/212/1224
	19	23b	57.1	65.9	-3p
	20	328	55.4	86.8	
	21	24-1	60.0	58.5	**MiRNAs:
	22	183	52.6	50.0	15b/135b/1224-3p
	23	133b	85.7	60.0	
Shimizu, 2013	24	137	79.41	63.64	0.816 (0.693-0.938)
	25	124-2	79.41	90.91	0.797 (0.660-0.934)
	26	124-3	58.82	100	0.901 (0.807-0.995)
	27	9-3	76.47	72.73	0.797 (0.660-0.934)
Snowdon, 2013	28	Panel[3]	80	100	Not available
Tolle, 2013	29	520e	70.0	63.2	0.679 (0.510-0.819)
	30	618	70.0	68.4	0.629 (0.524-0.819)
	31	1255b 5p	85.0	68.4	0.764 (0.601-0.885)
Yun, 2012		200a:			
	32	Invasive	55.1	72.7	0.679
	33	Non-Invasive	54.4	65.7	0.638
		145:			
	34	Invasive	84.1	61.1	0.790
	35	Non-Invasive	77.8	61.1	0.729
Zhang D., 2014	36	99a	78.0	85.7	0.800 (0.715-0.866)
	37	125b	84.8	76.2	0.813 (0.729-0.897)
	38	Combination	86.7	81.1	0.876
Zhang X., 2015	39	155	80.2	84.6	0.804 (0.756-0.845)
		Combination of Cytology and 155	85.8	Not available	Not available
Zhou, 2014	40	106b	76.8	72.4	0.802

MiRNA: micro RNA; 95%-CI: 95% Confidence Interval; AUC: Area Under the Curve.
[1]: MiRNAs: 200, 93, 940, let7b, 191, 21, 15a, 10a, 26a.
[2]: MiRNAs: 187, 18a*, 25, 142-3p, 140-5p, 204.
[3]: MiRNAs: 125b, 126, 143, 200a.

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CORRECT ANSWERS TO THE EXAM QUESTIONS

Question 1: answer C

Increased intake of unsaturated fatty acids will increase the production of cholesteryl esters (cholesterol + unsaturated fatty acid) by the intracellular ACAT enzyme. Consequently, cholesterol concentrations inside cells that contain ACAT will drop. To keep intracellular cholesterol concentrations constant, cells will try to increase uptake of cholesterol by increasing the expression of the LDL-receptor. Increased LDL-receptor expression will lead to increased LDL-uptake by the cells and thereby decrease the plasma LDL concentration.

Question 2: answer B

Jaundice with increased levels of conjugated bilirubin point in the direction of a hepatic or post-hepatic etiology, since conjugation of bilirubin is performed only in hepatocytes. Increased ALP more specifically suggests a post-hepatic etiology, because this enzyme is particularly concentrated in cells of the bile duct. The conjugated, water-soluble bilirubin will be excreted by the urine. Urinary urobilinogen will be decreased because bile will not reach the intestinal flora, preventing the transformation of conjugated bilirubin to urobilinogen.

Liver damage seems absent in this patient, since AST and ALT are normal. ALT is found predominantly in the liver, and will be elevated in case of liver damage. AST is present in the liver as well, but is also expressed in muscle, kidney, brain and red blood cells. Consequently, elevated levels of AST may reflect pathology in organs other than the liver.

The exam questions can be found back on page 5 in this journal.

SUMMER SCHOOL NEUROSURGERY RAMS 2016 WINNING ABSTRACT

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This year's RAMS Summer School was held at the department of Neurosurgery on the 11th and 12th of July. The programme, which was devised in collaboration with the NCCN (Neurochirurgisch Centrum Nijmegen), provoked a lot of enthusiasm from both participating students as well as lecturers. A novelty of this year was the mixture of backgrounds of the 25 participants. Bachelor or master, biomedical sciences or medicine, psychology or molecular life sciences, all synergizing with their unique views on the subject.

The event started on Monday morning with an official opening by Prof. Bartels. In the following two days, the students were submerged in a full programme consisting of lectures and practically oriented education. The programme included a refreshing neural anatomy course in the dissection rooms, excellent lectures by four different neurosurgeons from Radboudumc and two eye-opening workshops. At the end of the two days, small groups of students were given two hours to prepare a mini-review plus 5-minute pitch on a scientific subject chosen by themselves.

The final pitches were held at the conference room of Radboudumc Director's Board. The level of the pitches was high, especially when the short preparation time was taken into account. The students also covered a great variety of subjects, from optogenetic research techniques to peripheral nerve stimulation as a treatment for an overactive bladder, and from impulse control disorders in Parkinson to deep brain stimulation for epilepsy. The last group decided to concentrate primarily on the content, thus using their time to write a five page review in two hours instead of making a powerpoint. This won them the award for the best group of the RAMS Summer School 2016. Afterwards, we asked them to write an abstract of their (mini-)review, which can be read below.

The RAMS Summer School 2016 was organized by four (former) RAMS members. They thank Guido de Jong and Dylan Henssen from the NCCN for their organizational help.

Background

Although the majority of patients with tonic-clonic seizures are completely seizure-free with new anti-epileptic drugs, a large amount of patients are drug resistant and therefore still suffer from daily seizures which heavily reduce their quality of life. Alternative intervention methods such as deep brain stimulation (DBS) are found to be effective in reducing symptoms in a variety of neurological disorders, but the current state of literature on the effects of DBS on tonic-clonic seizures is unknown.

Methods

We systematically searched for studies on the effect of DBS on tonic-clonic seizures, regardless of the cause of the seizures, in PubMed. We excluded any article that wasn't written in English. Studies in both humans and animals were included.

Results

A total of three articles were found showing inconsistent findings due to great methodological differences. The first study performed DBS in the substantia nigra reticulate in rats, showing no effects of DBS in reducing evoked tonic-clonic seizures. The second study stimulated the hippocampal region using DBS in three patients with tonic-clonic seizures. The tonic-clonic seizures disappeared completed and no side effects were reported. Finally, a third study performed a randomized controlled trial in which the cerebellum was stimulated in four patients. DBS caused a reduction in seizure rate even after a 2-year follow-up.

Conclusions

Current research gave little but promising evidence regarding DBS in reducing tonic-clonic seizures. However, previous studies vary greatly in their methodology with regards to study design and the target brain area of DBS. Therefore, more randomized controlled studies are needed in order to show the potential effects of DBS in the treatment of tonic-clonic seizures.

All Summer School Neurosurgery RAMS 2016 participants.

SAFETY AND EFFECTIVENESS OF THE HUMAN PAPILLOMAVIRUS VACCINE

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ABSTRACT

BACKGROUND: Cervical cancer is one of the most common types of cancer in women. The human papilloma virus (HPV) can be detected in almost all the cases of cervical cancer. The uncertainty regarding the safety and effectiveness of the HPV vaccine in the media has probably lead to low vaccination rates in the Netherlands.
OBJECTIVE: We performed a systematic review to evaluate the effectiveness and safety of the HPV vaccines Cervarix, Gardasil and Gardasil-9 in boys and girls.
METHODS: Embase and PubMed databases were searched on our domain, determinant and outcome with their synonyms. We included case-reports, trials and cohort studies with outcomes of antibody titers and adverse events. We selected on the availability of the full text and on Dutch and English language.
RESULTS: In our analysis we included 5 case reports, 12 cohort studies and 26 trials. In Cervarix and Gardasil, Geometric Mean Titer (GMT) levels rise after vaccination and peak at month seven (GMT for Cervarix, HPV16: 2176 IU and HPV18: 97.6 IU; Gardasil, HPV16: 271.7 IU and HPV18: 57.2 IU). After the seventh month, GMT levels decrease and seem to stabilize after 24 months. The most frequently mentioned adverse effects are local pain, erythema and swelling or systemic effects like fatigue, headache and myalgia. These symptoms did not last and were considered not serious.
CONCLUSION: We found high antibody titers, especially when vaccinated with Cervarix. We also found no serious adverse events and therefore can conclude that vaccination with HPV vaccines seems to be effective and safe. More research is needed on long-term safety and effectiveness of HPV vaccines.

WHAT'S KNOWN: HPV vaccination programs are implemented across the world to protect girls and women against cervical cancer. There have been concerns about the safety and effectiveness of the HPV vaccine.

WHAT'S NEW: The HPV vaccine Cervarix causes high antibody titers against HPV types 16 and 18 for at least 72 months. Serious adverse events have hardly been reported.

KEY WORDS: HPV Vaccine, Safety, Effectiveness, Cervical Cancer

*Supplementary material is marked with * and can be found online at www.ramsresearch.nl*

Background

Cervical cancer is a common type of cancer in women. Each year, in the Netherlands, about 700 women are diagnosed with cervical cancer and the annual mortality of cervical cancer is 200 [1, 2]. In 99.7 percent of the cases the Human Papillomavirus (HPV) can be detected [3]. Although the presence of HPV in women with cervical cancer implies a probable cause, it does not necessarily mean HPV is the (only) cause of the cancer.

More than 170 types of HPV exist, but not all of them infect the anogenital area [4]. In fact, only about 40 types of the virus infect the anogenital area and can therefore have a role in the development of the cervical cancer [5]. Those are categorized into 'High Risk' and 'Low Risk' types, of which the High Risk causes cancer. In 70% of the cases of cervical cancer HPV16 and HPV18, examples of High Risk types, are responsible. The virus codes for viral proteins, the most important are named E6 and E7. These proteins interact with the p53 gene and retinoblastoma (Rb), which can lead to disruption of the cell cycle, uncontrolled cell proliferation and eventually cancer [6]. After being exposed to HPV, it can take up to 10-15 years to develop cancer. Additional mutations in for example oncogenes or genes that suppress cell proliferation, caused either by environmental influences or de novo mutations,are necessary, for the disease is multifactorial. Besides cervical cancer, the virus can also cause penile cancer, anal cancer, genital warts and other lesions [5]. This is why the safety and effectiveness of the HPV vaccine in males is also important to investigate. The vaccination of males could also be beneficial for achieving herd immunity, since the virus is transmitted sexually. To transmit the virus, however, penetration is not required [6].

HPV is often the cause of cervical cancer, thus preventing infection would be useful. A possible way to prevent infection with HPV is the use of a vaccine. A HPV vaccination program has already been implemented in many countries across the world. The vaccine contains a virus-like particle (VLP), which is an encapsulating protein of the virus [6]. As the vaccine does not contain any viral DNA or RNA, it cannot infect cells and replicate. The immune system recognizes the protein as pathogenic and initiates an immune response against the virus, producing antibodies and developing memory cells. The three most used vaccines are Cervarix, Gardasil and Gardasil-9 [7]. The bivalent vaccine Cervarix contains proteins of HPV types 16 and 18, the quadrivalent vaccine Gardasil also contains types 6 and 11, and the 9-valent vaccine Gardasil-9 also contains proteins against type 31, 33, 45, 52 and 58. Beside the VLP, the vaccines contain an adjuvant, consisting of a protein and aluminium hydroxide, for a stronger immune response. Vaccination comprises three doses of the vaccine, given at day 0, month 1 or 2 and month 6.

In the Netherlands, the vaccination rate for HPV is low compared with other vaccines (61% vs. >90%) [8]. A possible reason for the low vaccination rate is the high coverage of the topic in the media, regarding the safety and effectiveness of the vaccine. This could have influenced people's opinions into thinking the vaccine is unsafe and ineffective. We wanted to know whether these accusations were true. Thus, we performed a systematic review on the safety and effectiveness of the HPV vaccines Cervarix, Gardasil and Gardasil-9.

Research question

What is the effectiveness and safety of the HPV vaccines Cervarix, Gardasil and Gardasil-9 in males and females?

Methods

Search strategy and selection

For this systematic review we searched in PubMed and Embase. We used Mesh terms and title and abstract (tiab) for relevant synonyms for the domain: males and females, determinant: HPV-vaccine (2-, 4-, or 9-valent) and outcome: effectiveness and safety. The final search strategy can be found in table 1*. In our process we revised our initial search strategy by adding a few terms. With the final search strategy, we found 67 additional articles ("additional search" in table 1*). We removed all the duplicate articles. These were screened on title and abstract for relevance concerning the domain, determinant and outcome. Exclusion criteria are mentioned in figure 1. Afterwards we selected the articles based on full text. The inclusion criteria are the following: a trial in which antibody titers or Geometric Mean Titer (GMT) was an outcome; a trial in which side effects or adverse effects of the HPV vaccine were monitored; cohorts concerning the long-term side or adverse effects; cohorts monitoring antibody titers or GMT; case reports. All articles we found were divided over the five of us and the selection was done independently, without checking each other's selection.

Critical appraisal

The articles we included were trials, cohort studies or case reports. To assess the quality and risk of bias of the trials we used the Cochrane risk of bias tool and for the cohort studies we used the Newcastle-Ottawa scale (NOS). The assessment of the risk of bias can be found in figure 2. Each person critically appraised one fifth of the selected articles, which means that each article was critically appraised independently by one person. We did not discuss each other's critical appraisal.

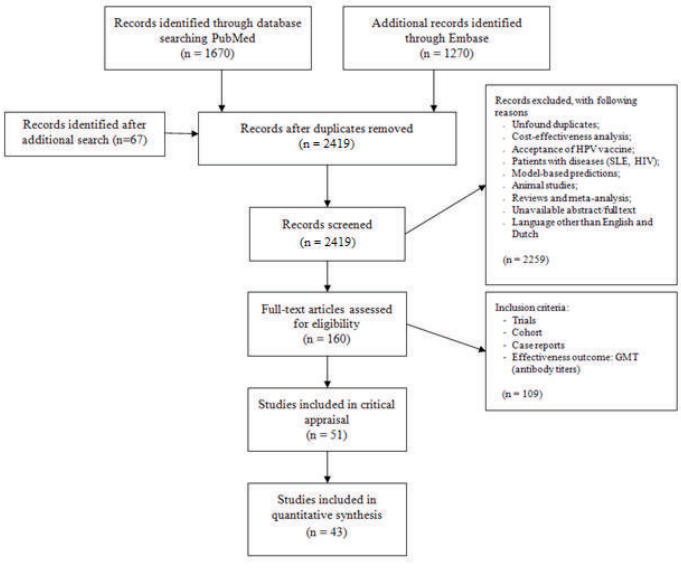


Figure 1: *Flowchart of the search and selection strategy. Our initial search strategy gave us 2419 articles. Halfway our process we revised our initial search strategy and added a few terms, which lead to finding 67 new articles. This gave us a total of 2419 without duplicates. When using the final search strategy in Table 1, all 2419 articles will be found.*
GMT: Geometric Mean Titer

Effectiveness: data analysis

The GMT for HPV types 16 and 18 was extracted from the selected studies. We chose to only include data from HPV types 16 and 18, because all vaccines (Cervarix, Gardasil and Gardasil-9) protect against these types. To be able to compare all the data, we converted it into International Units (IU), because GMT levels can be measured in EU/mL and mMU/mL [9]. For each articles the weighted mean of the GMT was calculated, if not mentioned by the article. MS Excel 2007 was used for the analysis. First, we split the data for the bivalent, quadrivalent and 9-valent vaccine. Then, we analysed the bivalent, quadrivalent and 9-valent vaccines separately. No subgroups were made, because of the heterogeneity of the study populations in the different articles. During data analysis, we found that one study did not describe the amount of people within each subgroup, thus we were not able to calculate the weighted mean of the GMT levels for this study. This is why we excluded it.

Safety analysis

We collected data from the cohort studies, trials and case reports. From the cohort studies and the trials, the articles were divided into two and each half was analysed by one person individually. The number of local and systemic side effects and the vaccine-related serious adverse events (SAE) were counted. Additionally, we described the events mentioned in the different case reports and cohort studies investigating the association between HPV vaccination and several diseases in more detail.

Results

We found 1670 and 1270 records in PubMed and Embase respectively. Halfway our process we revised our initial search strategy and added a few terms, which lead to finding 67 new articles. This gave us a total of 2417 without duplicates. When using the final search strategy in table 1*, all 2417 articles will be found. Based on the critical appraisal a few articles were excluded because they did not contain the proper information or because they had a very high risk of bias. In our analysis we included 5 case reports, 12 cohort studies and 26 trials. The study population consisted of males and females of all age groups. An overview of all studies and their characteristics can be found in table 2*.

Effectiveness

The four graphs in figure 3 show the effectiveness of Cervarix and the Gardasil against HPV types 16 and 18. The 9-valent vaccine was excluded from this figure, due to a minimal amount of articles which examined this vaccine. Gardasil-9 will be discussed later. The weighted mean of the GMT levels rises after vaccination and peaks at month seven, the first measuring point after the third dose of the vaccine (GMT for Cervarix, HPV16: 2176 IU; Cervarix, HPV18: 973.6 IU; Gardasil, HPV16: 271.7 IU; Gardasil, HPV18: 57.2 IU). After the seventh month, the GMT levels start to decrease and seem to stabilize after approximately 24 months.

The GMT levels after vaccination are higher than the reference levels after a natural infection (5.8 (1.8 - 18.6) IU and 2.6 (1.8 - 3.7) for HPV16 and 18, respectively) [51], with exception of the GMT levels against HPV18 after vaccination with Gardasil (figure 3d). Figure 3 also shows that the GMT levels for both HPV types are higher after vaccination with Cervarix than with Gardasil.

Only two studies researched the effectiveness of Gardasil-9. These studies only measured at month seven of the vaccination program [10, 11]. Castellsague, X., et al [10] showed GMT levels against HPV16 and 18 of 225.8 IU and 55 IU, respectively. Van Damme, P., et al [11] showed GMT levels against HPV 16 and 18 of 515.1 IU and 159.1 IU, respectively.

Safety

The most frequently mentioned local side effects are pain, erythema and swelling. For the systemic side effects, the most frequently mentioned are fatigue, headache or myalgia (table 3). These symptoms did not last and were considered non-serious. The frequency of the local effects is higher in the HPV group. There are no relevant differences in the frequency of systemic effects between the HPV group and the controls. There are a few SAE related to the vaccine, as specified by the article, but these occurred equally across the two groups.

Arnheim-Dahlstrom, L., et al [12] studied the association between the HPV vaccine and autoimmune diseases, neurologic diseases and venous thromboembolism. This cohort study included vaccinated girls and unvaccinated girls in Denmark and Sweden. For various diseases they calculated the risk in the two groups and rate ratios (RRs). Out of the 23 RRs for autoimmune diseases, only three were significantly increased. These three RRs are associated with Behcet's syndrome, Raynaud's disease and type 1 diabetes. For the RRs we refer to the article. For the other outcomes, neurological diseases and venous thromboembolism the RRs were not significantly increased.

Schurink-van't Klooster, T.M., et al [43] examined the association between the HPV vaccine and migraine. In the months following HPV vaccination, the incidence rate of migraine in the vaccinated group was compared to the incidence rate of migraine in the unvaccinated group, and incidence rate ratios (IRR) were calculated for each month. The IRRs for migraine ranged from 0.0 to 3.0 and none of the IRRs were statistically significant.

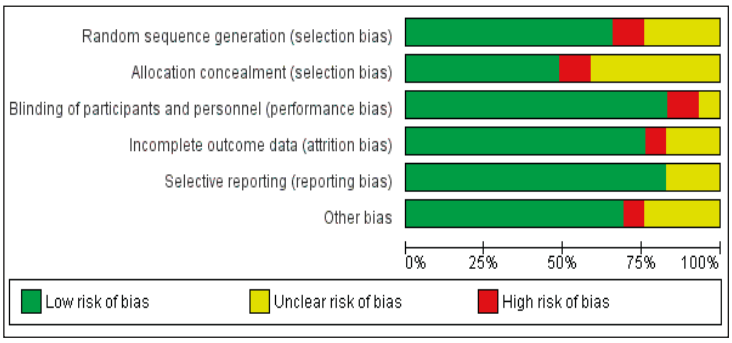
Case reports

A 17-year-old girl had a hypersensitivity reaction after injection with Gardasil (Badiu, I., et al) [14]. This hypersensitivity was caused by the excipient polysorbate 80 (an excipient is a substance that is often added in vaccines or drugs for long-term stabilization of the product). Severe reactions due to polysorbate 80 are infrequent.

A different case report describes the persistent bilateral visual loss and left hemiparesis in a 16-year-old girl, 10 days after her second Gardasil vaccination (DiMario Jr., F.J., et al) [23]. It was very likely she had demyelinating encephalomyelitis (DE), triggered by the vaccination. DE can also be triggered by a viral infection. The authors mention that also other vaccines have been associated with acute DE, but causality has not been demonstrated.

Article	Selection	Comparability	Outcome	Total
Arnheim-Dahlstrom, L., et al (2013)	***	**	*	6/9
Castellsague, X., et al (2011)	****	**	**	8/9
de Ridder, M.A.J., et al (2014)	***	-	**	5/9
Jain, K.M., et al (2013)	**	-	-	2/9
Klooster, T.M.V., et al (2011)	***	-	-	3/9
Konno, R., et al (2014)	****	**	***	9/9
Schwarz, T.F., et al (2014)	***	**	***	8/9
Naud, P.S., et al (2014)	***	**	**	7/9
Schwarz, T.F., et al (2012)	***	**	***	8/9
Schwarz, T., et al (2014)	***	-	***	6/9
Villa, L.L., et al (2006)	***	*	**	6/9
Schurink-Van't Klooster, T.M., et al (2015)	***	*	**	6/9
Average	***	*	**	6/9

a)



b)

Figure 2: *The critical appraisal of (a) cohort studies with NOS and (b) trials with the Cochrane risk of bias tool. In figure 2a, the higher the amount of stars means a lower the risk of bias. For "selection" a maximum of four stars could be awarded. For "comparability" this was 2 stars and for "outcome" it was a maximum of three stars. In total, a maximum of 9 stars could be given.*

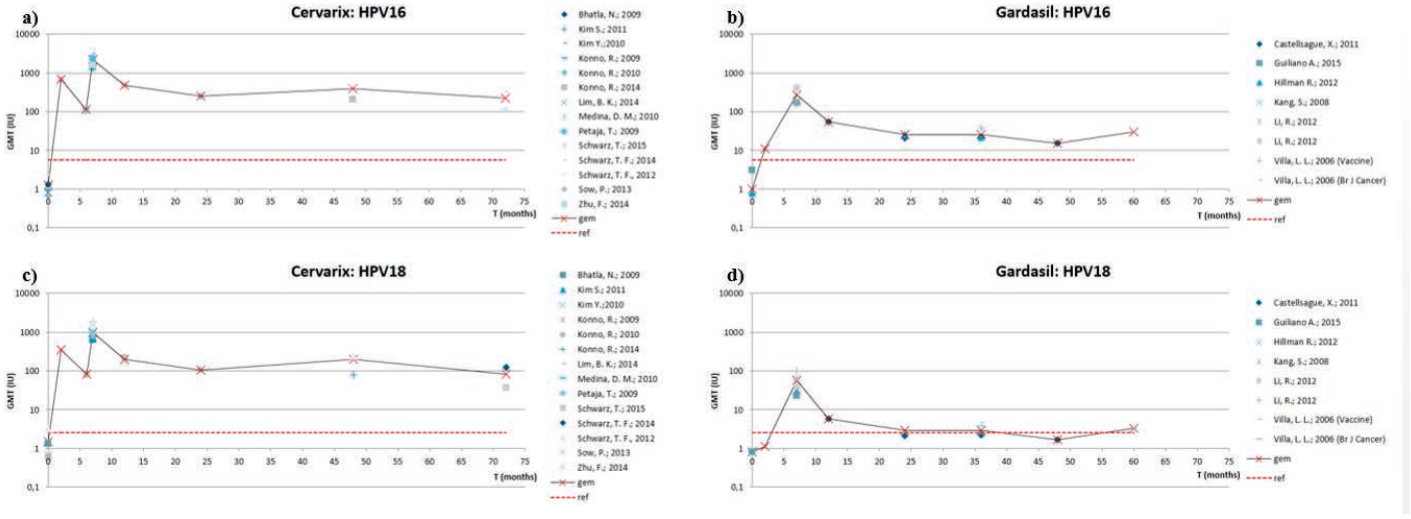


Figure 3: *Effectiveness of Cervarix and Gardasil against HPV16 and 18. GMT levels after vaccination with Cervarix showing anti-HPV16 (a) and anti-HPV18 (b) or GMT levels after vaccination with Gardasil showing anti-HPV16 (c) and anti-HPV18 (d). GMT = geometric mean antibody titer in International Units (IU). T = time in months. The first vaccination was given at month 0, the second at month 1 or 2 and the third at month 6. Weighted mean: mean of the GMT levels at a certain point of time adjusted for study size. GMT level after a natural infection (HPV-16: 5.8 (1.8 - 18.6); HPV-18: 2.6 (1.8 - 3.7)) Lim BK, Ng KY, Omar J et al. [51].*

Table 3: *The number of side effects occurring in patients and controls in absolute numbers and percentages [13, 16, 17-21, 25-42, 44-48, 51, 52].*

Side effects	Number of studies	Patients *(n)*	Frequency in patients *n* (%)	Control (*n*)	Frequency in controls *n* (%)
Local					
Pain	20	19105	13341 (69.8%)	10994	5334 (48.5%)
Erythema	15	14288	4210 (29.5%)	10390	1812 (17.4%)
Swelling	16	18988	4252 (22.4%)	11418	1827 (16.0%)
Other*	6	10977	6231 (56.8%)	9572	5373 (56.1%)
Systemic					
Fatigue	17	17492	2738 (15.7%)	9374	1774 (18.9%)
Fever	20	20092	1542 (7.7%)	11028	823 (7.5%)
Headache	18	18886	3222 (17.1%)	10449	2407 (23.0%)
Nausea	5	5915	240 (4.1%)	3323	59 (1.8%)
Myalgia	10	9553	2795 (29.3%)	6437	2527 (39.3%)
Dizziness	5	6310	285 (4.5%)	4706	18 (0.4%)
Other*	7	11147	4032 (36.2%)	10600	3616 (34.1%)
SAE related to vaccine**	4	12847	14 (0.1%)	9325	6 (0.1%)

Other side effects were not specified in the articles.
****Serious Adverse Events (SAE) as mentioned in the articles.***

The third article (Martinez-Lavin, M., et al) [50] describes two cases of fibromyalgia-like illness. Both patients (11-year-old and 14-year-old girl) experienced severe pain, paresthesias, allodynia, insomnia and chronic fatigue. The article mentions that similar cases have been described after other types of vaccination.
The fourth article presents 3 cases of systemic lupus erythematosus (SLE) following HPV vaccination (Soldevilla, H.F., et al) [15]. This happened in a 17-year-old girl, a 45-year-old woman and a 58-year-old woman. The HPV vaccination has, as also mentioned in the other case reports, triggered the immune system and therefore lead to a case presentation of SLE. There was no mention of which vaccine was used.

The fifth and final case report demonstrates a case of interstitial lung disease (ILD) in a woman in her 40's three months after her third Cervarix vaccination (Ymamoto, Y., et al) [22]. Other vaccines, as the influenza vaccines, can also induce ILDs.

Discussion

Regarding the results of the effectiveness analysis of the HPV vaccines, it seems that Cervarix is effective against HPV16 and 18. Gardasil seems to be effective against HPV16, although it does not appear to have an effect against HPV18. This is not in agreement with other literature [7]. This result could be explained by bias in our studies or by other sources of bias. Those sources being the bias mentioned above, but faults could also have been made in converting the GMT. The article [9] mentions a converter for GMT levels for HPV16, thus it could be possible that this conversion cannot be used for HPV18 GMT levels, but this is highly unlikely.

Furthermore, it was remarkable that the GMT levels for Cervarix were higher than the GMT levels for Gardasil. This stronger immune response could be explained by the fact that a different and a higher dose of the adjuvant is used in Cervarix than in Gardasil [24, 31]. Also, a different dose of HPV16 and 18 VLP was used in the vaccines. Another possible reason for this difference in GMT levels between Cervarix and Gardasil is the timing of the three doses given. The review of Di Mario, S. et al. (2015) [53] shows that the bivalent vaccine in naïve girls has a higher efficacy. This corresponds with our findings.

Some studies were unclear in reporting the frequency of side effects. Also, some studies did not divide the side effects into subgroups and only mentioned 'local effects' or 'systemic effects'. The cohort study about migraine did not show a causal effect between the HPV vaccine exposure and the incidence of migraine. The other cohort study found three significantly increased RRs, which means that being exposed to the HPV vaccine gives a higher risk of gaining an autoimmune disease than not being exposed. Autoimmune diseases, however, occur also more often after other vaccines. Overall, the HPV vaccines appear to be well-tolerated.

There are a few discussion points to note in our review. First, we did not double check each other's selection and critical appraisal: this could result in selection bias. It was impossible to do this differently due to time limitation. Secondly, we did not include the article if the full text was not available and we also excluded articles based on language. This can cause selection bias as well. During data analysis, the data is analysed as one group and no subgroups were made. Possible subgroups were males and females. A distinction between different age groups or seropositive and seronegative could also have been made. This is shown in another review [53], which finds a difference in efficacy between naïve girls and women previously infected; for these women the vaccine was not effective.

We only found two studies examining Gardasil 9, which only measured at month seven, and therefore we cannot draw any conclusions about this vaccine type. Another point of discussion is our effectiveness outcome. We described antibody titers, assuming that high antibody titers prevent an HPV infection. The studies had an average follow-up period of 5-6 years. Ideally, we would look at the development of dysplasia or cancer to describe the outcome of effectiveness, which would require a follow-up period of at least 10 years. This was not yet possible as a consequence of the latency period.

Conclusion

We conclude that Cervarix is effective against HPV16 and 18, but Gardasil only seems to be effective against HPV16. We recommend further research to find out why Gardasil seems less effective than Cervarix and not

effective at all against HPV18. Also, additional research should be done in subgroups. The HPV vaccines are well-tolerated and thus considered safe. Accepting your child's invitation for the vaccination is advised. More research regarding the long term effectiveness, especially the incidence of cervical cancer, and safety of the vaccines is recommended.

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BIG, BIGGER, BIG DATA

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Introduction

In August, the VU University Medical Centre Amsterdam and the OLVG got huge media coverage after they announced using Big Data in treating patients with sepsis. Last April, IBM finished the €2.4 billion acquisition of Truven Health Analytics, a company that specializes in healthcare data management. Truven gives IBM data on hundreds of types of costs, quality and outcome information derived from healthcare institutions all across the United States. Both examples indicate that the future of scientific research lies in analyzing large datasets. Especially when nearly everyone nowadays has all kinds of equipment that monitors health status, like iPhones with Healthkit, smartwatches and blood pressure monitors.

We were curious what is done with these developments in Radboudumc and decided to talk to Marleen van Gelder and Marion Biermans about this matter. Marleen van Gelder works as Assistant Professor in Reproductive Pharmacoepidemiology for the Department for Health Evidence. She is the scientific coordinator of the PRIDE (PRegnancy and Infant DEvelopment) study [1], a long-term study among pregnant women that focuses on short-term and long-term effects of several factors on the health of mother and child. This study has already lead to multiple publications. Furthermore, Marleen van Gelder works for Radboud REshape Innovation Centre that developed REach which we will discuss later. Marion Biermans has set up the Technology Centre for Electronic Health Record-based research [2]. She is currently working as Research Program Leader Vascular Damage at the Department of Primary and Community Care and as a Behavioural Scientist at the Postgraduate Training in General Practice. She promoted on the subject of using data from EHRs (Electronic Health Records) for epidemiologic research.

Could you tell us about your recent work?

Marleen: We started with the preparations for the PRIDE Study in 2008. It took some time to get permission from the Medical Ethical Committee, because it was one of the first studies that would make use of web-based questionnaires. The committee wanted to make sure that the data was stored securely and would not end up being additional burden on participants. How does the study work? Pregnant women receive a flyer with information about the study from their midwife or gynaecologist. They can visit the website to register after which they will be able to access the questionnaires to participate in the study. The web-based questionnaires have some unique aspects compared to paper ones. Since drug use is one of the priority exposures, it is vital to receive information about all medications women took during their pregnancy. Respondents tend to underreport their use of medication, that is why we included modules that ask whether the respondent had a certain medical indication during the pregnancy. If she did, a pop-up appears showing some of the most commonly used medications for that indication. By now we have included more than 4,000 women. This data is currently used by several interns and PhD candidates who are looking into the use of contraceptives, occurrence of depression, and diet in relation to pregnancy outcomes. Although we aim for an inclusion of around 100,000 women, we can certainly be glad with the number of participants there have been so far.

Marion: First, let me tell you something about the general practices we work with. At this moment we can extract data from practically all EHR systems in general practice, and have data available from more than 200 practices, which accounts for around a million patients. That is huge. This data is used by many (bio)medical students for their scientific internship. An example of a large study that we are currently working on is the PAGODE study [3]. This study focuses on the question whether general practitioners should screen for primary hyperaldosteronism: a condition that causes the inflicted to have a higher chance of cardiovascular diseases because of elevated aldosterone levels. We included around 60 practices and extracted data from their EHRs. The participating general practitioners invited patients with a new diagnosis of high blood pressure to take part in the study and measured their aldosterone and renin levels. If the aldosterone-to-renin ratio turned out to be elevated, a patient was referred to Radboudumc for further diagnostic workup. This is a wonderful example of transmural research.

Another research line focuses on chronic kidney disease. In the CONTACT study [4], we extracted data from 47 practices, providing an overview of the quality of care for this group of patients. General practitioners documented chronic kidney disease in only a third of the patients with this disease. The rest was not diagnosed as chronic kidney disease patient. It is clear that the quality of care for these patients in primary care can be improved.

Those results also point out a weak spot in using patient records for data mining: the issue of underdiagnosis. What are the advantages of using EHRs?

Marion: One of the major advantages of EHRs is that you can keep a helicopter view. We did research on patients with diabetes in primary care [5]. We found that 85% of all patients had at least one chronic comorbid disease. We found high prevalence and incidence density rates for both concordant and discordant comorbidity. The latter may interfere with diabetes management. I believe that you would not have had this result with a clinical trial. Clinical trials require researchers to have a narrower focus.

The biggest advantage of Big Data is obviously the scale. We studied whether gout is an independent risk factor for cardiovascular diseases [6]. At first, when data was used from 4 general practices, gout did not prove to be an independent determinant for the development of cardiovascular diseases. Later, we repeated the study but included far more patients. This time gout turned out to be a substantial and independent risk factor for cardiovascular diseases (hazard ratio 1.44). I believe that the difference in outcome had everything to do with the number of included patients.

Are there also disadvantages of using EHRs?

Marion: You have already mentioned the issue of underdiagnosis. When certain information is not documented, you miss data. This can cause

you to draw a wrong conclusion. Since the data is observational and collected in the daily practice, the quality of data will be lower compared to data from an RCT. Choosing your data sources carefully and deciding what to do with missing data are both essential for drawing useful and valid conclusions.

What are the recent technological advances in the field of big data?

Marleen: With REshape we developed REach. It is a platform that enables patients and researchers to compose digital questionnaires. These questionnaires can be integrated into apps, which can be found in the app store. Patients or healthy individuals can download this app and contribute to research in that way. Another great aspect of this platform is that it can extract data from the patient's phone after giving consent. HealthKit is a function on iPhone which can store all sorts of health information. An individual can allow REach to retrieve information from HealthKit and make this available to a third party such as a research group. This method uses Apple's ResearchKit, an open source framework that allows researchers and developers to create apps for medical research. This has already proven to be very successful: researchers in the United States included more participants by using such an application in a couple of hours than they had before in months using traditional recruiting methods. That was a real eye-opener!

For the time being we have only worked with Apple because iOS made it easier to develop REach. In addition, Radboudumc was the first University Medical Centre (UMC) from outside the United States to have been asked by Apple to conspire on the topic of 'health'. That was announced on the Worldwide Developers Conference in 2014.

Marion: I think that using a TTP (Trusted Third Party) to combine datasets can be really helpful. Linking datasets from primary and secondary care would certainly give us the possibility to gain edge scientific knowledge. To protect the privacy of patients, researchers have no access to identifiable patient data from EHRs, such as names, addresses and citizen service numbers, which are necessary for linkage of data. Instead, researchers can invoke a TTP to perform this linkage for them. This solution is generally accepted as "state of the art" to process and link data in a safe way which is compliant to privacy guidelines. The downside is that a TTP can be quite expensive.

Using data collected by commercial parties might increase the amount of analyzable data enormously. If possible, would you think the use of such data to be ethically sound?

Marleen: I do not know if tech companies have direct access to consumers' health data, but some app developers do. As a researcher I would not use data without the explicit permission from concerning individuals. We have thought long and hard on safety when we developed REach. That is also one of the reasons why REach is only available on iPhone. HealthKit data are only stored locally on the iPhone, not in the cloud.

Marion: I would not want to collect data in such a way either. We do not sell or buy data. Being trusted by doctors and patients is vital to a researcher. Unfortunately, the current regulations assume that researchers want to know about individuals. That is not true. I am only interested in the scientific yield of large scale data sets. One should not worry too much about researchers peeking into an individual's data. If a researcher would make personal data of a famous individual publicly available without their permission, the researcher's career would be over. I think the major risk is that databases would be hacked by criminals. Digital security is an important issue.

What should we expect from the future?

Marleen: We would first like to expand REach and make it compatible with other operating systems such as Android. The PRIDE study database will be enriched with data collected from other sources, such as EHRs and Perined, a foundation committed to the improvement of perinatal care. In addition to that, within the PRIDE Study, we try to include variables that were not primarily measured for research purposes. You could think of data on air pollution and e-cigarettes. The study will generate large amounts of data. We can use these to look for correlations and test hypotheses. In the future we could also try to generate hypotheses instead of just testing hypotheses.

Marion: I really advocate that Radboudumc sets up a TTP construction in the near future, which would permit combining data extracted from EHRs in general practice and data from Epic. This would offer the unique possibility to follow patients from the onset of symptoms in primary care to the subsequent diagnosis and treatment in secondary care. That could give great insights. It would be even more valuable if patients could transfer health data from their phones or smartwatches to their EHR. Such options would enormously expand the amount of information that could be analyzed.

Another major development is data pooling. Every UMC has a network of general practices affiliated to them from which they extract data. Sharing this data provides great opportunities for research. Especially if you want to investigate rare diseases, data pooling is essential for obtaining data on a sufficient number of patients.

Conclusion

Analyzing Big Data has already proven itself to be an invaluable method to find new correlations and test hypotheses. Despite some shortcomings, Big Data has the potential to grow in its possible applications as well as in the amount of data combined and analyzed. Implementation of platforms and constructions as REach and TTP might play an important role in expanding the amount of useful data acquired. Hopefully, researchers from Radboudumc will soon be able to combine EHRs from primary care with data from Epic to gain new, profound insights.

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HYDROGEN CYANIDE EMISSION BY MONO- AND CO-CULTURES OF *PSEUDOMONAS AERUGINOSA* AND *ASPERGILLUS FUMIGATUS*

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ABSTRACT

BACKGROUND: Cystic fibrosis (CF) is often complicated by chronic respiratory infections and, inherently, lung damage. Chronic infection with *Pseudomonas aeruginosa* (PA) is common in CF-patients. Co-infection with both PA and *Aspergillus fumigatus* (AF) results in a significant reduction of lung function in CF-patients, and a higher rate of exacerbations and hospitalization compared to patients infected with only AF. Over the past decade, several studies have indicated hydrogen cyanide (HCN) as biomarker for PA infection in exhaled breath.
OBJECTIVE: To investigate whether AF can influence the production of HCN by PA in vitro, using laser-based photoacoustic spectroscopy
METHODS: Four PA strains and three AF strains were selected for this study. Different combinations of PA and AF strains were tested. A laser-based photoacoustic instrument was used to measure HCN levels. Production by co-cultures was compared with those of PA mono-cultures.
RESULTS: All PA strains produced HCN, but only the ATCC 27853 strain produced more HCN in co-culture with AF than in the mono-culture. Furthermore, in co-culture environment, commercially available strains seem to produce higher levels of HCN and in a shorter period of time compared to clinical isolates.
CONCLUSION: The presence of AF can increase or decrease the HCN production by PA strains investigated in this study. However, the mechanism is not clear. It can be speculated that these PA strains exhibit different virulence. *Aspergillus fumigatus* does not produce HCN, but it seems to be affected by the toxicity of HCN. In addition the growth of AF is impaired and therefore PA can overgrow AF.

WHAT'S KNOWN: Hydrogen cyanide could be used as a specific biomarker for early detection of infection with PA in patients with cystic fibrosis.

WHAT'S NEW: *Aspergillus fumigatus* affects the production of hydrogen cyanide by PA and is therefore a confounding factor in early detection of PA infection.

KEYWORDS: Cystic fibrosis, *Pseudomonas aeruginosa, Aspergillus fumigatus*, Co-cultures, Hydrogen cyanide, Laser-based photo-acoustic spectroscopy

Introduction

Cystic fibrosis (CF) is a systemic disease caused by a genetic mutation of which the deltaF508 (ΔF508) gene mutation is the most common variant. This defect leads to various phenotypes of impaired cellular chloride-channels in respiratory cells that cause mucus to become abnormally viscous and trap bacteria resulting in chronic respiratory infections and severe lung damage [1]. Determination of the pathogen causing a respiratory infection in CF-patients is currently based on cough swabs (which is often inconclusive), sputum cultures, or an invasive bronchoalveolar lavage (BAL) [2]. However, not all patients are able to perform an induced cough, and a BAL is an invasive procedure. Therefore, a non-invasive diagnostic test is desirable.

A common pathogen causing chronic respiratory infections in CF-patients is *Pseudomonas aeruginosa* (PA) [1, 3] and is classified as an opportunistic pathogen. This applies to CF-patients who have a local weakened immune system inside the lungs due to less effective mucus evacuation. Moreover most of the PA strains are multi-drug resistant which makes treatment more difficult [4, 5].

Over the past decade, several studies have suggested that hydrogen cyanide (HCN) is a biomarker for PA infection in exhaled breath [6-10]. The reason why cyanide is produced by PA is still unclear, but it might have a beneficial effect on the competition with other pathogens [8]. Cyanide is highly toxic and diffuses fast through tissue. It then irreversibly binds to terminal oxidases and inhibits aerobic respiration [8]. *Pseudomonas aeruginosa* itself seems to counter these toxic effects of cyanide by active detoxification mechanisms [8]. Furthermore, it has developed a respiratory chain with terminal oxidases insensitive to cyanide so the bacteria can still use aerobic respiration as an energy source [8].

Studies measuring HCN in vivo report lower levels compared to those reported in in vitro studies [7, 10]. Besides PA, other pathogens can be frequently found in sputum cultures of CF-patients, for example Burkholderia cepacia and *Aspergillus fumigatus* (AF) [8-10]. Burkholderia cepacia is proven to produce HCN and so far, HCN production has not been detected in AF cultures [8-11]. However, it is known that a co-infection with both PA and AF causes a significant reduction in lung function of CF-patients [9]. We hypothesize that this might be related to a higher HCN production by PA in the presence of AF, which leads to a greater amount of lung-tissue damage due to cyanide toxicity. Therefore, the aim of this study is to investigate whether AF influences the production of HCN by PA in vitro.

Methods

Four PA strains and three AF strains were selected for this study. Two commercially available PA strains (ATCC 27853 and ATCC 10145), and two clinical isolates from CF sputum samples (B11094822 & B11097119) were tested. All three AF strains were clinical isolates from sputum samples collected from CF-patients (AZN 8196, V152/81 and V154/62). Different combinations of AF and PA strains were tested (table 1).

Cultures

Pseudomonas aeruginosa was inoculated into 50 mL of Brain Heart Infusion (BHI) broth, a commonly used growth medium for bacteria, in a Erlenmeyer flask. The initial concentration of the PA was approximately 3.5x106 colony forming unit (CFU)/mL.

Aspergillus fumigatus was inoculated on Sabouraud dextrose agar supplemented with 0.02% chloramphenicol for 2 x 7 days at 37oC. Using a swab, the spores were collected and suspended into 5 mL of BHI broth plus 0.1% Tween 80 until a concentration of approximately 2.6x106 CFU/mL was reached. This was verified using a spectrophotometer measuring the transmission at 530 nm. Thereafter, the suspension was further diluted (10x) in BHI broth to 2.6x105 CFU/mL, in a silicon coated Erlenmeyer flask.

Co-cultures with both PA and AF were prepared from a culture of AF as stated above. *Pseudomonas aeruginosa* was added 15 hours after the inoculation of AF, because PA would overgrow the fungus if both pathogens are inoculated at the same time.

Headspace sampling

To measure HCN production in an Erlenmeyer flask, the air above the culture, on the bottom of the flask, needs to be collected. On top of each flask a glass T-piece (headspace) is placed to collect the air. The same setup was used as described by Neerincx et al. [9]. Briefly, all cultures were placed in an environmental chamber at 37 oCand continuously shaken on an orbital shaker at 100 revolutions per minute (rpm). All Erlenmeyer flasks were closed using a glass stopper which contained two Teflon valves to use as the inlet and outlet. A bacterial filter was placed on the inlet to prevent contamination of the samples. The headspaces of all cultures were constantly flushed with 3.5 L/h of clean air. Each experiment was performed in duplo.

Hydrogen cyanide measurements

Detection of HCN production from each culture was performed by a laser-based photoacoustic detector [9, 11, 12]. In short, headspace samples were led to an absorption cell where the HCN molecules interact with the laser beam. The laser beam was set at a specific wavelength, 3286.5 to 3288.0 cm−1 (3041.4–3042.8 nm), where the HCN molecules present absorption features. The light absorption results in expansion of the HCN molecules. The laser beam is switched on and off very rapidly, so that the gas inside the cell heats up and cools down, generating a periodical pressure change, i.e. sound waves. The amplitude of the sound wave is proportional to the concentration of the probed molecules and can be measured by a microphone. Using this system, a detection limit of 0.4 parts per billion volume (ppbv) can be reached for HCN. Calibration of the system is done with a standard mixture of 5 parts per million (ppm) HCN in nitrogen. In addition, a sterile BHI medium sample is used as a control. The headspace, from up to 6 Erlenmeyers at once, was measured within an automated system including electronic Teflon valves. Using this valve system, samples were analyzed over a period of time in equal conditions per experiment, by switching from one sample to another after approximately 12 minutes.

Data analysis

The data from the laser-setup was analyzed with a LabView program (Superfitting). Hydrogen cyanide, H2O, NH3 and CO2 all absorb light in the same wavelength region. Superfitting ensures that the measured HCN

Table 1: *Overview of combinations of mono- and co-cultures of Pseudomonas aeruginosa and Aspergillus fumigatus used in this study.*

Pseudomonas aeruginosa **Mono-cultures**	***Aspergillus fumigatus*** **Monocultures**	**Co-cultures**
PA ATCC 27853	AF AZN 8196	PA ATCC 27853 + AF AZN 8196
	AF V152/81	PA ATCC 27853 + AF V152/81
	AF V154/62	PA ATCC 27853 + AF V154/62
PA ATCC 10145	AF AZN 8196	PA ATCC 10145 + AF AZN 8196
PA B11094822	AF AZN 8196	PA B11094822 + AF AZN 8196
PA B11097119	AF AZN 8196	PA B11097119 + AF AZN 8196

PA: Pseudomonas aeruginosa
AF: Aspergillus fumigatus
Pseudomonas aeruginosa strains ATCC 27853 and 10145 are commercially available, the other two PA strains are clinical isolates collected from sputum samples of CF patients.
All AF strains are clinical isolates.

values are free of interference from other gases produced by the biological samples. To calculate the production rate of HCN, values in ppbv were multiplied with the airflow of 3.5 L/h and divided by 1,000 to obtain production rates of microliters per hour (µL/h). The area under the curve (AUC) was used to determine the total HCN production over 60 hours, starting at t=5h till t=65h after inoculation with PA since this period contained the high peak of HCN production for most samples. Due to technical errors of the laser setup, two separate experiments were performed with PA strain B11097119. Therefore two sets of data were available (data not shown). The AUCs for each separate set of PA B11097119 and an average of these AUCs was calculated.

Statistical analysis
The AUCs of each sample was calculated and averaged with the AUC of their corresponding duplicate measurement. Subsequently, the mean total production of mono- and co-cultures for each PA strain was compared with other strains, by using a Student's T-test for paired samples with SPSS 20.0.0. Differences of the means (d) with 95% confidence interval (95%-CI) were calculated and significance was described as a p-value below 0.05.

Results

In each experiment, containing two duplicate samples of a mono- and co-culture and a control sample, HCN production was measured.
All co-cultures of PA and AF started to produce HCN within 15 hours after inoculation of PA, with variation between strains. Production of HCN fell below detection limit at different points in time, although some strains still produced HCN at 85 hours after inoculation with PA.

An overview of the graphs for the mono-culture with PA strain ATCC 27853 and corresponding co-cultures of this strain is shown in figure 1. In all panels the mono-culture of AF is shown which does not produce any HCN. This figure shows an increase in HCN production by PA strain ATCC 27853 in co-culture, with an up to 8-fold increase in a co-culture with AF strain V152/81.

An overview of the graphs for co-cultures with AF strain AZN 8196 in combination with the commercially available PA strains ATCC 27853 and 10145 is shown in figure 2. In the graph the mono-culture of AF is shown which does not produce any HCN. The experiment with the clinical PA strains B11094822 and B11097119 showed lower HCN production values ranging from 0.2-0.5 µl/h (data not shown).

The results show that only the co-culture with AF AZN 8196 and PA ATCC 27853 had a higher mean HCN production compared to the mono-culture with the corresponding PA strain, but this was not significant (d= -4,00 [-32,48 - 24,48], p= 0.33). The co-culture with AF AZN 8196 and PA B11094822 differed significantly from the mono-culture of the same PA strain (d= 12,37 [4,60 - 20,13], p= 0.03).

A barplot of all mean AUCs per PA strain with their corresponding co-cultures is shown in figure 3. The barplot shows a graphical view of the difference in calculated mean AUCs and shows the influence of different AF strains on HCN production by different PA strains.

Discussion

In this study, we assessed the influence of AF on the in vitro HCN production of PA using laser-based photoacoustic spectroscopy as a proof of principle. Neerincx et al (2015) [9] have previously reported HCN production levels for mono-cultures of commercially available and clinical isolates of PA strains. In comparison with the study by Neerincx et al [9] the dynamics of the HCN production in this study are similar, but the absolute values of the peak height in HCN production are lower. There are some factors that can account for this difference. Firstly, the PA strains used in this study could produce less HCN due to biological variance. Nevertheless, the results may be of interest for further investigations. Secondly, it is known that cyanide is highly toxic and it might suppress growth of other pathogens, that are not able to withstand the toxic effect of cyanide [8, 11]. The levels of HCN produced by PA might even affect lung tissue, causing more damage which could result in the chronic PA seen in CF-patients [8]. To our knowledge, this is one of the few studies to combine PA and AF in a co-culture to examine the effect on HCN production by PA. According to recent studies by Chippendale et al (2014) [13] and Neerincx et al [9, 11], HCN is not produced by AF and can therefore be used as a biomarker for PA even if the patient is infected with AF.

To investigate the influence of AF on HCN production by PA in a wider range, more strains of PA and AF should be tested. The experimental conditions are different from in vivo conditions in patients, where multiple pathogens can be present inside their lungs. It could therefore be interesting to test multiple pathogens in different combinations with PA to examine their influence of HCN production. The results show no HCN production above detection limit after approximately 65 hours after inoculation with PA, a limitation of our study. It is likely that this decrease in HCN production is caused by depletion of nutrients of the medium inside the Erlenmeyer flask. A solution would be to have an infinite source of medium or to be able to add medium into the flask without disconnecting the tubes. This will maintain the same environment inside the Erlenmeyer. In spite of these limitations, the results seem to indicate an effect of AF on HCN production by PA. In relation to earlier studies [7-10], this study shows a real-time production of HCN by PA in a co-culture with AF. The results show a high, sharp peak in HCN concentration by PA strain ATCC 27853 in the co-cultures. However, the involved mechanism is not clear. A hypothesis could be that AF is affected by the toxicity of HCN produced by this strain of PA. Therefore, growth of AF is partially impaired. Another hypothesis would be that clinical isolates compete at a different rate with AF due to genetic variations and difference in virulence. As stated earlier, a co-infection with both pathogens results in a significantly lower lung function, which might be related to higher HCN production. In this study we included two clinical strains of PA which did not produce higher levels of HCN compared to commercial strains. To investigate whether virulence influences HCN production by PA, more clinical strains have to be tested and compared with HCN production by commercial strains.

Conclusion

We conclude that AF affects the HCN production by PA and that AF can increase or decrease HCN production. To investigate this phenomenon, further research is needed to assess the relationship between virulence and the capacity to produce HCN, and will require more combinations of strains of both microorganisms.

Acknowledgements
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Conflicts of interest
None.

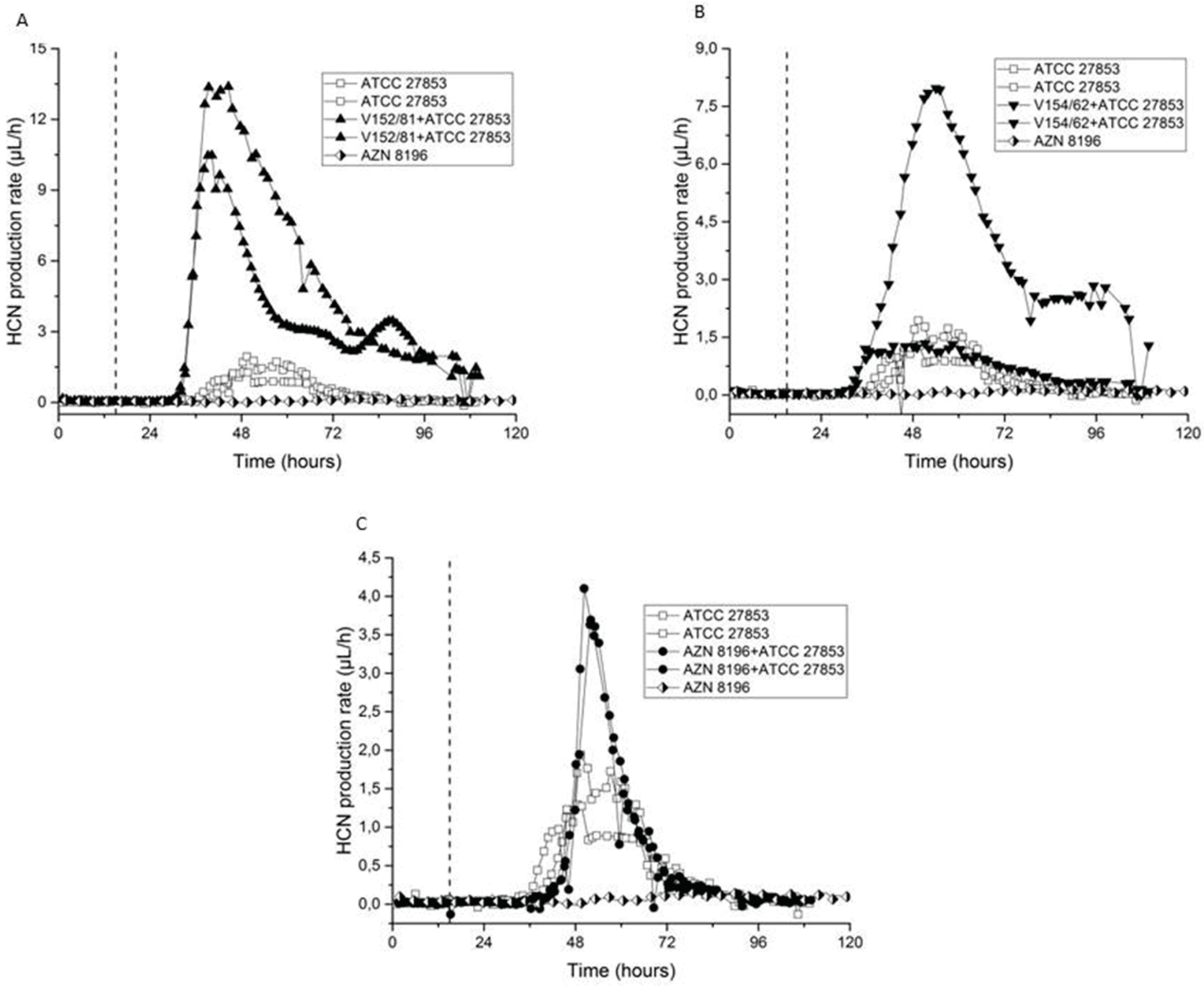

Figure 1: *Overview of Pseudomonas aeruginosa (PA) strain ATCC 27853 in combination with Aspergillus fumigatus (AF) strains V152/81 (A), V154/62 (B), AZN 8196 (C). Hydrogen cyanide (HCN) production rate in microliters per hour (µL/h) is plotted against time in hours. The dashed line represents time of inoculation with PA at 15 hours.*

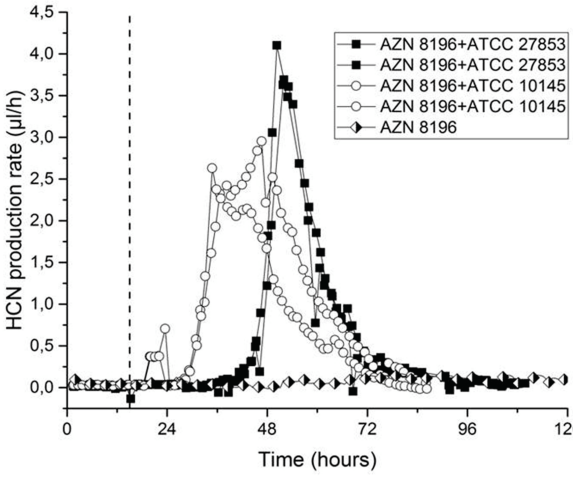

Figure 2: *Overview of Aspergillus fumigatus (AF) AZN 8196 in combination with commercially available Pseudomonas aeruginosa (PA) strains ATCC 27853 and ATCC 10145. Hydrogen cyanide (HCN) production in microliters per hour (µL/h) is plotted against time in hours. The dashed line represents time of inoculation with PA at 15 hours.*

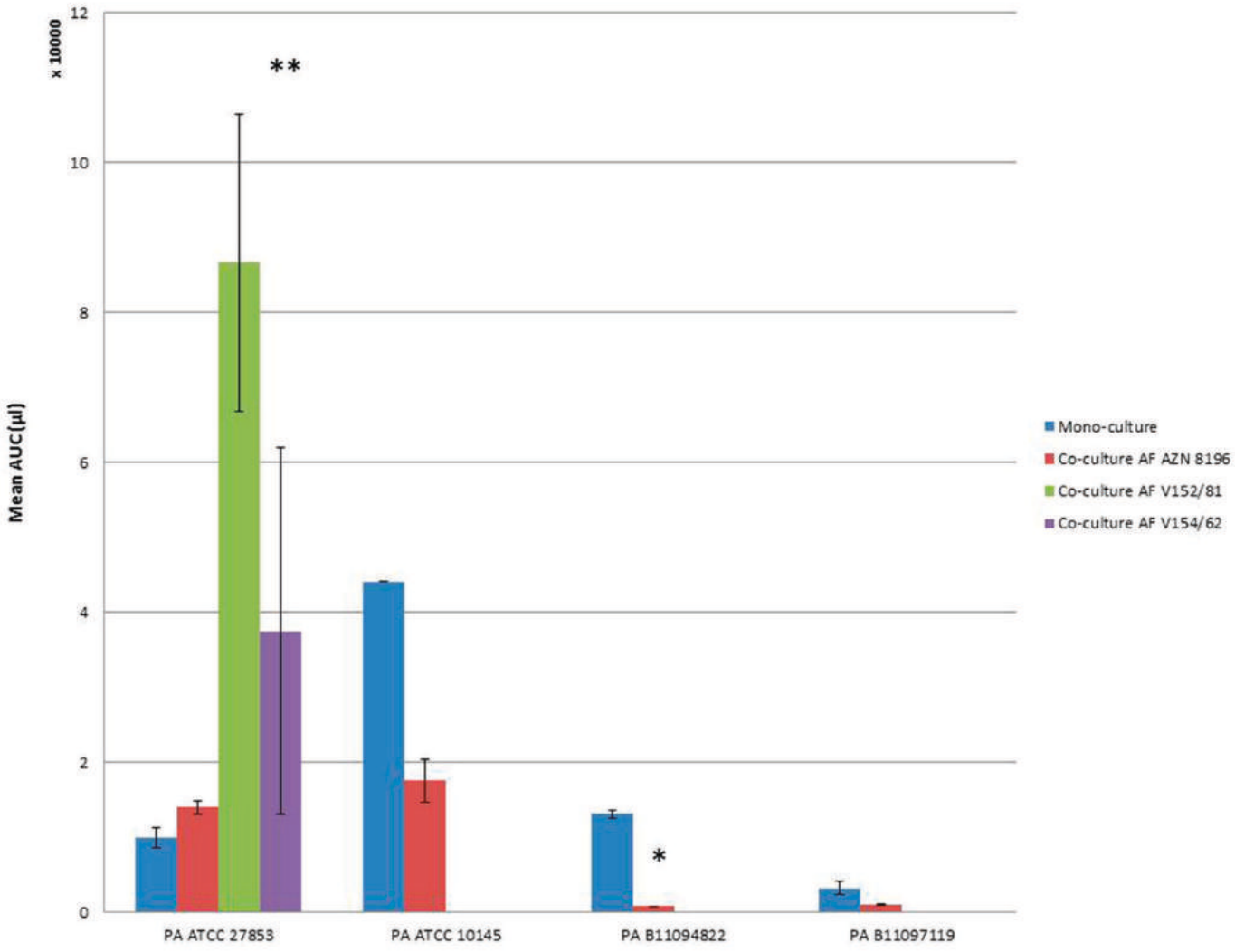

Figure 3: *Barplot of all mean area under the curve (AUC) per Pseudomonas aeruginosa (PA) strain with their corresponding co-cultures. The bars are shown with 95% confidence interval of the mean. *: significant difference (p <0.05) between mean AUC of the mono-culture of PA B11094822 and the corresponding co-culture with Aspergillus fumigatus (AF) AZN 8196. **: significant difference (p <0.10) between mean AUC of the co-culture of PA ATCC 27853 and AF V152/81 and the co-culture with PA ATCC 27852 and AF V154/62.*

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RECENT HIGH-IMPACT PAPERS PUBLISHED BY RESEARCHERS FROM RADBOUDUMC

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

Paternal versus maternal *de novo* mutations

It was already known that spermatocytes carry more mutations compared to oocytes. Spermatocytes constantly multiply during puberty, inevitably accompanied by mistakes during replication, whereas oocytes are already present from birth. As a result, spermatocytes develop one extra mutation per year, which is four times more than oocytes. Using whole-genome sequence data from 816 parent-offspring trios, researchers from the department of Human Genetics and colleagues in the USA investigated the parental origin of *de novo* mutations (DNMs) and the underlying mutational mechanisms. Goldmann et al. found differences between maternally and paternally derived mutations and these differences increase with paternal age. The number of maternally derived mutations also increases with maternal age, but to a lesser extent. Additionally, the researchers discovered genome regions that are enriched for maternally derived DNMs, and could thus be important for oogenesis and oocyte survival. [2]

New candidate genes for intellectual disability

DNMs are of particular interest in identifying the cause of certain diseases such as intellectual disability (ID). Using novel statistical analyses, researchers from the department of Human Genetics and colleagues in Groningen en Maastricht discovered ten new candidate genes causative for ID. Lelieveld et al. combined data from 820 patient-parent trios with previous research, which resulted in 2,104 individuals with 2,637 DNMs across 1,990 genes. Meta-analysis revealed ten genes that carry more DNMs than theoretically expected. Individuals with DNMs in these genes showed strong phenotypic overlap. The newly identified disease genes also proved to be 'intolerant', meaning that genetic variation in these genes often leads to ID. A similar analysis was performed on sequence data from patients with various neurodevelopmental disorders, including autism spectrum disorder, schizophrenia and epileptic encephalopathy. Interestingly, this analysis yielded five disease genes that had also been identified in the previous analysis of ID patients, of which two genes harboured additional mutations. The latter suggests that DNMs in these genes may be responsible for a broader phenotype than ID alone. [3]

The role of EHMT1 clarified

In 2006, researchers from Nijmegen - Kleefstra and Van Bokhoven - discovered the gene responsible for the Kleefstra syndrome: EHMT1. How mutations in this gene lead to the neurodevelopmental disorder remained unknown, however, until recently.

Normally, neurons constantly adapt their firing rate to preserve neural network stability. This can be achieved through synaptic scaling. Synaptic scaling refers to the process of balancing excitation and inhibition by negative feedback, in case of prolonged unidirectional neuronal activity. Although an epigenetic basis was already established, the exact modifications and mechanisms underlying synaptic scaling remained unknown. Researchers from the department of Cognitive Neuroscience, Human Genetics and Molecular Biology - under whom Kleefstra and Van Bokhoven themselves - now indicated a key role for the EHMT1 protein. The EHMT1 protein appears to contribute to an epigenetic repressive genetic program required for synaptic scaling. The discovery of this mechanism might aid in finding drugs that target the EHMT1 gene, or genes that are regulated by EHMT1. [4]

Infection risks: It's in your DNA

Hardly anything is known about the interindividual variation of cytokine responses to different pathogens in healthy individuals. Researchers from the department of Internal Medicine and the Radboud Center for Infectious Diseases, analysed peripheral blood mononuclear cells from 197 healthy volunteers, showing that the cytokine response is dependent on the individual and also on the type of pathogen. Genetic analysis revealed relationships between genetic variations and cytokine responses to a particular pathogen. For example, variations in GOLM1 were associated with higher susceptibility to candidemia through modulated IL-6 production. In the future, genetic information might thus be used to predict an individual's response to a specific pathogen. Eventually, uncovering the molecular link between genetic variations and an increased susceptibility to infection could aid in the development of drugs to strengthen immunity. [5]

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RAMS

A Word from the Board of RAMS

Dear reader,

First of all, I would like to thank you for holding your attention to this very last page. In the fifth edition our just retired chairman Lars Gallée paid tribute to the editorial board and general board of RAMS 2015-2016. As the former vice-chairman and current chairman of RAMS, I am very proud to pronounce that the new editorial and general board have made a great start in continuing and improving all RAMS activities.

It is important to emphasize the word 'activities', as RAMS organizes more than solely the editing and management of a scientific medical journal. The RAMS Summer School, RAMS Symposium and RAMS Masterclasses are the best examples of these activities, in which science is the common denominator. Our goal is to enthuse students about science in general and to stimulate them to participate in research during their studies.

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Bas Vreugdenhil
Chair RAMS 2016-2017

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