FACULTY OF MEDICINE AND HEALTH SCIENCES

The microbiome in relation to cancer risks

A systematic review on non-gut microbiome

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THE MICROBIOME IN RELATION TO CANCER RISKS

ABSTRACT

Background
The human microbiome is a complex and dynamic entity situated inside the human body and outside. It consists of many different microbiota with a diverse range of functions (for instance in nutrition and digestion, production of metabolites, development of the immune system etc.) but some of these effects might also be related to the development of cancer. This link might offer new opportunities for cancer screening, diagnosis and maybe even cancer treatment.

Objectives
To assess the relation between several non-gut microbiomes and the risk of cancer.

Search methods and selection criteria
Relevant articles were searched up until the third of November 2016 in the following databases: PubMed, EMBASE and the Cochrane Library. All case control studies, cohort studies and RCT's who were written in English and investigating a possible link between a non-gut microbiome and cancer were included. Studies with children as subjects, in vitro studies and studies with animals were excluded from this review.

Data collection and analysis
Two investigators independently selected titles and abstracts from the bibliography retrieved by the search strategy according to inclusion and exclusion criteria. When needed, the opinion of a third independent person was requested. Also the quality of the included articles was assessed with the Newcastle – Ottawa quality assessment scale.

Main results
A total of thirty-one articles were included. Thirty studies were case controls, including one nested case control study, and one was a cohort study. The year of publication ranged from 1982 to 2016 and the origin of the publications was very divers, from all over the world. The included studies investigated the following microbiomes: the bile duct microbiome, the cervical and intrauterine microbiome, the esophagus and gastric microbiome, the laryngeal microbiome, the lung

microbiome, the oral microbiome, the skin microbiome and the urine microbiome. The oral microbiome was the most studied.

All of the articles, besides one article of 1983, showed certain differences between the cases and the controls. These were differences either in the presence of certain microbiota or in their abundances. While some of these findings were consistent across certain studies, many differences in results were noticed as well since various known and unknown factors influence the microbiome (for instance ethnicity, smoking behavior, food intake and habits but also the stage of the cancer, the type of cancer etc.). For some bacteria an association with cancer was very likely, while for others this was only suggestive.

The most relevant and consistent outcomes were the following. Two studies investigating the cervical microbiome both found a decrease of *Lactobacillus crispatus* in the cervical cancer cases. This bacteria is considered as a beneficial organism that helps sustaining the healthy microbiome by various mechanisms. Next, of all the articles studying the oral microbiome, five articles observed a changed abundance of *Streptococcus* in the cases with oral cancer, four of them observed a changed abundance of *Prevotella* and two of them an increase in *Rothia*. There is not much known of this latter bacteria, but *Streptococcus* and *Prevotella* have been studied previously. These two are thought to play an antagonistic role against each other thus while *Streptococcus* is decreasing during tumor development, the anaerobic *Prevotella* can grow effortlessly at the tumor site. Furthermore, two articles both noticed a decrease of *Neisseria* in the oral microbiome of pancreatic cancer cases and this decrease was also found in the oral cancer cases from another study. At last, three studies investigated the tongue coating images and samples of patients with several different cancers (colorectal cancer, lung cancer and gastric cancer), yet in all of them a decrease in *Neisseria* again and *Haemophilus* was seen. However, their mechanisms of cancer initiation or development remain unclear.

Conclusion

No clear and confirmed new relation between a certain microbiota and cancer was found, but many possible relations were observed. Thus, further research with newer techniques and a more uniform sample collection is required to establish such a possible link. Cancer-microbe causality remains a great challenge here. Even when there is no causal link between a bacteria and a certain cancer, those microbiota might still be useful as a tool for adjuvant treatment as well as a biomarker for screening, diagnosis or for those most at risk for treatment-related complications.

ABSTRACT (Dutch version)

Achtergrond

Het menselijk microbioom is een complex en dynamisch geheel gelokaliseerd binnenin en op het menselijk lichaam. Het bestaat uit vele verschillende microbiota met diverse functies (bijvoorbeeld voeding en vertering, productie van metabolieten, ontwikkeling van het immuunsysteem enzoverder) maar sommige van deze effecten zouden eveneens bijdragen tot de ontwikkeling van kanker. Dit verband zou nieuwe mogelijkheden kunnen bieden voor de screening van kanker, evenals de diagnose en eventueel zelfs de behandeling.

Doel

Het verband tussen verschillende microbiomen (behalve het darm microbioom) en het risico op kanker beoordelen.

Zoekmethode en selectie criteria

Relevante artikels werden opgezocht tot drie november 2016 in de volgende databasen: PubMed, EMBASE en de Cochrane Library. Alle case controle studies, cohorte studies en RCT's die geschreven zijn in Engels en een mogelijk verband onderzochten tussen het microbioom (met uitzondering van het darm microbioom) en kanker, werden geïncludeerd. Studies met kinderen als proefpersonen, in vitro studies en studies op dieren werden uitgesloten van deze review.

Data collectie en analyse

Twee onderzoekers selecteerden elk afzonderlijk titels en abstracts van de bibliografie die verkregen werd met de zoekstrategie volgens de in- en exclusiecriteria. Indien nodig, werd de opinie van een derde onafhankelijke persoon gevraagd. Eveneens werd de kwaliteit van de geïncludeerde artikels beoordeeld met de Newcastle – Ottawa quality assessment scale.

Belangrijkste resultaten

Een totaal van éénendertig artikels werden geïncludeerd. Dertig waren case controle studies, waarvan één een nested case controle, en één was een cohorte studie. Het publicatiejaar van de artikels reikte van 1982 tot 2016 en ook de afkomst was heel divers, van over de hele wereld. De geïncludeerde studies bestudeerden de volgende microbiomen: het microbioom van de galwegen, het cervicaal en intra-uteriene microbioom, het slokdarm en maag microbioom, het laryngaal microbioom, het long microbioom, het oraal microbioom, het microbioom van de huid en het urinair microbioom. Het oraal microbioom was het meest bestudeerd.

Alle artikels, behalve één artikel uit 1983, toonden significante verschillen tussen de cases en de controles. Het ging om verschillen in de aanwezigheid van bepaalde micro-organisme of verschillen in hun aantallen. Terwijl sommige bevindingen vrij consistent waren doorheen de studies, werden er toch eveneens veel verschillende resultaten waargenomen doordat vele gekende en ongekende factoren het microbioom kunnen beïnvloeden (zoals etniciteit, rookgedrag, voedselinname en gewoontes, maar ook het stadium van de kanker, het type kanker etc.). Voor sommige bacteriën was een verband met kanker zeer waarschijnlijk, terwijl het voor andere louter suggestief was.
De meest relevante en consistente bevindingen waren de volgende. Twee studies die het cervicaal microbioom onderzochten, ontdekten beiden een afname van *Lactobacillus crispatus* in de cases met cervix kanker. Deze bacterie wordt gezien als een voordelig en gunstig micro-organisme dat bijdraagt tot een gezond microbioom op verschillende manieren. Vervolgens, van alle artikels die het oraal microbioom bestudeerden, bemerkten vijf studies een verandering in het aantal van *Streptococcus spp*., vier ervan merkten een verandering in het aantal van *Prevotella spp*. en twee ervan een toename in *Rothia*. Over deze laatste bacteria is er weinig gekend, maar *Streptococcus* en *Prevotella* werden wel reeds bestudeerd. Vermoedelijk spelen deze twee bacteriën een antagonistische rol ten opzichte van elkaar, dus wanneer *Streptococcus* afneemt ten gevolge van carcinogenesis, kan de anaerobe *Prevotella* probleemloos verder prolifereren in de omgeving van de tumor. Verder hebben twee artikels een afname in *Neisseria* waargenomen in het orale microbioom van patiënten met pancreaskanker en deze afname was ook opgemerkt in de patiënten met een orale tumor van een andere studie. Tot slot, drie studies onderzochten in patiënten met verschillende tumoren (colorectale tumor, long tumor en maag tumor) het uitzicht van de tong en de stalen hiervan, en opnieuw werd een afname van *Neisseria* alsook een afname van *Haemophilus* waargenomen. Echter, het mechanisme van deze bacteriën dat aanleiding zou kunnen geven tot het ontstaan of het verder ontwikkelen van kanker blijft vaag.

Conclusie

Er werd geen duidelijk en overtuigend nieuw verband vastgesteld tussen een bepaald micro-organisme en een kanker, maar vele mogelijke verbanden werden wel opgemerkt. Aldus, verder onderzoek met de nieuwere technieken en met een meer uniforme verzameling van de stalen is nodig om zo een verband te kunnen vaststellen. De causaliteit tussen een microbe en kanker aantonen blijft een uitdagende zaak. Zelfs wanneer er geen causaal verband aanwezig zou zijn, kunnen deze microbiota mogelijks nuttig zijn als een hulpmiddel voor adjuvante therapie alsook als biomerker voor screening, diagnose en voor zij met het hoogste risico op bijwerkingen van behandeling.

INTRODUCTION

The microbiome and human health

The human microbiome includes the collective genome of all bacteria, viruses, archaea, fungi and protists found in and on the human body [1]. It is quite a well-defined habitat that has its own distinct physicochemical characteristics and it has gained worldwide interest during the last few years [2]. Since the development of high-throughput approaches using next-generation sequencing and 16S ribosomal RNA or DNA sequence reads, the knowledge and insights concerning the human microbiota, even the nonculturable microorganisms, has increased enormously [3, 4]. But also the complexity of the human microbiome became revealed. The human body harbors over 10^{14} microbial cells and the number of microbial genes is over 100 times higher than the number of human genes [3, 5]. Especially the gut microbiome has become well known but microorganisms inhabit all the barrier surfaces of the human body including the skin, the oral cavity, the nasopharynx, the esophagus and stomach, but also the vagina, the urinary tract and the lung [5].

The many functions of the microbiome demonstrate its importance: it plays a role in regulating nutrition and digestion, in metabolism and the production of high energy metabolites, detoxification, development and function of the innate and adaptive immune system, inflammation and homeostasis [5, 6]. Some authors consider the human microbiome another “organ”, while others call the human being together with its microbiome one metaorganism [5, 6]. But not only the presence of those microorganisms is essential for the human health, also the composition of microbiota is crucial [6]. That composition varies depending the anatomical site in or on the human body and it is very divers between individuals [3]. The microbiota form a dynamic entity and can change within an individual in response to diet and other external factors such as medication, environment and lifestyle [3, 6].

This microbiota diversity or the lack of, is related with health and disease [6]. An altered microbiome linked to a disease, has been named dysbiosis [1]. This microbial imbalance may play critical roles in diseases such as increased susceptibility to infection, autoimmune conditions and cancer [5]. For example, microbes can cause an infection in a direct way by attacking our human cells and targeting our immune system in various ways, just like *Helicobacter Pylori* causes gastritis [6]. But they might also be part of an indirect cause of infection, for example an acute upper respiratory tract infection with rhinovirus can alter the microbiome in such a way that it is suggested that the change of composition leads to an increased susceptibility to infections elsewhere in the respiratory tract such as otitis media and pneumonia [2].

This latter shows that although the gut microbiome might be the most studied and most often linked with diseases, also microbiomes elsewhere can be pathogenic. Formerly the lungs were considered a sterile site, but since the development of the new culture independent techniques, they seem inhabited by relatively diverse microbes [2]. Recently is found that the lungs of patents with cystic fibrosis is chronically colonized with pathogenic organisms and much more diverse than previously expected. Further research is necessary but it is reasonable to assume that the microbe interactions in this environment might be as important as the interactions within the gastrointestinal tract [2].

Furthermore, multiple studies show that in patients with inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, the gut microbiome is different than in patient without IBD. There is not one microbial pathogen in these diseases, but the intestinal microbiome itself has been considered to be pathogenic and contributing to the dysregulated inflammatory response in predisposed hosts [2]. Besides the role of the microbiome, there is also a genetic susceptibility for the development of IBD. Thus, IBD is a truly microbiome related disease because both host and microbe and thus also their interactions with each other are altered in this condition. This mechanism might not only lead to IBD in the gut, but to divers cancers anywhere in the body [2].

From microbiota to cancer

Cancer is a worldwide leading cause of death. It is associated with a tremendous social and economic burden [3]. Moreover, the number of cases is likely to increase because of the obesity epidemic and the aging of the population. Luckily, the therapeutic options are also improving thanks to targeted therapy such as imatinib and trastuzumab [3]. But these therapies are not suited for every cancer and not for every patient. The majority of cancer are still treated with conventional chemotherapeutics with varying degrees of efficacy and adverse effects [3]. Therefore, cancer prevention is a major topic. And the microbiome might play an important role in it, as well as in expanding or ameliorating the therapeutic options [1-3].

The barriers of the human body are constantly subjected to environmental insults and injuries. In most individuals, these infections, trauma and mutations are rapidly repaired and so the homeostasis is restored. However, when the host is impaired or a virulent microorganism is present, this may lead to persistent barrier breach and a failure to restore homeostasis [7]. This barrier breach doesn't has to be physical, it can also be an alternation of the barrier permeability or a physiological communication of the organism and the microbiome trough an intact membrane [5]. In that situation of barrier break down, there are three broad categories of how microbiota contribute to carcinogenesis: by altering the balance of host cell proliferation and cell death; by guiding the

function of the immune system; and by influencing the metabolism of host-produced factors, ingested foods and pharmaceuticals [7]. For example, *Helicobacter pylori* produces proteins called cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) which interact with the β catenin pathway and so activates cell proliferation, survival, migration and angiogenesis. All these processes are central in carcinogenesis [6, 7]. *Bacteroides fragilis* can have a similar effect by the production of *Bacteroides fragilis* toxin (Bft) that also stimulates the β catenin pathway, but on top of that Bft elicits high levels of reactive oxygen species (ROS). This ROS damages the host DNA and can overtake the DNA damage repair system leading to persistent DNA damage and mutations. This DNA instability can be an important part of carcinogenesis [3, 7]. Furthermore the virulence factors CagA and VacA of *Helicobacter pylori* activate the NF-κB pathway, the master regulator of cancer associated inflammation, leading to host inflammatory responses [6, 7]. At last *Butyrivibrio fibrisolvens* forms an example of the third category, but in a positive way. This bacteria is able to ferment fibers into butyrate, which functions as a tumor-suppressive metabolite by inhibiting histon deacetylase in cancerous colonocytes [3].

These examples take place at the gut, but the principles count for any barrier in the body. When there is a barrier breakdown, there is a loss of the intended symbiosis and microbes can damage the human cells and influence the human immune responses in a pro-inflammatory or tumor suppressive way [7]. This loss of appropriate boundaries is a critical step in the development of certain tumors [7]. For example, the oral microbiome has the capacity to convert ethanol to acetaldehyde after alcohol consumption. This acetaldehyde is a genotoxic and human carcinogen, and plays an potential role in oral and gastrointestinal carcinogenesis when it affects the DNA of the host cells [4].

Furthermore, the effects of this barrier breakdown don't remain local. Especially the oral microbiome is linked with distant cancers such as lung cancer and pancreatic cancer due to systemic effects [1, 4]. After mastication, tooth-brushing and dental procedures oral bacteria appear in the systemic blood circulation and can provide a source of ligands for toll-like receptors, which in turn leads to activation of the immune system and inflammation [4].

This inflammation might play a major role in the pathogenesis of cancer [6]. About 10-20% of all cancers has an involvement of microorganisms and up to 20% of the cancers is preceded by a chronic inflammation [6, 7]. Chronic inflammation with his many cytokines and growth factors not only promotes tumor development, it also creates a tumor microenvironment which influences tumor progression and metastasis [6]. Now recently, it is known that the microbiome is an essential regulator of inflammatory responses [6]. For example, as mentioned above, *Helicobacter pylori* activates the NF-κB pathway by CagA and VacA, but many microbes associated with cancer,

appear to activate this pathway. This occurs by signaling through pattern recognition receptors such as toll-like receptors (TLR) and nucleotide-binding oligomerization domain-like receptors (NOD) [7]. This activation leads to the production and release of many cytokines (Il-6, IL-8, IL-17, IL-22, etc.) and growth factors (TNF, GM-CSF, CSF-1, etc.) that recruit and activate many inflammatory cells (myeloid cell, T-helper cells, fibroblasts, etc.). Especially the Th1 and Th17 inflammatory response would be important for the development of cancer [6]. So, when the human barriers are breached, the microbiome damages the host cells and can trigger immune responses that are able to promote cancer development and progression. These cancer cells in turn can also produce pro-inflammatory cytokines and chemokines activating pro-inflammatory cells, which maintains the pro-tumorigenic environment [6].

Besides the effect of the microbiome, there is a consensus that high intake of saturated fats and obesity increases the cancer risk. Obesity seems to be an inflammatory state and the combination of obesity, inflammation and the microbiota might be an in inseparable trio that fuels cancer [7].

The obstacles in the microbiome studies

The number of epidemiologic studies that associate the human microbiome and cancer is rising, however it is challenging to obtain hard evidence. First, it stays difficult to determine the cancer-microbe causality [1, 7]. When a bacteria is enriched at a tumor site, there are other possible reasons for that association besides a causal relation. For example, the microbe can take advantage of the tumor's oxygen tension or carbon sources, or find an underused nutritional niche where they can grow freely [7]. Does the carcinogenic process changes the local environment and creates new niches for microbes, or does an alternation of the microbial composition and its function contributes to the carcinogenesis? Reverse causation is a great concern [1]. Another difficulty is that different microbes might contribute at the different stages of the carcinogenesis [3, 5, 7]. In the initial stage, the microbiome can lead to genetic mutations and chronic inflammation, but they are also involved in creating other tumor-promoting environments as obesity and the metabolic syndrome [5]. Afterwards, other microbiota can be responsible for the tumor growth, angiogenesis and metastasis [5, 7]. Since cancer development is a process that takes many years, it is possible that by the time the cancer is diagnosed, the initial causal microbe might already have passed because of the later-stage tumor environment [3, 7]. On top of that, there is more than only the 'one microbe-one disease' neoplasms [3]. It is well known that *H. pylori* leads to gastric cancer and there are 9 other microorganisms that are designated by the International Agency for Cancer Research to be carcinogenic to humans [3, 7]. But some microbes might have modest and subtle contributions to the cancer development, what will make

it harder to detect them. It is also likely that some of those modest contributions depend on the genetic background of the host including polymorphisms, sex and age; as well as other factors such as smoking, alcohol consumption, diet and physical activity [1, 3].

Gnotobiotic facilities, where germfree animals are raised and carefully colonized by human-derived microbiota in a rigorously controlled manner, might provide a solution to some of the mentioned difficulties. Of course they have the disadvantage that humans cannot be used in this type of experiments, but they may offer insights in the chronologic role of microbes in the carcinogenesis [3]. These insights in the interactions inside the microbiome and between the microbiome and the human body might completely change the way we prevent and treat cancer today [1, 3-7].

Microbiota as therapeutic target

The insights and knowledge concerning the human microbiome has already led to new therapeutic options. First of all, there are the prebiotics and probiotics. Prebiotics are defined as indigestible food ingredients that selectively stimulate the growth and/or activity of certain gut microbiota that confer a health benefit. Most of them are carbohydrates that can only be metabolized by specific members of the microbiome [2, 3]. For example the dietary fiber inulin, that promotes the growth of Bifidobacteria, is already implicated in cancer prevention [3]. Because such a therapy presumes that the needed microbiota are present, the 'synbiotic' was created. This contains both the relevant microorganism and the prebiotic carbohydrate [2].

Probiotics on the other hand are living organisms present in certain food or dietary supplements that also confer a health benefit in adequate amounts [2, 3]. The best know is probably Lactobacilli from yoghurt. There is not much evidence for cancer prevention, but it is used for many other health outcomes such as improved digestion [3]. Improving the probiotics by engineering their DNA might lead to stronger beneficial effects. For example, *Lactobacillus acidophilus* is a beneficial bacteria, but it also has pro-inflammatory effects through activation of TLR and cytokine production. A mice study showed that when this bacteria was engineered by a deletion in the DNA strain responsible for the inflammatory reaction and then dosed to mice with colonic polyps, it resulted in a regression of the polyps [3]. This illustrates the possible impact of a simple oral intake of probiotics. Also antibiotics affect the microbiome and they have shown to decrease the tumor burden in certain mouse models. But since they kill commensal bacteria and support the antibiotic-resistant problem, they are not (yet) good candidates for the purpose of chemoprevention [3]. Although new and more specific antibiotics are being developed with less microbiota disrupting potential (like fidaxomicin for *Clostridium difficile* infection), our current elementary understanding of the structure-function relations of the microbiome leads to a lack of precision in this approach [2]. Instead, it would be

better to maintain or restore the beneficial microbial composition. This is the basis for fecal microbiota transplantation, which can be considered a probiotic treatment [3]. The fecal transplantation has shown a remarkable success for *C. difficile* infections, but this has not been seen in other conditions [2].

Other mentioned therapeutic options are: the use of bacteriophages that target specific bacterial pathogens [2]; the development of specific inhibitors against potentially oncogenic properties of commensal bacteria without disrupting the delicate balance between microbial families [6]; bacterial based vaccines that express tumor antigens [7]; and, probably the easiest way of all, aspirin and NSAID to inhibit the tumor-elicited inflammation [6]. This latter already showed a decrease in colorectal cancer risks [6].

Besides new therapeutic options, affecting the human microbiome is also important to ameliorate existing therapies. For example, the microbiota has shown to treat the common and sometimes severe diarrhea of the chemotherapeutic Irinotecan in mice studies. It also contributes to the effect of the platinum-based chemotherapy Oxiplatin, and starts antitumor responses in combination with Cyclophosphamide [7]. More understanding of the specific roles of the microbiome is required to fully see the microbiome as an adjuvant therapy that enhances efficacy or attenuates the toxicity of chemotherapies [5, 7]. Moreover, it is possible that the microbiome influences the host's responsiveness to immunotherapy, to total body irradiation and even adoptive T-cell transfer [5, 7]. Taken together, the microbiome might have several crucial uses in the clinical practice of the future.

OBJECTIVES

The purpose of this study was to assess the role of the non-gut microbiome (lung microbiome, skin microbiome, oral and etc.) in the development of cancer at the site of the microbiome and beyond.

METHODS

Criteria for considering studies for this review.

Type of studies

Studies that were utilizing the following designs, were considered for inclusion:

1. Observational studies: case control studies, cohort studies.
2. Interventional studies : RCT's.

Reviews, case reports and other studies without a comparison, cross sectional studies, reports from conferences or annual meetings, editorials, opinions, in vitro studies and studies with animals were excluded from this review.

Type of participants

Adult humans with or without any type of cancer. Children (< 18 years) were excluded from this review.

Type of interventions

No specific interventions required.

Type of outcome measurements

The incidence of cancer and its association with the non-gut microbiome (lung microbiome, skin microbiome, oral and etc).

Search methods for the identifications of studies

PubMed, EMBASE and the Cochrane Library were databases used for research. There was no limitation in date of publication. Studies not written in English were excluded from this review. Also, references in the selected publications were checked for further studies. For comprehensive search strategies, see appendix 1-3.

Data collection and analysis

Selection of studies

Resulting hits from the search strategy were entered in EndNote library. The authors (Astrid Loobuyck, Zeger Vandenbulcke) each independently selected titles and abstracts from the

bibliography retrieved by the search strategy according to the inclusion and exclusion criteria. Full text copies from studies that fulfilled the criteria after this selection were obtained. In case of a disagreement between the two authors or when fulfilled to the criteria was unclear, the article stayed included and the full text was checked. When the disagreement was still present, the opinion of a third independent person was requested (Nathalie Michels).

Qualtity of the articles

For analyzing the quality of the articles, the Newcastle – Ottawa quality assessment scale was used. This assessment scale consists of three categories: selection, comparability and exposure; of which each could receive up to four stars for selection, two stars for comparison and three stars for exposure. This Newcastle – Ottawa quality assessment scale was found on http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [8] on the fifth of September in 2017.

RESULTS

Study design and population characteristics of the included studies

A total of thirty-one articles were included and used for this review (see Methods and appendix 4. Flowchart). Of the included articles thirty studies were case controls, including one nested case control study [9], and one was a cohort study [10]. No RCT's were found.

Six of the articles were executed in the U.S.A.; three in Mexico; another six articles were published in Europe; one in South Africa; fourteen in Asia, from which eight articles were published in China, and one in Australia.

The year of publication ranged from 1982 to 2016 among the thirty-one articles. The majority of them (twenty-six articles) were published between 2010 and 2016. The five others were published in 1982 [11], 1983 [12], 1989 [13], 1996 [14] and 2000 [15].

The sample size of the studied populations varied tremendously. Only one article had a sample size smaller than 10 [16], five articles had a sample size ranging from 10 to 24, six a population size from 25 to 49 and seven articles a population size from 50 to 99 while twelve articles studied over 100 subjects.

Since children were excluded from this review (see Methods), all the studies researched a population of adults with an overall age ranging from 18 to 96 years old. In most of the studies, the cases tended to be a bit elder than the healthy controls. Eight articles showed an average age of

the overall population above 60 years, another eight articles showed an average age of the overall population above 50, but under 60 years old.

Type of microbiomes and cancers

The following microbiomes were considered in the included studies (for the gut microbiome we refer to the thesis of Zeger Vandenbulcke):

- the bile duct microbiome,
- the cervical and intrauterine microbiome,
- the esophagus and gastric microbiome,
- the laryngeal microbiome,
- the lung microbiome,
- the oral microbiome,
- the skin microbiome,
- and the urine microbiome.

The most often studied microbiome (in fourteen articles) was the oral microbiome. Five of these articles used oral cancer as the outcome, one article used oral, pharyngeal and laryngeal cancer as outcome, three articles used pancreatic cancer, one article used colorectal cancer in specific while another article had colorectal, lung and gastric cancer as outcome, one article looked at head and neck cancer, another one at esophageal cancer and a last one used gastric cancer. One article did research about the laryngeal microbiome and laryngeal cancer. Six articles studied the gastric and/or esophageal microbiome. Three of them compared the microbiome of healthy controls with that of patients with gastric cancer, two of them compared it with the microbiome of patients with esophageal cancer and one article did not use cancer but esophagitis and Barret esophagus as outcome. Three articles did research about the cervical microbiome using cervical cancer as outcome and one article studied the intrauterine microbiome in general, analyzing endometrial swabs and tissues. This latter article compared healthy controls with patients with chronic endometritis and/or endometrial polyps. The bile duct was researched in two studies using cholangiocarcinoma as outcome. Another two articles were studying the lung microbiome with lung cancer as outcome. One article studied the urine microbiome and compared the microbiota of healthy controls with that of patients with breast cancer [11]. Finally, the last article did research on the skin microbiome and general cancer cachexia [17].

Table 1. The microbiomes and the possible links with cancer researched in this review.

MICROBIOME	LINKED WITH THE FOLLOWING CANCER(S)	# ARTICLES
Bile duct microbiome	Cholangiocarcinoma	2
Cervical microbiome	Cervical cancer	3
Intrauterine microbiome	Chronic endometritis	1
Esophagus microbiome	Esophagus cancer	1
Gastric microbiome	Gastric cancer	3
	Esophagus cancer	1
Esophagus and gastric microbiome	Barret esophagus	1
Laryngeal microbiome	Laryngeal cancer	1
Lung microbiome	Lung cancer	2
Oral microbiome	Oral cancer	5
	Head and neck cancer	1
	Oral, pharyngeal and laryngeal cancer	1
	Esophagus cancer	1
	Pancreatic cancer	3
	Gastric cancer	1
	Colorectal cancer	1
	Colorectal, lung and gastric cancer	1
Skin microbiome	Cancer cachexia	1
Urine microbiome	Breast cancer	1

Quality of the studies

For analyzing the quality of the articles, the Newcastle – Ottawa quality assessment scale was used. This scale was found on http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp [8] on the fifth of September in 2017. Most of the articles scored well for selection: nineteen articles got four stars and nine got three stars. Only two articles got two stars [18, 19] and one got just one star [16], due to lack of information about the selection of cases and controls and thus their representativeness for the community. The study of Seo 2014 [19] used the same cancer patients as controls by taking samples of adjacent normal gastric tissue, but it is not mentioned or not known if there was a (history of) disease at that spot.

Also for comparability, eighteen articles scored the maximum of two stars, while six got one star and seven did not get any star. These last articles did not perform any correction for confounders between cases and controls of any kind (matching cases to controls, statistical analysis considering possible confounders such as age, gender, smoking status, alcohol consumption etc.). This decreases the comparability between the patient group and the healthy controls. For the third category, exposure, twenty-seven articles got two stars while only four articles got one star. Low scores on exposure were mostly because the studies used another method of ascertainment between cases and controls, for example resection for the cases with cancer while biopsy for the healthy controls.

Overall significant findings and difficulties in comparisons

All of the articles, besides one article from 1983, showed certain significant differences (p value < 0.05) in microbiome between cases and controls. This is due to several reasons. First, some studies used different kind of samples from the same subject for researching the microbiome. For example, the study of Amir 2013 [20] collected samples of esophageal tissue and gastric tissue, finding only a significant difference in microbiome of the gastric fluid. Second, most articles used several aspects to describe and compare the microbiome: alpha diversity, beta diversity, relative abundance, absolute abundance and some articles even cluster microbiota into communities or make ratios comparing the abundance of two microbiota taxonomic groups. Furthermore, the microbiome could have been analyzed at different levels, mainly the phyla, the families and genera were studied. Thus, some articles show no significant difference in the presence of certain phyla but they do show a difference in more specific taxonomic levels. For example the study of Nasrollahzadeh [21] found the same five most abundant phyla between the cases with esophageal cancer or dysplasia and the healthy controls, but different abundances of the orders Clostridiales

and Erysipelotrichales. At last, the researchers sometimes divide the cases and/or controls in multiple subgroups to reveal more specific links. These subgroups can show significant differences with the microbiome of the controls/cases, but they can also be combined to explore such differences. So this method makes comparisons possible between cases and several subgroups of the controls, but also comparisons between the controls and the several subgroups of the cases. And furthermore, it also allows comparisons between the several subgroups of the cases or the controls themselves. The study of Gong 2013 [22] used two subgroups depending on the location of the laryngeal cancer, a supraglottic versus glottic tumor group, but there were no significant differences found between the microbiomes of these groups.
Some findings were consistent with others, but this was not always the case. Differences in results could be due to different tissue sampling (different method or different site), different gene analysis or the specific population (e.g. genetic and environmental differences like nutritional intakes and habits, oral hygiene, air pollution etc.). Furthermore, different stages of the cancer might harbor different microbiomes and this could be a cause of non-consistent results. This illustrates the complexity of the human microbiome and their interactions.

Overall results from the studies

See appendix 5 for a general overview of the most frequently found phyla and bacteria in these studies, see appendix 6 – 7 for the design and results of the included studies.

Bile duct microbiome

Only the cholangiocarcinoma has been investigated in relation with the bile duct microbiome by two articles, but one article only focused on the extrahepatic variant.
The study of Avilés-Jiménez 2015 [23] found a higher abundance of Fusobacteria, Acidobacteria and Planctomycetes in patients with an extrahepatic cholangiocarcinoma (ECCA) compared to the healthy controls with a benign biliary pathology. Both showed a microbiome dominated by Proteobacteria. Twenty-six operational taxonomic units (OTU) were significantly different in the ECCA patients, but five of them were considered contaminations. So, the ECCA patients showed an increase of Methylophilaceae, *Fusobacterium*, *Prevotella*, *Helicobacter* and *Campylobacter* but a decrease in *Nesterenkonia*, *Rothia* and *Mesorhizobium*.

Chng et al. 2016 [24] compared samples from cholangiocarcinoma (CCA) tissue with the non-neoplastic adjacent liver tissue and with normal liver tissue from healthy controls. The bile duct tissue of the CCA patients showed Dietziaceae, Pseudomonadaceae and Oxalobacteraceae as the most abundant bacteria and these were also present in the normal hepatic tissue of the healthy controls. Comparing the normal hepatic tissue of these controls with the normal adjacent hepatic tissue of the CCA patients, showed significant differences in Enterobacteriaceae, Lachnospiraceae, Bifidobacteriaceae and Sphingomonadaceae. On the other hand, comparing the normal adjacent hepatic tissue of the CCA patients with the CCA tissue only showed a difference in *Stenotrophomonas* that was enriched at the tumor tissue.

The α- and β- diversity of the samples

Avilés-Jiménez et al. found no difference in microbiome diversity between the ECCA patients and the controls, but a partial separation of microbiota composition between the two groups was significant (PCoA with unweighted UniFrac distances and Adonis test) [23]. Likewise, Chng et al. noticed that the microbiome diversity of the tumor tissue was similar to the diversity of adjacent normal tissue, but the comparison of the microbiome of the controls with the microbiome of the normal adjacent tissue from the cases showed a significant difference (PCoA with weighted and unweighted UniFrac distances). The intra patient microbiomes (tumor and adjacent normal tissue) were more similar relative to the inter tumor microbiomes [24].

Cervical and intrauterine microbiome

Cervical microbiome in relation to cervical cancer

Lactobacillus crispatus and *Lactobacillus iners* are often recurring species showing relations with a healthy cervical microbiome. The study of Audirac-Chalifour 2016 [25] noticed that *Lactobacillus crispatus* was decreased in the patient group with cervical cancer compared to the healthy controls, while *Lactobacillus iners* was completely undetectable in the patient group. In the healthy control group this *Lactobacillus iners* was clearly present. But the study of Oh 2015 [26] found slightly different results. They found that the risky microbial pattern for cervical intraepithelial neoplasia (CIN) consisted of *Atopobium vaginae*, *Gardnerella vaginalis* and *Lactabacillus iners.* This risky pattern also showed a low abundance of *Lactobacillus crispatus,* in agreement with the findings of Audirac-Chalifour et al. The relative abundances of *Lactobacillus crispatus* and *Lactobacillus iners* were not different between women with CIN and healthy women in the study of Oh, but some women with CIN showed a high proportion of *Lactobacillus iners*, which is totally different than the study of Audirac-Chalifour. One possible explanation for this difference is that Audirac-Chalifour

investigated Mexican women while Oh did their research on Asian women in Korea. That Audirac-Chalifour researched samples of cervical cancer and Oh used samples of CIN, could be another explanation. Similar to the study of Oh et al., Seo 2016 [27] found that patients of South Korea with a *Lactobacillus iners*-dominant microbial type had a higher risk of CIN compared to patients with a *Lactobacillus crispatus*-dominant microbial type. Also the *Atopobium vaginae*-dominant microbial type was related with a higher risk of CIN.

Audirac-Chalifour et al. also noticed other bacteria such as *Sneathia spp.*, *Megasphaera elsdenii*, *Shuttleworthia satelles* and *Fusobacterium necrophorum*. *Sneathia spp.* was characteristic for patients with a HPV infection and squamous intraepithelial lesions (SIL), since these patient groups showed the highest abundance, but it was also found in patients with cervical cancer. *Megasphaera elsdenii* and *Shuttleworthia satelles* were usually associated with bacterial vaginosis and not with SIL, but not much is known of these two microorganisms. On phyla level, the late stages of cervical cancer showed a significantly higher abundance of the *Fusobacterium spp.* with *Fusobacterium necrophorum* that had only been observed in the cervical cancer group. In the microbiome of the cervical cancer patients, they did not find bacteria of the Bifidobacteriaceae family (mainly *Gardnerella vaginalis* in healthy controls) [25].

Intrauterine microbiome in relation to chronic endometritis

The study of Fang 2016 [18] investigated the vaginal and mainly the intrauterine microbiome of patients with chronic endometritis and endometrial polyps. The intrauterine microbiome of the diseased patients showed a higher abundance of *Lactobacillus*, *Bifidobacterium*, *Gardnerella*, *Streptococcus*, *Alteromonas* and *Prevotella* compared to the healthy controls and a decreased abundance of *Pseudomonas*. On phylum level, this means a higher relative abundance of Firmicutes sequences and lower Proteobacteria sequences [18].

The α- and β- diversity of the samples

The studies also tested α- and β-diversity. The cervical cancer group in Audirac-Chalifour showed a higher α-diversity (Shannon index) and a higher β-diversity (principal component analysis (PCoA) with weighted UniFrac distances: cancer patients were significantly separated from the healthy controls [25]. Also in patients with endometrial polyps, α-diversity (Shannon index and OTU number) was significantly higher [18]. In the same direction, patients with CIN had a higher operational taxonomic unit (OTU) number [26].

Esophagus and gastric microbiome

The esophageal and gastric microbiome in relation to Barret esophagus/esophageal cancer

In the Australian population with heartburn, Amir et al. [20] found that the esophageal microbiome was mainly dominated by Proteobacteria and Firmicutes. But they did not find significant differences in the microbiome between the patients with a Barret esophagus (BE) and the healthy controls with heartburn. In 1983, Mannell et al. [12] had found similar results. In the esophageal microbiome of patients with esophageal cancer *Streptococcus viridans* was the most common, followed by *Streptococcus faecalis*, *Haemophilus influenzae* and *Neisseria catarrhalis*. They did not find any significant difference between the microbiome of the patients with esophageal cancer and the healthy controls with no evidence of esophageal disease [12].

The gastric fluid also mainly consisted of Proteobacteria and Firmicutes plus Bacteroidetes. At genera level, the gastric microbiome patients with BE showed that the Enterobacteriaceae and Methylobacteriaceae were enriched while Pasteurellaceae and Porphymonodaceae were decreased compared to the healthy controls with heartburn [20].

The gastric microbiome in relation to esophageal cancer

Nasrollahzadeh et al. [21] investigated the gastric tissue microbiome of patients with esophageal squamous cell carcinoma (ESCC). They also found Firmicutes, Bacteroidetes and Proteobacteria as the main components of the microbiome and the phyla composition was consistent across the cases and controls. The patient group with ESCC did show a higher abundance of *Clostridiales* and *Erysipelotrichales* and a lower abundance of *Helicobacteraceae* compared to the healthy controls.

The gastric microbiome in relation to gastric cancer

Avilez-Jiminez et al. [28] investigated the gastric tissue of patients from Mexico with non-atrophic gastritis (NAG), intestinal metaplasia and gastric cancer. The microbiome of these gastric tissues again showed a dominance of Proteobacteria and Firmicutes. The patients with gastric cancer had a microbiome with decreased abundance of two taxa of TM7, two Porphyromonas and one Neisseria while there was an increase in *Lactobacillus coleohominis* and Lachnospiraceae [28]. Also in Korea, researchers explored the gastric tissue microbiome of patients with gastric cancer and healthy controls. Eleven gastric microbiomes out of the sixteen were dominated by *Helicobacter pylori*, which showed a higher abundance in the adjacent normal tissue of the gastric cancer patients than in the tumor site itself [19]. Besides *Helicobacter pylori*, the microbiome of the

gastric cancer patients showed a decrease in *Propionibacteirum spp*., *Staphylococcus spp.*, and *Corynebacterium spp*. and an increase in *Clostridium spp*. and *Prevotella spp*. [19].

In 1989, Japanese researchers already found that *Campylobacter pylori* (renamed in 1989 as *Helicobacter pylori*) was present in 85% of all the subjects, patients with several diseases and healthy controls. There were higher detection rates for *Helicobacter pylori* in the patients with gastric cancer and the patients with ulcera compared to the healthy controls. All of the patients with gastric cancer were positive for this bacteria, but there was a small sample size and a dissociation between methods [13].

The α- and β- diversity of the samples

A- and β-diversity were also tested in multiple studies. The gastric fluid microbiomes were well separated between patients with BE and healthy controls (principal component analysis with unweighted UniFrac distances) which indicates a high β-diversity [20].

Likewise, the PCoA based on unweighted UniFrac distances showed a clear separation between the gastric tissue microbiome of the patients with cancer and the controls with NAG in the study of Avilez-Jiminez et al. [28]. The diversity was significantly different between these two groups and they observed that the microbiome from NAG to the intestinal metaplasia to the gastric cancer group showed a trend of diminishing diversity [28].

The study of Nasrollahzadeh et al. also conducted a weighted and unweighted UniFrac which resulted in a significant difference between the ESCC group and the healthy controls. The α-diversity (measured by Chao1) was not significantly different between the ESCC cases and controls [21].

Laryngeal microbiome

Only laryngeal cancer has been investigated in relation with the laryngeal microbiome.

The study of Gong et al. 2013 [22] found that the laryngeal microbiome mainly consisted of Firmicutes, Fusobacteria, Bacteroidetes, Proteobacteria, and Actinobacteria while at genera level *Streptococcus*, *Fusobacterium*, *Prevotella*, *Neisseria* and *Gemella* were predominant. The patients with laryngeal squamous cell carcinoma (LSCC) showed a higher abundance of *Fusobacterium*, *Prevotella* and *Gemella*, but a lower abundance of *Streptococcus* and *Rothia* compared to the controls.

The α- and β- diversity of the samples

In general, the samples from LSCC and the controls could clearly be separated thus indicating a high β-diversity [22].

Lung microbiome

Only lung cancer has been investigated in relation with the lung microbiome.

The study of Carpagnano 2014 [29] was able to demonstrate the presence of fungi in the lungs of 12 patients of the 43 patients with non-small cell lung cancer. Most of them were colonized by *Aspergillus niger,* the others *by Aspergillus ochraceus* and by *Penicillum spp*. None of the healthy controls showed a colonization with fungi.

When analyzing these lung cancer patients no difference was found in smoking habit, inclusive pack years or time since quitting smoking, in subjects with fungal colonization.

The study of Hosgood 2014 [30] had a different viewpoint: they investigated buccal and sputum samples of lung cancer patients who had never smoked and the possible effect of household pollution when burning polycyclic aromatic hydrocarbons (PAH)-rich coal. Lung cancer patients had an enrichment of *Streptococcus*, *Granulicatella* and *Abiothrophia* compared to the controls.

The α- and β- diversity of the samples

The diversity of the oral bacterial community found by buccal samples was similar between the lung cancer patients and the healthy controls, but the sputum samples showed clear differences (PcoA with weighted UniFrac distances: the sputum samples from the cancer patients were significantly closer to each other than they were to the sputum control samples) [30].

Oral microbiome

The oral microbiome in relation to oral cancer

The study of Schmidt et al. [31] showed that the oral microbiome mainly consists of Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria. This was equal for the patients with oral cancer, but they found an increased abundance of Fusobacteria and Bacteroidetes and a decreased abundance of Actinobacteria and Firmicutes in these patients with oral cancer compared to the healthy controls. At the genus level, there was a decrease in *Streptococcus* and *Rothia*, but an increase of *Prevotella*.

Guerrero-Preston et al. [10] found the same five most abundant phyla, but in a slightly different order of frequency. They used oral rinses and not oral swabs like Schmidt et al. for sample

collection. In their study group, the patients with head and neck squamous cell carcinoma (HNSCC) had an oral microbiome with a higher abundance of Firmicutes and a lower abundance of Bacteroidetes and Proteobacteria compared to the healthy controls. At the genus level, *Streptococcus* and *Prevotella* were dominant across all samples. There was a higher abundance of *Streptococcus* and *Lactobacillus* but a lower abundance of *Aggregatibacter*, *Neisseria*, *Haemophilus*, *Prevotella*, *Lautropia*, *Leptotrichia* and *Gemellacellae* in the patients with HNSCC. Distinguishing the patients with oral squamous cell carcinoma (OSCC) from those with oropharyngeal squamous cell carcinoma (OPSCC) was possible by the *enterobacteriaceae* and the O*ribacterium*. Their OTU network shows that the total abundance of *Streptococcus*, *Veillonella* and *Dialister* can be used to separate tumor samples from controls. Treatment and HPV status effected the composition of the microbiome. HPV infection mainly changed the abundances of *Veillonella*, *Prevotella* and *Streptococcus* [10].

Oral swabs from patients with an OSCC had a higher frequency of yeast colonization, a higher fungal burden and more yeast cells compared to the healthy controls in the study of Berkovitz et al. [32]. *Candida* was the most prevalent fungal genus in OSCC patients and controls, but the OSCC patients did show a higher diversity of yeasts compared to the controls (based on MALDI-TOF-MS analysis).

Henrich et al. [16] did research on the oral microbiome of patients with Fanconi Anaemia (FA) and oral cancer. As controls, they used a healthy control group and another FA patient. The microbiome of the cancer patients was dominated by Bacteroidetes, Firmicutes, Proteobacteria and Tenericutes. This was different for both control groups where Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria were the main phyla. In the FA patients with oral cancer, *Mycoplasmataceae,* mainly *Mycoplasma salivarium,* dominated at the tumor site and the adjacent gingiva while *Pseudomonadaceae,* mainly *Pseudomonas aeruginosa*, was frequent at all sites except the tumor site. In the control group, the oral microbiome showed a dominance of *Streptococcus*, *Veillonella* and *Neisseria*. *Streptococcus* was significantly reduced in the FA cancer patients (some species were increased, other decreased) and *Rothia mucilaginosa* was even completely absent compared to the control groups [16]. This is similar to the findings of Schmidt et al. [31]. Both FA oral cancer patients tested positive for Candida in almost all samples [16].

The oral acetaldehyde production in relation to oral, pharyngeal and laryngeal cancer

Three studies tried to explore the mechanism of oral cancer by analyzing the link between increased salivary acetaldehyde levels and the oral microbiome. Especially smoking and heavy drinking lead to high levels of the first metabolite of ethanol, namely the toxic and carcinogenic

acetaldehyde which is produced by the oral bacteria [15, 33]. Normally, the healthy human saliva does not contain a measurable level of acetaldehyde [33]. Salivary samples with high acetaldehyde levels showed a higher abundance of *Streptococcus salivarius*, *Streptococcus viridans*, C*orynebacterium sp.*, *Stomatococcus sp*. and yeasts. Furthermore they also showed a slightly higher total amount of anaerobes, but this was not significant for one specific species. Homann et al. did not find a difference in the acetaldehyde production between the patients with oral cancer and the controls [15].

This is confirmed by the study of Marttila et al. [33] who did not find a significant difference in acetaldehyde production between the patients with OSCC and the controls, nor a correlation between the acetaldehyde levels and the amount of bacteria in any patient group. They did detect a significantly higher number of microbes in the lesion site of the OSCC patients. Both the aerobe and the anaerobe bacteria were more prevalent than in the healthy controls. The samples from OSCC patients also had a higher frequency and amount of *Candida*. These OSCC patients who were positive for colonization with *Candida*, did have significantly more frequent a mutagenic production of acetaldehyde than the patients without this colonization [33].

The third study, published already in 1996 [14], did not confirm the statement of the other two studies. Jokelainen et al. did find a significant higher acetaldehyde production capacity in the mouth washings of patients with oral, pharyngeal or laryngeal cancer after in vitro incubation with ethanol compared to the healthy controls. The consumption of alcohol and cigarettes was almost two times higher in the cancer patients than the controls, highlighting that these two are considered major risk factors [14].

The oral microbiome in relation to esophageal cancer

However, the oral microbiome is not exclusively linked with oral cancer. Chen et al. [34] investigated the relation between the oral microbiome and patients with ESCC. They found the same five most abundant phyla i.e. in order of frequency: Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria and Actinobacteria. Patients with ESCC showed an increase in *Prevotella*, which is similar to the study of Schmidt researching oral cancer patients, and also an increase in *Porphyromonas and Streptococcus*. Oppositely, Schmidt et al. saw a decrease of *Streptococcus* in the oral cancer patients [31]. Furthermore, in the oral microbiome of the patients with ESCC there was seen a decrease of sixteen different genera, primarily of *Atopobium*, *Actinobacillus*, *Aggregatibacter*, *Corynebacterium, Dialister* and *Peptococcus* [34].

The oral microbiome in relation to pancreatic cancer

Other studies researched the oral microbiome and its relation to the pancreatic adenocarcinoma. The nested case-control study of Fan et al. [9] found that carriers of *Porphyromonas gingivalis* and carriers of *Aggregibacter actinomycetemcomitans* had a higher risk of developing pancreatic cancer. For *Porphyromonas gingivalis* there was even a dose-response relationship with high carriers showing a higher risk. An oral microbiome with Fusobacteria and its genus *Leptotrichia* was related with a lower risk of pancreatic cancer [9].

Farrell et al. [35] investigated the same relation. In their population, they found sixteen species/clusters with a significant difference between the oral microbiome of the patients with pancreatic cancer and the healthy controls. These species/clusters belonged to 6 different genera namely *Streptococcus*, *Prevotella*, *Campylobacter*, *Granulicatella*, *Atopobium* and *Neisseria* (by HOMIM array results). The microbiome of the cancer patients showed a decrease of *Streptococcus* and *Neisseria*, but an increase in *Granulicatella* (confirmed by qPCR). Using these bacteria as biomarkers resulted in a 96,4% sensitivity and 82,1% specificity for *Neisseria* and *Streptococcus* in separating patients with pancreatic cancer from the healthy controls. The other combinations resulted in a slightly lower sensitivity but much lower specificity [35].

A more recent study from Torres et al. [36] found slightly different results. They found the same five dominant phyla in the oral microbiome as the other studies mentioned previously, but the microbiome of the patients with pancreatic cancer had lower levels of Proteobacteria compared to the healthy controls. *Neisseria* and *Porphyromonas* were present in lower abundances in the patients with cancer, while *Leptotrichia* were present in higher abundances compared to the controls. In contrast with Farrell et al., the levels of *Streptococcus* and *Granulicatella* showed no difference between the cases and controls. The decrease in *Neisseria* was observed in both studies [36].

The oral microbiome in relation to colorectal, lung and gastric cancer

In Asia, three studies investigated the value of tongue diagnosis as a method of the traditional Chinese medicine. Han et al. [37] used tongue images and the tongue diagnostic information acquisition system to divide their patients with colorectal cancer in a thick tongue coating group and a thin group. These two groups had their own microbial characteristics, sometimes overlapping and sometimes not. In the thick group there was a higher abundance of *Leptotrichia*, *Prevotella* and *Actinomyces*, but a lower abundance of *Gemella* and *Parvimonas* compared to the other groups. There were nine other genera who were present in different abundances compared to the other groups. The thin group showed a significant lower abundance of *Veillonella*. On the other

hand, *Enhydrobacter*, *Janthinobacterium* and *Yersinia* were higher in the thin group but lower in the thick group compared to the healthy controls. Combining these two groups, the oral microbiome of the colorectal cancer patients showed a higher number of *Streptococcus* and a lower number of *Haemophilus* than the controls [37]. So most of the bacteria were consistent across the subjects, but unique species could be observed in every group.

The second study did research on the value of tongue diagnosis in patients with gastric cancer [38]. Also here, the patients were divided in a thick group and a thin group based on tongue images and the tongue manifestation acquisition instrument. There was a significant difference in thickness of tongue coating between the patients with gastric cancer and the healthy controls, who showed thinner tongue coatings. Both patients and controls showed the same six dominant phyla: Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria and TM7. The relative abundance of Proteobacteria was decreased in the microbiome of the gastric cancer patients, while the relative abundance of Actinobacteria was increased compared to the healthy controls [38]. This decrease in Proteobacteria was also found in the pancreatic cancer patients of Torres et al. [36] . At the genus level, the five most prominent species in the thick gastric cancer patients were *Prevotella*, *Streptococcus*, *Actinomyces, Veillonella* and *Leptotrichia*. The thin group was dominated by *Prevotella*, *Veillonella*, *Leptrotrichia*, *Lactococcus* and *Streptococcus*. This was different for the controls where *Prevotella*, *Neisseria*, *Streptococcus*, *Haempophilus* and *Fusobacterium* showed the highest relative abundances. So, the microbiome of the patients showed a lower abundance of *Fusobacterium*, *Neisseria*, *Haemophilus* and *Porphyromonas* [38]. Han et al. also noticed this decrease in *Haemophilus* in their colorectal patient group while the decrease in *Neisseria* and *Porphyromonas* was also seen in the pancreatic cancer population of Torres et al. [36, 37]. Furthermore, there were differences between the thick and the thin group: the thick group had higher abundances of *Streptococcus* and *Actinomyces* while in the thin group *Lactococcus* and *Leptotrichia* were present [38].

Another study of Han [39] expanded the group of patients to patients with several types of cancer namely lung cancer, gastric cancer and colorectal cancer [39]. These patients had clearly distinguished physical characteristics of the tongue compared to the healthy people. Furthermore, the patients showed a lower number of *Neisseria*, *Porphorymonas*, *Fusobacterium* and *Haemophilus* than the control group. This is similar to the two previous studies [37]. At species level, it were *Fusobacterium periodonticum*, *Haemophilus parainfluenzae, Peptostreptococcaceae bacterium*, *Prevotella aurantiaca*, *Prevotella salivae* and one species from TM7 that were statistically different between cancer patients and controls [39].

The α- and β- diversity of the samples

Many of the studies measured the α-diversity of the groups. The microbiome of the HSNCC patients showed a significant lower α-diversity (measured by the Chao1 richness estimator and Faith's Phylogenetic Diversity index) than the healthy controls [10]. The α-diversity (Shannon index) of the FA patients with oral cancer was also lower than the control groups in the study of Henrich et al. [16]. Testing the α-diversity in the oral microbiome of the ESCC patients revealed a significant difference between the ESCC patients and the healthy controls. The ESCC patients had a lower OTU richness and diversity as well (measured by Chao1 and Shannon index) [34]. Furthermore, the Asian patients with colorectal cancer and a thick tongue coating showed a lower OTU number than the healthy controls or the thin group (measured by the abundance-based coverage estimator, Chao and Shannon) [37]. Hu et al. confirmed these results for their patients with gastric cancer and a thick tongue coating (abundance-based coverage estimator, Chao and Shannon index) [38]. The study of Torres, that compared patients with pancreatic cancer with diseased controls (but without cancer) and healthy controls, did not find any difference in α-diversity (Chao1) [36].

In the American study of Schmidt et al., the weighted and unweighted UniFrac showed significant microbiome differences for patient identity, but there were no differences between the microbiome of the lesion site and of the control normal site within the same cancer patient. This highlights the inter-individual differences of the oral microbiome [31]. This was confirmed by another American study where Guerrero-Preston et al. showed that the microbial communities in HNSCC samples could clearly be separated from normal samples (using non-metric multidimensional scaling and PCoA) [10]. The microbiome of the FA patient with oral cancer as well was clearly separable from the other control groups (weighted UniFrac and PCoA) [16]. Also Chen et al. found significant differences between the microbiome of the ESCC patients and the healthy controls (PCoA with unweighted and weighted UniFrac distances). These results were similar even after correction for age, sex, education, smoking, alcohol drinking, family history of ESCC, number of missing and filled teeth, times of tooth brushing and daily consumption of vegetables and fresh fruit [34]. The β-diversity (analysis of similarities ANOSIM with weighted and unweighted UniFrac) between the pancreatic cancer patients and the diseased and healthy controls was not significant [36].

Skin microbiome

The study of Li 2014 [17] found that the main phyla in the microbiome of the axillary fossa were Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes. There was a decreased abundance of the Actinobacteria in the skin microbiome of patients with cancer cachexia compared to the

healthy controls. At the genus level, this means the *Corynebacterium* spp. showed a lower relative abundance. Besides the *Corynebacterium* spp., the *Staphylococcus* spp. was also a main bacterium in all subjects [17].

The α- and β- diversity of the samples

Li et al. noticed a lower α-diversity (measured by non-parametric ACE and Chao1 algorithm) in the skin microbiome of the patients compared to the healthy controls. But there were no significant differences in Shannon index, Simpson index or the Shannon measure of evenness. The number of OTU's were also lower for the cancer cachexia patients. Furthermore, the intra-group similarity of the cancer cachexia patients was higher than for the controls (the DGGE profiles analysis) [17].

Urine microbiome

Adlercreutz et al 1982 [11] investigated the excretion of urine lignans in healthy postmenopausal women and women who had been treated for breast cancer. These lignans are formed by bacteria in the intestinal tract out of precursors in the diet and excreted in a cyclical pattern during the menstrual cycle. They found that the urinary excretion of enterolactone was significantly lower in the women who had been diagnosed with breast cancer. This latter group also showed a lower enterodiol excretion compared to the vegetarian healthy controls. Also the enterolactone excretion to fibre intake ratio was lower in the women who had been diagnosed with breast cancer.

DISCUSSION

Bile duct microbiome and cholangiocarcinoma

Similarities and dissimilarities in results

The two studies found the same dominant phyla in the controls namely the Proteobacteria and Actinobacteria. Avilés-Jiménez et al. found twenty-one OTU's that were increased or decreased in the controls but only two of them overlapped with the study of Chng et al. i.e. increased Sphingomonadales and Xanthomonodales. For this latter order, the two articles assigned the increase to different families: Sinobacteriaceae [23] or *Stenotrophomonas* of the Xanthomonadaceae family [24].

Three reasons for these differences in outcome can be revealed. First, Avilés-Jiménez only researched cases with an extrahepatic cholangiocarcinoma, so their discovered microbiome can be expected to be more similar to the microbiome of the small intestine than to the hepatic microbiome. In the population studied by Chng et al., only sixteen of the sixty cases had a strictly extrahepatic cholangiocarcioma. Their findings of the microbiome showed several shared families with the hepatic microbiome like Dietziaceae, Pseudomonadaceae and Oxalobacteraceae. On the other hand, they did find an increase in enteric bacteria in those cases with a cholangiocarcinoma associated with *Opisthorchis viverrini*. Second, there were slight differences in the 16S rRNA gene analysis: targeting the V4 or the V3-V6 regions. At last, the origin of both populations was quite different: Mexico [23] versus Thai, Chinese, Caucasian, Malay and Indian [24].

Possible underlying pathways

The study of Avilés-Jiménez et al. mainly focused on the higher abundance of *Helicobacter pylori* in the cases and a significant enrichment of the virulence genes (VacA) of these bacteria suggesting an association with ECCA. As mentioned in the introduction, VacA and CagA stimulate the β catenin pathway thus activating cell proliferation, survival, migration and angiogenesis [6, 7]. Also Fusobacterium, Prevotella, and Campylobacter were increased in the ECCA patients, as previously seen in other gastrointestinal tumors. Indeed, an obstruction in the bile duct might lead to a retrograde bacterial reflux from the small intestine or there might just be a general dysregulated immune response. At last, there were three bacteria found that were seen as unusual for human flora: Methylophilaceae, *Nesterenkonia* and *Mesorhizobium*. Methylophilaceae showed a higher abundance in the ECCA patients, *Mesorhizobium* on the contrary showed a higher abundance in the controls with benign biliary pathology. *Nesterenkonia* was present in all patients and most abundant in the controls. These two bacteria with lower concentrations in ECCA support the hypothesis that they might be involved in early changes of the microbiome developing towards a cancerous environment [23, 24]. Finally, Methylophilaceae was also reported in a patient with fulminant pulmonary illnesses: a thirty-six white male had developed acute respiratory distress syndrome in five days and a few weeks later he died without any etiologic cause identified. A re-initiated microbial culture from a cryopreserved broth of blood sample was conducted. This revealed the first *Mesorhizobium* isolated from humans. It is suggested that it can invade human cells and survive within them, not susceptible for antibiotics [40].

In Asia, *Opisthorchis viverrini* (OV) is considered a major risk factor for cholangiocarcinoma rather than *Helicobacter pylori.* Only the non-OV patients showed an enrichment of *Stenotrophomonas* at the tumor site compared to the adjacent normal tissue, suggesting different etiologies of the cancer

between these two subgroups. The OV-patient group had a large enrichment for Bifidobacteriaceae, at the tumor and the normal adjacent tissue. So if these bacteria affect carcinogenesis, the pathway is unlikely via direct mechanisms. The possible mechanism might be found in the gut. Bifidobacteriaceae are known as inhabitants of the gut microbiome and this gut microbiome is able to produce several carcinogens as ammonia and bile acids. These have been implicated in colorectal cancer progression. Thus the observation that the OV-microbiomes showed a higher abundance of Bifidobacteriaceae and a higher potential for producing ammonia and bile acids, suggests similar carcinogenesis in the OV-patients. Finally, combining both groups, the intra patient microbiomes (tumor site and adjacent normal tissue) were found to be more similar than the intra tumor microbiomes (across patients) suggesting that each person has an individual specific bile duct microbiome. A carcinogenic process or an OV infection will change the entire microbiome at tumor site and adjacent tissue [23, 24].

General conclusions

The bile duct microbiome is a complex microbiome with influences from both the hepatic and gut microbiome. The microbiome of the cholangiocarcinoma cases is clearly different from the controls, but there are also microbiome differences within the cases depending on the tumor etiology. Further research is needed to reveal these etiologies and the involvement of bacteria.

The cervical and intrauterine microbiome

Similarities and dissimilarities in results

Three studies investigated the cervical microbiome in relation to cervical cancer and CIN. Audirac-Chalifour found a decrease of *Lactobacillus crispatus*, *Lactobacillus iners* and *Gardnerella* in the cervical cancer cases, but an increase of Fusobacteriales (with a presence of *Fusobacterium Necrophorum*). The microbiome of the cases was clearly different from the controls and different from the cases with SIL. These cases with SIL showed a microbiome characterized by three bacteria: *Sneathia spp.*, *Shuttleworhia satelles* and *Megasphaera elsdenii*. These are not mentioned by the other articles [25].

The other two Korean articles investigated cases with CIN, trying to reveal a risky microbial pattern for developing cervical cancer. Both found that a microbiome dominated by *Atopobium vaginae* was related with a higher cancer risk. Furthermore, a synergistic effect between this microbiome type and a semi-western diet (lower intake of fibers, carotenes, vitamin C etc.) was observed with an odds ratio of 20.8. (95% CI 2.21-195.6) [27]. Another risky microbial pattern was a microbiome

dominated by *Lactobacillus iners*. This seems controversially with the findings of Audirac-Chalifour at first sight, but Oh et al. also found a very high abundance of *Lactobacillus iners* in some cases with CIN. They summarized that a microbiome with *Atopobium vaginae, Gardnerella vaginalis, Lactobacillus iners* and a low abundance of *Lactobacillus crispatus* was related to a higher risk of cervical cancer [26]. This inconsistency in results might be due to the different populations i.e. Mexican [25] versus Korean [26, 27] or a difference in 16S rRNA analysis i.e. the use of a primer targeting the V3-V4 region [25] versus targeting the V1-V3 region [26, 27].
Moreover, the HPV status of the subjects was checked. Higher abundances of Bacteroidetes, Actinobacteria, Tenericutes and Proteobacteria were present in the HPV-positive women than in HPV-negative. These HPV-positive microbiomes lost the normal composition with *Lactobacillus crispatus* and *Lactobacillus iners*, which was present in the HPV-negative patients. Even the OTU number of this HPV-positive group was lower, although these women had a higher abundance of *Lactobacillus iners* but a lower abundance of *Lactobacillus crispatus* and *Gardnerella,* which shows quite an overlap with the previous described risky microbial patterns [25]. The last study only stated that the rate of high risk HPV infection was higher in the cases with CIN than the controls [25-27].

The intrauterine microbiome showed similarities with the cervical microbiome. Intrauterine, Proteobacteria were predominant followed by Firmicutes and Actinobacteria. At genera level, the most abundant was again *Lactobacillus* as well as *Enterobacter* and *Pseudomonas* (both not seen in the cervical microbiome) and *Gardnerella*. There were significant differences between the cases with chronic endometritis and polyps versus the controls. These cases showed a higher α-diversity and a higher abundance of Firmicutes, but a lower of Proteobacteria [18]. Although the cases of this study did not have cancer, this investigation is still very useful. Endometrial polyps have been related to several inflammatory factors stimulating this overgrowth of endometrial tissue [18, 41, 42]. This hyperplasia of the endometrial tissue as well as the inflammation, present in the chronic endometritis cases and possible cause of the hyperplasia, are both crucial processes in carcinogenesis.
Furthermore, they also compared the intrauterine microbiome with the vaginal microbiome. In this microbiome Firmicutes were predominant, followed by Actinobacteria, which is different from the intrauterine microbiome but similar to the cervical microbiome as found by Oh et al. [18, 26]. This shows that the intrauterine microbiome might be associated with intrauterine lesions independent of the composition of the vaginal microbiome. Moreover, it reveals that the cervical microbiome has more overlap with the vaginal microbiome (dominated by *Lactobacillus* and *Gardnerella*) than the intrauterine microbiome [25-27].

Possible underlying pathways

In all stages of cervical cancer the microbiome is significantly different [25]. Especially the decrease of *Lactobacillus crispatus* and changes of *Lactobacillus iners* in CIN cases and cervical cancer cases were noticed by the three articles. *Lactobacillus crispatus* has been considered as more beneficial due to the competition for adhering sites with pathogens and the production of antimicrobial compounds (such as hydrogen peroxide, lactic acid and bacteriocin-like substances) that helps sustaining a healthy microbiome, while *Lactobacillus iners* was more often found in bacterial vaginosis [43]. Also *Megasphaera elsdenii* and *Shuttleworthia satelles* were generally associated with vaginosis, but not much is known of these two microorganisms. Both these bacteria were mainly found in the SIL cases, as well as *Sneathia spp*. which was predominant. On the other hand, in the cervical cancer cases, *Fusobacterium spp.* was predominant. These are both bacteria of the phylum Fusobacteria and this phylum is a possible microbiological biomarker for HPV infections [25].

In specific, *Fusobacterium spp.* are part of the healthy oral and gut microbiome, but they also have been noticed as opportunistic pathogens in inflammatory diseases at both sites. These species have even been associated with colorectal cancer by modulating the E-cadherin/β-catenin pathway and activating NF-κB. This leads to changes in the environment and thus *Fusobacterium spp.* might be causing a immunosuppressive environment, dominated by anti-inflammatory cytokines (IL-4, IL-10, etc.) and T cells (Treg cells, Th2 cells, etc.). Other bacteria, like *Atopobium vaginae* and *Gardnerella vaginae,* can also affect the immune system inducing a higher expression and secretion of these T cells by a chemotactic cytokine called regulated on activation, normal T-cell expressed and secreted (RANTES). This cytokine recruits T cells and activates NK cells amongst many other things [44]. Furthermore, several factors contribute to this immunosuppressive environment stimulating the cervical cancer development. For instance a HPV infection, with the oncoproteins E6/E7, has shown a synergistic effect on cancer risk when combined with a risky microbial pattern [25, 26].

In addition, also dietary factors play a role [27]. The semi western diet (with more red meat and less vegetables and fruit) showed a synergistic effect combined with a microbial pattern of *Atopobium vaginae.* Also the amount of fibers and vitamins (A and C) have been associated with cervical cancer risk. An unhealthy diet might result in an imbalance of nutrients and lower concentrations of several vitamins. This lack of vitamins and nutrients can lead to DNA damage and affect the immune system thus resulting in a higher cervical cancer risk. When a woman with

this risky dietary pattern is infected by HPV, this might facilitate the incorporation of the HPV genes into the human genome and increase DNA damage and the presence of oncoproteins [27].
Fang et al. found that women with chronic endometritis and/or endometrial polyps showed a significantly different microbiome than the controls. Wether these endometrial polyps influence the microbiome or vice versa remains unclear. Another possibility is that both interactions occur. Several bacteria have been linked with a possible cell proliferation or apoptosis inhibiting effect, but they remain suggestions. Remarkable, *Lactobacillus* and *Bifidobacterium* (observed in the bile duct microbiome) are again suspects of increased carcinogenic risks [18].

General conclusions

Several differences in the cervical microbiome can be seen during cancer development, with specific characteristics for each stage. Many of these bacteria are not only associated with cancer, but are even thought to play a causative role. Other factors as dietary patterns and HPV infections are confirmed to be important risk factors. However, results are not always consistent results across studies because of various factors (menstrual cycle, hygiene practices, etiology, sample techniques etc.) influencing the cervical microbiome.
Furthermore, the healthy uterus is not a sterile place. The intrauterine microbiome harbors many bacteria and shows clear differences compared to the cervical microbiome. The cervical and vaginal microbiome are showing an analogous composition.

Esophagus and gastric microbiome

Similarities and dissimilarities in results

Two studies investigating the esophageal microbiome and esophagal disease found that this microbiome was dominated by Proteobacteria and Firmicutes. There were no clear differences between the cases and the controls, including no changed abundance of *Streptococcus* or *Prevotella* in the cases [12, 20]. Other studies did find these changes in the cases with a BE [45]. In the included studies, this lack of a difference between the groups is remarkable since most microbiomes do show significant changes when a pathologic process is evolving [46].
Amir investigated cases with Barret esophagus by studying their gastric fluid while Nasrollahzadeh used stomach biopsies of cases with esophageal cancer [20, 21]. Hence, their findings were rather different. Nasrollahzadeh noticed the same predominant phyla i.e. Firmicutes, Bacteroidetes and Proteobacteria, but in another order of frequency. Also at genera level, they observed different changes: an increase of Enterobacteriaceae and Methylobacteriaceae with a decrease of

Pasteurellaceae and *Porphyromonas* [20] versus an increase of Clostridiales and Erysipelotrichales with a decrease of Helicobacteriaceae [21]. Besides the difference in characteristics of the cases and the samples, additional factors for these diverse results might be due to the studied population (study of Australia [20] versus study of Iran [21]) or the use of primers targeting other 16S rRNA gene regions (region V6-V7 [20] versus V3-V4 [21]). Furthermore, some bacteria were found in the gastric fluid that are also common in the oral microbiome (for instance, Streptococcaceae and Veillonellaceae) [20].

The gastric microbiome of the cases with gastric cancer showed a significant difference compared to the controls, even if these control samples were taken within the same cancer patient [19, 28]. Furthermore, this difference remained when *Helicobacter pylori* was not taken into account [19]. The phyla Firmicutes and Proteobacteria were again found predominant. At genera level, this were Lachnospiraceae (from Clostridiales) and Streptococcaceae [28]. The Mexican study found twelve species that were changed across the groups. Five of them showed a decreasing trend from cases with NAG to IM to gastric cancer (amongst them *Neisseria* and *Porphyromonas*), while two showed the opposite trend by increasing towards the gastric cancer (i.e. *Lactobacillus coleohominis* and Lachnospiraceae) [28]. Next, a Korean study found a decrease of *Helicobacter pylori*, *Propionibacterium* spp., *Staphylococcus* spp. and *Corynebacterium* spp. in the gastric cancer cases and an increase of *Clostridium* and *Prevotella* [19]. Only the increase of *Clostridum* might be seen as similar to the Mexican study since it belongs to the Clostridiaceae family. As well the Clostridiaceae as the Lachnospiraceae belong to the order of Clostridiales. So in both studies, one family of the Clostridiales was increased in the cases [19, 28]. Both studies used 16S RNA gene analysis but no regions were mentioned. Additionally, a Japanese study focused on *Campylobacter pylori* (renamed as *Helicobacter pylori* in 1989). This bacteria was found in 85% of all the biopsies of the subjects, but in a significantly higher abundance in those patients with ulcers. There was a higher detection rate in gastric cancers of *H. pylori* but due to small sample size, this was not significant. The gastric fluid of the controls consisted of Lactobacilli and facultative anaerobes, while in the gastric cancer groups it consisted of *H. pylori* (present at any pH level), the facultative and obligate anaerobes. The latter group showed a higher total bacterial count [13].

Comparison with other reviews

Since the discovery of the importance of *Helicobacter pylori* in the development of gastric cancer, several studies started to investigate the possible relation between certain microbiota and gastric cancer. For the totality of this review, a comparison with other reviews and their results was made.

An Australian article stated that positive serology for *H. pylori* increases the cancer risk with a RR of 5.91 (CI 3.41 – 10.3) and 74% of the non-cardia gastric cancers were attributable to *H. pylori.* But also other bacteria might be important in the carcinogenesis. The microbiome of these gastric cancer cases showed a predominance of Firmicutes and at genera level mainly *Streptococcus*, *Lactobacillus*, *Veillonella* and *Prevotella* [47]. In consistency, another review noticed a higher prevalence of *Streptococcus parasanguinis*, *Streptococcus mitis*, *Lactobacillus*, *Veillonella* and *Prevotella* in the cases, but a lower prevalence of Helicobacteraceae. Although there should be mentioned that these reviews are partly based on the same original studies. Furthermore, this latter review reported that in a recent study the gastric cancer cases showed a higher bacterial load with an increase of *Escherichia*, *Shigella*, *Nitrospirae* and *Burkholderia fungorum* and as well of *Lactobacillus* and Lachnospiraceae. The presence of *H. pylori* barely changed the composition and relative proportions of the gastric microbiome [48]. Remarkably, the abundance of *H. pylori* was lower in the gastric cancer cases [19,47,48]. At last, Dias-Jácome et al. found the same most abundant phyla present in the gastric microbiome, mainly Proteobacteria and Firmicutes. Although *H. pylori* is the strongest risk factor for gastric cancer, they focused on the role of non-*Helicobacter pylori* bacteria in the tumor development. From their analysis of thirteen cross-sectional studies, they concluded that the gastric carcinogenesis might be associated with an increase of many bacteria, for instance *Lactobacillus coleohominis*, *Klebsiella pneumonia* or *Actinobacter baumannii*, as well as a decrease of many others, like *Porphyromonas*, *Neisseria*, *Prevotella pallens* or *Streptococcus sinensis* (see appendix 8 for detailed results) [49]. This is partly consistent with the results from the included studies of this review, as mentioned above, but with a clear difference concerning *Prevotella*. The study of Seo mentioned that the abundance of *Prevotella* was increased in the cancer cases and previous reviews also mentioned this increase [19,47,48]. Hence, there is a lot of inconsistency among the different studies but among the reviews as well.

Possible underlying pathways

In the patients with heartburn, the reflux with gastric acid, bile acids and microbiota might contribute to inflammation in the esophagus [20]. Enterobacteriaceae might be responsible for this inflammation since they have also been associated with inflammatory bowel disease (IBD) and irritable bowel syndrome. They would act by activating Toll-like-receptors and stimulating TNF-α production, thus a pro-inflammatory environment arises [20, 50, 51]. Furthermore, little is known about the etiology of the changes in the esophagus. Several factors such as reflux and medication for example, might be influencing the esophageal microbiome therefore making clear associations difficult. An important note is that the esophageal microbiome would mainly be derived from the

oral microbiome, so oral hygiene and dental status could be playing a role as well [21, 46]. Additionally, the need of invasive techniques to recruit esophageal samples leads to limited research about this topic [46].

The most abundant family in the gastric microbiome was Lachnospiraceae [28]. This family consists of obligate anaerobes, which can only grow in an environment without oxygen. Genera of the Lachnospiraceae have been found in the mouth and the gut and they have been associated with a protective role against carcinogenesis. They have shown to decrease in patients with inflammatory diseases such as IBD due to increased bile acid levels and increased reactive oxygen stress caused by the inflammation. Their sensitivity to inflammation might make them a useful biomarker [52]. The gastric cancer cases showed a lower diversity but controversially, a higher abundance of Lachnospiraceae [28]. Therefore, further research of the specific species might be required or research of the mechanisms and interactions of Lachnospiraceae. On the other hand, *Lactobacillus coleohominis* was also increased in gastric cancer and was found before in the urine and the vaginal microbiome, but not specifically related with any diseases [28].
Helicobacter pylori and its role in gastric cancer has been studied extensively. Only 1-3% of the infected patients might develop a gastric cancer, mostly decades after the infection. *H. pylori* colonizes the gastric tissue and leads to an inflammatory process evoking a host immune response. This response leads to the release of interleukins and TNF-α changing the acidity in the stomach by inhibition of the parietal cells. Also bacterial enzymes contribute to this change, sustaining the survival of *H. pylori.* Then, during that colonization, *H. pylori* can break down the tight junctions between the gastric cells, invading the gastric mucosa and inserting their own genetic information into the cells. This suppresses the host immune response and leads to epigenetic changes as well as the production of VacA and CagA [53]. Additionally, also other bacteria can profit of these changes, showing interactions between *H. pylori* and others. First of all, the ammonia and bicarbonate produced by *H. pylori* can be used by other bacteria [19]. Secondly, when the pH rises, *Clostridium* has been noticed to increase as well [19]. Clostridiales in general, have been shown to change the pathogenicity of *H. pylori* by recruiting T cells to the gastric mucosa [21]. Thus the presence of Clostridiales or other bacteria might make the difference between carcinogenesis and chronic inflammation [21, 28]. This might also explain why there was a lower abundance of *H. pylori* in the cancer cases. In Japanese populations, *H. Pylori* has been investigated as well and has shown prevalence's of 39-76%. Those prevalences were significantly higher in patients with gastroduodenal diseases such as ulcera, gastritis and cancer [13].

The pathways of *Prevotella*, *Streptococcus* and *Porphyromonas* might also be relevant for the gastric and esophageal cancers, but they are mentioned and explained at the underlying pathways of the oral microbiome.

General conclusions

Concerning the esophageal microbiome, the studies investigate different populations and different samples leading to many different results. Standardized protocols are needed for sample collection and for gene analysis, as well as more research especially for the influencing factors.
Also in the gastric microbiomes, many changes have been observed and many bacteria have been associated to inflammatory processes. Only *Helicobacter pylori* has been studied extensively, but more bacteria might be involved and even needed in cancer development.

Laryngeal microbiome and laryngeal cancer

Similarities and dissimilarities in results

The laryngeal microbiome was dominated by Firmicutes, Fusobacteria, Bacteroidetes, Proteobacteria and Actinobacteria. At genera level, fifteen changed abundances were noticed with most importantly an increase of *Fusobacterium*, *Prevotella* and *Gemella* and a decrease in *Rothia* and *Streptococcus*. The abundance of *Prevotella* and *Solobacterium* was significantly higher in the cases with a T3-T4 tumor than the T1-T2 tumors [22].

Possible underlying pathways

Since the large overlap between the laryngeal and the oral microbiome (see Results), the underlying pathways will discussed at the section concerning the oral microbiome.

General conclusions

There is a large overlap between the laryngeal and oral microbiome. LSCC cases show clear differences in microflora compared to the controls and this disruption might be contributing to cancer development in a similar way as in the oral microbiome.

Lung microbiome and lung cancer

Similarities and dissimilarities in results

In Italy, 27.9% of the lung cancer cases were colonized by fungi i.e. *Aspergillus niger*, *Aspergillus ochraceus* and *Penicillum spp..* None of the controls showed this colonization. In the cases with colonization, no difference was found in smoking habits, pack years and time since quitting [29].

Another study of investigated the lung microbiome of female, never smoking cases from two Chinese cities. These cases showed a higher abundance of *Granulicatella*, *Abiotrophia* and *Streptococcus*. Furthermore, the microbiomes could be clearly separated. Comparing the inhabitants of the two cities, those who used smokeless coal in the households showed a higher abundance of Proteobacteria and *Neisseria*, but a lower abundance of *Bacilli* and *Streptococcus* compared to the other city using PAH-rich coal. No fungi colonization was mentioned [30].

Possible underlying pathways

Aspergillus was found in 17.6% of the lung cancer cases. Aspergillus is a mold that can grow in crops, dried fruits etc. and that can form conidia [29, 54]. These conidia are present in the air and can end up in the lungs by inhalation. Because of their small size, they can bypass the mucociliary clearance mechanisms of the respiratory epithelium. Thus they get phagocytosed by the alveolar macrophages in the terminal airways. Of course, these conidia can develop again in full grown hyphae when the circumstances are favorable, mostly in immunosuppressed patients. Hence, these *Aspergilli* present in the human lungs could start producing mycotoxins such as aflatoxin, ochratoxin A and fumonisins. Furthermore, some *Aspergilli* own β-glucan polymers on their surface which activate Dectin-1 signaling. Also several Toll-like receptors (TLR) get activated and all together, this leads to an inflammatory response in the lungs. Taken together, the toxins or the infection of *Aspergillus* itself both might contribute to carcinogenesis, but further research is recommended [29, 54-56].

The mechanisms of the other bacteria are much more unidentified. Since the inhabitants using PAH-rich coal show a higher risk of lung cancer and microbial differences between the inhabitants of the two cities have been reported, it is suggested the microbiota might play an important role in how our body interacts with environmental exposures. On the one hand the microbiome might influence the ability of the body to cope with these exposures while on the other hand, the environment can effect the composition and functions of this microbiome. Furthermore, *Granulicatella*, *Abiotrophia* and *Streptococcus* are all three seen as pathogens and since lung

cancer is driven by chronic inflammation, there might be an etiologic link. Of course, other studies are needed to confirm these findings and reveal the possible mechanism [30].

General conclusions

Only few studies have investigated the microbiome of the lung. The lung is not at all a sterile environment with clear differences between the microflora of the lung cancer cases and the healthy controls. The evolution and the effects of this cancer associated microbiome remain hypothetical and require further investigations to understand the possible mechanisms.

The oral microbiome

Similarities and dissimilarities in the results of the oral microbiome in relation to oral cancer

Two American studies found that the oral microbiome in cases and controls were similar to the laryngeal microbiome and dominated by Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria but in a slightly different order of frequency [10, 31]. The first American study found a decrease of Firmicutes and Actinobacteria in the oral cancer cases compared to the controls and at genus level, a decrease of *Streptococcus* and *Rothia*. This *Streptococcus* and *Rothia* decrease was also present in the pre-cancer cases and in the LSCC cases (as mentioned before) [22]. On the other hand, they saw an increase of the genus *Fusobacterium* at the tumor site compared to the adjacent normal tissue. For Bacteroidetes, there were no consistent changes noticed, but there was an association between the cases and a higher abundance. Also *Prevotella* differed inconsistently among the subjects. Taken together, there was a clear separation between the three groups: cancer cases, pre-cancer cases and healthy controls. There was no separation possible between the microbiome of the tumor site and adjacent tissue highlighting the inter-individual differences [31]. The other American study found different results. The cancer cases showed a lower diversity compared to healthy controls. Oppositely, they saw an increase of Firmicutes and they did find consistent changes of Bacteroidetes i.e. a decrease in the cancer cases. Furthermore, they noticed a decrease of Proteobacteria. At genus level, all samples were dominated by *Streptococcus* and *Prevotella*. The cases showed a higher abundance of *Lactobacillus*, *Veillonella* and *Streptococcus,* which is in contrast to the first study. Remarkably, the abundance of *Lactobacillus* increased and *Streptococcus* decreased with the progression of the TNM stages. Again the several groups could clearly be separated [10].

This relation of *Streptococcus* and the TNM staging might be a first explanation for the differences in results. A second reason for this inconsistency might lie in the sample method. Schmidt et al.

used oral swabs while Guerrero-Preston et al. used tumor samples and salivary rinses. Next, they investigated different cancer types i.e. oral cancer [31] versus head and neck squamous cell carcinoma [10]. Then, another reason might be the difference in 16S rRNA analysis i.e. the use of a primer targeting the V4 region [31] versus the V3-V5 region [10]. Finally, Schmidt used the normal adjacent tissue from the cancer patient as control samples while Guerrero-Preston used healthy controls and this might be a fifth explanation [10, 31].

The study of Henrich et al. investigated a unique population of two patients with FA and oral cancer as well as five healthy individuals and two patients with FA and benign leukoplakia. They found Tenericutes as one of the most abundant phyla in the oral microbiome of the FA cases with cancer, next to Bacteroidetes, Firmicutes and Proteobacteria. This Tenericutes was most abundant at the tumor surface, but was not detected by the previous two studies. The FA cases showed a lower α-diversity than the FA controls with leukoplakia, but there was a high β-diversity allowing to separate both groups. In agreement with Schmidt et al., they noticed a decrease of *Streptococcus* (but an increase of several *Streptococcus* species) and a decrease of *Rothia*. In the FA cases *P. salivae* and *Prevotella spp.* showed an increased abundance whereas two *Prevotella* species showed a decreased abundance (*P. melaninogenica* and *P. nanceiensis*). Furthermore, Mycoplasmataceae were increased in the cases and especially *M. salivarium* was present in all cases with the highest load at the tumor surface. This bacteria was not present or in low abundances in the controls. At last, both FA cases were positive for *Candida albicans* and only one control with leukoplakia [16]. This study also used oral swabs, similar to the study of Schmidt, but targeted the V1-V2 regions for 16S rRNA gene analysis [16].

Another study investigating the oral yeasts in the microbiome of patients with OSCC found a higher yeast colonization and a higher average fungal burden in those cases. Also there were more yeast cells at the tumor surface compared to the swabs of the healthy epithelium. The cancer cases showed a higher diversity, with *Candida* as predominant fungus [32].

Similarities and dissimilarities in the results of the oral acetaldehyde production in relation to oral, pharyngeal and laryngeal cancer

Homann et al. found that oral cancer cases did not have a significant different acetaldehyde production from the controls. Then, the subjects were separated into ‘high’ and ‘low’ acetaldehyde producers. The saliva of these high acetaldehyde producers showed a higher count of aerobic microorganisms i.e. *Corynebacterium spp*., *Stomatococcus spp*., *Streptococcus spp*. (similar to Guerrero-Preston [10]), and yeasts (similar to Berkovits [32] and Henrich [16]). Smoking and heavy alcohol intake were strong independent risk factors for a higher acetaldehyde production as shown

by regression analyses, however in this study there were no significant differences in the proportion of heavy drinkers or smokers between the 'high producers' and the 'low producers' [15].
Also Marttila et al. did not find a significant difference in acetaldehyde production between their oral cancer cases and controls. They did find higher counts of microorganisms in the OSCC cases mainly aerobic species, consistent with the findings of Homann et al, and at the tumor site, they also found a higher count of anaerobes. But the number of microbes was not related to the acetaldehyde production. Furthermore, there was a higher frequency and density of *Candida* in the cases and these samples with *Candida* colonization showed more frequently a high acetaldehyde production with mutagenic levels. At last, the acetaldehyde production in smokers was found significantly higher than in non-smokers [33].
A third study investigated the acetaldehyde production in cases with oral, pharyngeal or laryngeal cancer after ethanol incubation. They did notice a higher acetaldehyde production capacity in the cancer cases while the other two studies did not found a significant link. The study of Jokelainen did not find any difference in the acetaldehyde production capacity between smokers and non-smokers [14].
The differences in the results could be due to the diverse cancers of the cases. While two studies only investigated cases with oral cancer, i.e. OSCC [33] and general oral cancer [15] (while 90% of the oral cancers are OSCC [31]), one study investigated cases with oral, pharyngeal or laryngeal cancer [14]. Next, they also used different sample methods i.e. the filter paper sampling method [33], stimulated whole saliva [15] and mouth washings with a physiological saline [14]. All studies used head space gas chromatography for measuring salivary acetaldehyde levels. Remarkably, one study added ethanol to the microbiomes and only they found an increase in the acetaldehyde production capacity in the microbiome of the cases [14]. Yet, this capacity did not correlate with alcohol consumption, nor smoking behavior. Thus, the cancer cases are characterized by changes in their microbiome and these changes result in higher levels of acetaldehyde after ethanol consumption.

Similarities and dissimilarities in the results of the oral microbiome in relation to esophageal cancer

Chen et al. observed a lower diversity in the patients with ESCC. They found the same five most abundant phyla i.e. Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria and Actinobacteria (as mentioned above, similar to [31]). An increased abundance of *Prevotella*, *Streptococcus* and *Porphyromonas* was noticed while almost all the other genera decreased including *Veillonella*, *Rothia*, *Corynebacterium* and *Dialister*. Some of these changings are similar to results of the

studies investigating oral cancer cases (increase of *Prevotella* and *Streptoccus*, decrease of *Rothia*). Also there were highly significant differences in the microbiomes of the cases compared to the controls even after adjustment for possible confounders [34].

Similarities and dissimilarities in the results of the oral microbiome in relation to pancreatic cancer

The study of Torres and the study of Farrell both confirmed the five most abundant phyla as mentioned above in the oral microbiome [35, 36]. Firmicutes was the most divers phyla and *Streptococcus* the most diverse genus. Sixteen species of six genera were found to be different between the pancreatic cancer cases and healthy controls i.e. *Streptococcus*, *Prevotella*, *Campylobacter*, *Granulicatella*, *Atopobium* and *Neisseria*. *Neisseria elongata* and *Streptococcus mitis* were decreased in the cases, while *Granulicatella adiacens* was increased. Combinations of these biomarkers were capable of clinical separating the groups. For distinguishing the cancer cases with the non-cancer group, using *G. adiacens* and *S. mitis* showed a sensitivity of 85,7% and specificity of 52,7% [35]. So, the combination of these two biomarkers results in a small number of false negatives and thus could clarify whether further research is needed for a specific patient or not.

The second study also noticed a decrease of *Neisseria*, as well as a decrease of *Porphyromonas* and an increase of *Leptotrichia*, which were not mentioned in the previous study. The cancer cases showed a higher *Leptotrichia*/*Porphyromonas* ratio compared to the healthy controls. Next, they found a low β-diversity among the groups and no differences in the α-diversity. Finally, this second study did not observe the differences of the previous one regarding the abundances of *Streptococcus* and *Granulicatella* [36].

The only analogous result between these two studies was the decrease in *Neisseria*. All of their other changes abundances were different. Both studies were executed in the USA using unstimulated salivary samples to investigate the oral microbiome of the cases [35, 36]. But they each studied another control population i.e. thirty patients with chronic pancreatitis and thirty healthy controls [35] versus seventy-eight patients with other diseases (pancreatic diseases and non-pancreatic, also non-digestive diseases) and twenty-two healthy controls [36]. Also the number of cases was highly different: thirty pancreatic cancer cases [35] versus eight [36]. Finally, Farrell et al. matched their cases to the controls for age, gender and ethnicity. Their subjects had several ethnicities, mainly Caucasian but also African American, Asian and Hispanic [35]. Torres et al. did not match their cases nor corrected for possible confounders. However this might have been necessary since also their population had several ethnicities i.e. Caucasian, Hispanic, Asian and unknown but all of their cases were Caucasian or Hispanic [36].

The third study investigating this link, was a nested case-control study using samples of the patients before their diagnosis [9]. Microbiomes with *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans* were associated with a higher risk of pancreatic adenocarcinoma. In contrast, carriage of Fusobacteria or *Leptotrichia* was associated with a lower risk. The risks related to *Porphyromonas gingivalis* and *Leptotrichia* remained even after the exclusion of cases that developed their cancer in the following two years after sampling. This reduces the probability of reverse causation [9]. Thus, *Porphyromonas* is associated with a higher risk of cancer [9], but in the cancer cases itself, it is found in a lower abundance [36]. Furthermore, they also found a low β-diversity among the groups [9].

Similarities and dissimilarities in the results of the oral microbiome in relation to colorectal, lung and gastric cancer

Hu et al. found the usual phyla present in the oral microbiome: Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria and also TM7. The gastric cancer cases were divided into a thick tongue coating group (51,35%) and a thin (48,65%) while the controls all had a thin tongue coating. This thick group showed a lower diversity compared to the other groups. Furthermore, there were different genera characterizing each group i.e. thick group, thin group and controls. Overall, the gastric cancer cases showed a lower abundance of Proteobacteria and a higher abundance of Actinobacteria. At genera level, there was a decrease of *Neisseria*, *Haemophilus*, *Fusobacterium* and *Porphyromonas* [38]. This decrease of *Neisseria* was also found in pancreatic cancer cases [35, 36].

Another study investigated cases with colorectal, lung or gastric cancer. Again these cases showed significant differences in the physical characteristics of the tongue. Their microbiome showed a decrease of *Neisseria*, *Haemophilus*, *Fusobacterium* and *Porphyromonas*, completely similar to the study of Hu. There was also a decrease of two *Prevotella spp*. [39]. The decrease in Proteobacteria, *Neisseria* and *Porphyromonas* was also seen in the study of Torres researching pancreatic cancer cases [36].

The last study investigated cases with colorectal cancer. They found that also these cases had thicker tongue coatings than the controls. Nine of the fourteen cases had a thick tongue coating (64%) and these latter group showed a lower diversity, similar to the study of Hu. Furthermore, the thick tongue group had a higher abundance of *Prevotella*, *Leptotrichia* and *Actinomyces* but a lower abundance of *Gemella*. The thin group showed a lower abundance of *Veillonella* compared to the other groups. Several other species showed changed abundances among the groups but they were mainly different than the study of Hu (see Results). Overall, the CRC cases had higher numbers of

Streptococcus and lower numbers of *Haemophilus* [37]. Comparing again with the study of Torres, both found a higher abundance of *Leptotrichia* in the cases [36].
Remarkably, despite the differences in their cases two studies had very analogous results, but the third one showed different changings. Only the decrease in *Haemophilus* was consistent among the three studies. The first and most obvious reason might be the difference in cancers of the cases. Moreover, there was a clear difference in sample size i.e. 37 gastric cancer cases and 35 controls [38], 286 cases and 100 controls [39], versus 14 CRC cases and 7 controls [37]. Sampling methods and primers used for the 16S rRNA gene analysis were equal among all the studies.

Possible underlying pathways

The observed decrease of *Streptococcus* in pre-cancer and cancer cases might reflect the early changings of the oral mucosa surface because of the tumorigenesis [16, 31]. During the process, *Streptococci* might lose their ability to adhere to this mucosa while other species might adhere better, for instance *Fusobacterium*. These bacteria were increased in the cancer cases [31]. *Fusobacterium nucleatum* is reported to activate the nuclear translocation of NF-κB leading to IL-8 production and a pro-inflammatory environment. But this translocation is inhibited by *Streptococcus* thus attenuating the pro-inflammatory responses induced by *Fusobacterium*. In conclusion, this decrease of *Streptococcus* combined with an increase of *Fusobacterium* might play a role in oral cancers [57]. *Fusobacterium nucleatum* already has been associated with colorectal cancer. In the gut, it might increase the reactive oxygen species (ROS) production and IL-10 production leading to an inhibition of the T-cells and the antitumor immunity [58]. Furthermore, some *Streptococci* can inhibit the oral colonization of certain bacteria as A*ggregatibacter actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis* on the oral epithelial surfaces [57]. This highlights the importance of interactions between the microbiota themselves and not only between microbiota and the human mucosa [57].
Analogous, in the LSCC cases, there was a clear decrease of *Streptococcus* and an increase of *Fusobacterium* but as well an increase of *Prevotella*. The study of Gong et al. also mentioned the antagonistic role of *Streptococcus* against *Fusobacterium* and against *Prevotella* too. Thus these three microbiota act synergistically and competitively within the same niche. They suggested that *Prevotella* and *Fusobacterium* might be initiating the formation of biofilms at the normal respiratory epithelium. These biofilms stimulate inflammatory responses with cytokine releases resulting in the start of pathologic process [22]. Furthermore, both *Prevotella* and *Fusobacterium* are anaerobic bacteria. Thus when a tumor develops and grows quickly without yet the adequate blood and oxygen supply, these two bacteria can grow effortlessly in these hypoxic environment, mostly

necrotic regions, producing IL-8 and TNF [59, 60]. This might explain why cases with T3-T4 LSCC showed a higher abundance of *Prevotella* than the cases with T1-T2 tumors and why *Streptococcus* decreased among higher TNM stages [10, 22]. Taken together, *Prevotella* and *Fusobacterium* might not only initiate carcinogenesis by biofilm formation but also worsen the microenvironment which contributes to further cancer development and progression [22]. Nevertheless, the exact mechanisms of *Prevotella* are less studied and well known than these of *Fusobacterium*.
Mycoplasma salivarium was dominant at the tumor surface of the oral cancer cases with FA [16]. Usually, *M. salivarium* was considered as a non-pathogenic bacteria, but it has been reported in infected areas before, for instance periodontal infections, brain abscesses and septic arthritis [16, 61, 62]. Nolan et al. tried to reveal the possible impact and pathways of *M. salivarium* in patients with a suspected ventilator-acquired pneumonia. They found that blood monocytes incubated with this bacteria showed an attenuated effect of lipopolysaccharide (LPS) with a lower TNF-α production, a reduced reaction of the monocyte-derived macrophages and with impaired phagocytosis. This resulted in a reduced production of Il-6 and IL-10 (important for growth regulation), but an increased production of IL-8 (with chemotactic function) by the monocyte-derived macrophages. Hence, *M. salivarium* seems to suppress the ability of the immune cells to respond to the stimulation of LPS, but further research regarding this topic is needed [63]. Whether this bacteria could play a role in tumorigenesis remains unclear. Quirk et al. investigated a possible link between *M. salivarium* and ovarian cancer, but they did not find any association [64].

A common member of the normal oral microbiome is *Candida*. Although it is frequently reported in the oral cavity of healthy people, *Candida* has also been associated with several diseases, mainly infections [32]. In multiple studies, it has been found in a higher frequency and density in the oral cancer cases [16, 32, 33]. *Candida* is seen as an opportunistic fungus that not only promotes carcinogenesis but also metastasis. There are several pathways that are affected by this fungus: it triggers inflammation (with TNF-α, IL-18, etc.), induces a Th17 response that activates neutrophils, produces carcinogenic products (nitrosamines, acetaldehyde,…) and it takes part in molecular mimicry of the complement receptor stimulating cell growth and survival. Taken together, *Candida* might be forming a new therapeutic target to minimalize cancer risk [65].
An increased acetaldehyde production is one of the results of a *Candida* infection and has been repeatedly associated with oral cancer [14, 15, 33, 65]. Patients with a *Candida* colonization showed more frequently a high acetaldehyde production [33]. Acetaldehyde is the first metabolite of ethanol. The human liver as well as the kidneys and other organs can metabolize ethanol by alcohol dehydrogenases, but also microbes of the oral microbiome and others can convert ethanol

to acetaldehyde by oxidation [14, 15, 33, 65]. This acetaldehyde is considered toxic, mutagenic and carcinogenic. It can bind macromolecules and proteins creating acetaldehyde adducts. These adducts can act as neoantigens by interfering with normal cellular functions, leading to cellular destruction and causing inflammation [14, 65]. Next, acetaldehyde causes mitochondrial damage, activates the NF-κB pathway and reduces the glutathione antioxidant activity leading to higher concentrations of ROS and DNA damage [65]. Furthermore, this acetaldehyde production is largely influenced by genetics, smoking behavior and alcohol consumption [14, 15, 33, 65]. Patients with rapid metabolizing alcohol dehydrogenases have a quicker and higher production of this toxic metabolite. On the other hand, patients with a low aldehyde dehydrogenase (metabolizing the acetaldehyde) have a longer exposure to acetaldehyde. Patients who own both these enzymes, are associated with a higher risk of cancer in the upper gastrointestinal tract [15, 66]. Finally, smoking and alcohol consumption are seen as major risk factors for oral cancers. Smokers have an increased production (and acetaldehyde can even be present in the smoke) as well as heavy drinkers and these two factors have a synergistic effect on the acetaldehyde production [15, 33]. Thus on the one hand, when patients own this altered microbiome and have a high alcohol consumption, they might be exposed to high levels of acetaldehyde, seen as mutagenic and carcinogenic. On the other hand, some patients own this altered microbiome but don't have high alcohol consumption. These patients might have a higher vulnerability for the acetaldehyde levels on a genetic base.

In the pancreatic cancer cases, *Porphyromonas* is mentioned to play a role. *Porphyromonas gingivalis* is associated with a higher risk of pancreatic cancer [9] but in the cases itself, a decrease has been reported [36]. *P. gingivalis* is a known periodontal pathogenic [9, 36, 67]. There has been suggested that an initial increase of *Porphyromonas,* for instance in periodontitis, leads to activating the immune system and the production of antibodies. This inflammatory response leads to a decrease of the bacteria in the oral cavity but might result into pancreatic cancer [36]. The mechanism of *P. gingivalis* to initiate such an inflammatory response is being explored. The bacteria could invade the host cells and degrade receptors and cytokines resulting in a disruption of the signaling pathways. Furthermore, it could also activate Toll-like receptor pathways increasing (abnormal) systemic inflammation [9, 68]. Also *P. gingivalis* could lead to an increased production of nitrosamines, which are potent pancreatic carcinogens in animal studies [68]. Remarkably, the study of Michaud et al. found that the risk of pancreatic cancer of patients with a high concentration of these *P. gingivalis* antibodies (>200ng/ml) was twice as high as the risk of patients with lower concentrations [69].

Another frequently found bacteria in the pancreatic cancer cases was *Leptotrichia*. Leptotrichiaceae are commonly found in the healthy oral microbiome, but the role of *Leptotrichia* in the human mouth remains unclear. It is considered an opportunistic pathogen that already has been reported in several infections as mucositis, abscesses and endocarditis. It seems to contribute to diseases when other risk factors, local or systemic, are present [9, 70]. Proteins similar to LPS, have been observed at the surface of *Leptotrichia* leading to production of IL-1β, IL-6, IL-8, and IL-10. These interleukins play a role in cell differentiation, proliferation and apoptosis as well as in the cellular immune response. This over-activation of the immune system might lead to hemorrhage, fever, tumor necrosis, fatal shock, and septicemia. Taken together, *Leptotrichia* has many effects on the human body, however the mechanisms of how this bacteria could lead to pancreatic cancer, need further research [70].

General conclusions

Many bacteria can be found in the human oral cavity and these are not only associated with oral cancers but even with cancers of the lung, the esophagus, the stomach, the pancreas and the gut. While some microbiota have shown that they might be useful as biomarker, combined with other microbiota or used in a ratio, for screening healthy individuals and defining those most at risk, others have been reported to play a crucial role in the carcinogenesis. However, it stays a complex microbiome subject to many other environmental and genetic factors and thus, articles often show inconsistent results.

The skin microbiota and cancer cachexia

Similarities and dissimilarities in results

Only one study investigating this link was found. The cancer cachexia patients showed a lower diversity than the healthy controls with a decrease of Actinobacteria and a decrease of *Corynebacterium* at genera level [17]. Remarkably, there was also a decrease of *Corynebacterium* noticed in gastric cancer cases and esophageal cancer cases [19, 34].

Possible underlying pathways

Bacteria at the skin surface might be associated with cancer related cachexia. Several cytokines that have been associated with cancer (for instance IL-1, IL-8, IL-6 and TNF-α) have been identified in the human sweat. In response to these cytokines, our sweat produces antimicrobial products as cathelicidins, β-defensins and dermcidin, regulating the skin microflora [17, 71]. Bacteria as

Corynebacterium have shown to be susceptible to these antimicrobial peptides (AMP). Therefore, since cachexia has been linked to systemic inflammation, the concentration of these AMP's might be higher in the cachexia cases leading to a decrease of *Corynebacterium* [17]. *Corynebacterium* is a skin and nasal commensal. This bacteria is reported to interact with *Staphylococcus aureus* and diminish the virulence of this bacteria (by altering its gene expression). In the presence of *Corynebacterium*, *Staphylococcus aureus* showed an increased adhesion to epithelial cells and decreased hemolysin activity thus shifting from a pathogenic to a commensal bacteria [72]. Taken together, a decrease of *Corynebacterium* might make the patient more vulnerable than he already is worsening the prognosis. Furthermore, cachexia is related to serious malnutrition and hypoanabolism. This could create changes in the skin conditions and hence in the skin microflora. Thus, the changes in this microbiome are more likely to be a consequence of the cachexia instead of the cause [17]. But some skin bacteria might be related to the systemic inflammation contributing to cancer development as well as to the cachexia. However the mechanism of this interaction between bacteria and cachexia still needs to be explored.

General conclusion

The skin harbors a diverse and complex microbiome, where bacteria interact with each other. But little is known of the effects of these skin microbiota. They might be associated with plenty diseases but this association is not likely to be causal. Further research is needed to reveal the possible mechanisms and interactions of this microbiome.

The urine microbiota and breast cancer

Similarities and dissimilarities in results

Adlercreutz et al. found that postmenopausal cases with breast cancer had a lower enterolactone excretion than the control group. The enterodiol excretion was also lower when compared to the healthy vegetarian controls. Since fiber intake correlates with excretion of both the lignans, an enterolactone excretion to fiber intake ratio was calculated. The breast cancer cases showed a significantly lower ratio [11].

Possible underlying pathways

Fiber-rich plants contain many polyphenols for instance plant lignans. These lignans are present in several plants, vegetables, fruits etc. After digestion, their precursors can be metabolized by the gut bacteria into enterolactone and enterodiol. These are absorbed and again released in the urine

in a cyclic pattern during the menstrual cycle and during pregnancy (influenced by hormones). These lignans have been thought to possess anti-cancer effects. Cases with breast cancer have shown a lower excretion in the urine, regardless the fiber intake, thus lacking this protective effect. On the other hand, women with a western diet with a lower intake of lignans have shown a higher risk of developing breast cancer [11]. Taken together, enterolactones are suggested to lower the risk of breast cancer [11, 73]. Their mechanism however needs further research. Some suggest that enterolactones could affect estrogen receptors acting as antiestrogens, while others think they do not induce the classical estrogenic nor the antiestrogenic effect in female reproductive organs. In vitro studies have shown that enterolactones can regulate the proliferation of estrogen-sensitive cells, but others found that the in vivo binding affinity between enterolactones and the estrogen receptors is rather low [73]. Further exploration of this association is strongly recommended since enterolactones could easily be enrolled as a biomarker or for the prevention of breast cancer when enough evidence for these interventions is found.

General conclusion

A high lignan concentration has often been associated with a reduced risk of breast cancer. The underlying pathway is being investigated, but no clear mechanisms have been found yet. This study did not measure the gut microbiome but, since they metabolize and produce the lignans, the gut microbiota certainly might be involved. This illustrates how the microbiome might not only affect the habitat that they are colonizing, but even far beyond.

General conclusion

The combination of a specific microbiome with malignant potential and a human barrier breach could be the main ingredients for a damaging outcome as cancer. In this way, the microbiome can damage host cells and influence the immune response to promote tumorigenesis.

On the one hand, the bacterial composition of the microbiome plays an important role. Changes in this composition are clearly related to several diseases including cancer. In almost every study and for every microbiome, bacterial changes were noticed in the diseased cases compared to the healthy controls. In some articles the difference in abundance and presence/absence of bacteria was enormous. For example, the study of Aviles-Jimenez found twenty-six OTU's showing a different abundance in the patients with extrahepatic cholangiocarcinoma compared to the healthy

controls [23]. The difficulty here lies in distinguishing those changes that are a clear contribution to the development of cancer and the banal changes that are not. Understanding the effects of the several bacteria and their underlying pathways will help making this distinction, but these are not always identified. Another important note here is that cancer development has a long time axis. So, one bacteria might be important at the beginning of that time axis initiating several processes but become meaningless once these processes are evolving. Understanding the time axis of cancer development as well as knowing the moment and the method of microbiome influences would be a great advantage for developing clinical uses. This requires large and longitudinal studies with an intensive follow-up of the subjects and molecular studies for revealing the pathways.

Despite all these studies, the cancer-microbe causality stays challenging. Some studies tried to reveal this causality by using cases with cancer as well as cases with the premalignant forms, looking for a specific trend developing from the healthy controls to the premalignant cases and finally the cancer cases. For example, the study of Aviles-Jimenez found five taxa of the gastric microbiome that showed a decrease from the healthy controls to the cases with intestinal metaplasia to the cases with gastric cancer and two taxa that showed an increasing trend across these groups [28]. This might be an interesting and useful solution for some microbiome-cancer relations if such a trend is found. Because when one specific species (and no other species of this family or phylum) increases more when the cancer develops further, it is very suggestive for a causal role of that bacteria. But this is not always the case. Researching the cervical microbiome, Audirac-Chalifour found three specific bacteria present in the cases with squamous intraepithelial lesions and absent in the healthy controls. However, in the cases with cervical cancer, these bacteria were present in a decreased abundance or not present at all, while another bacteria (*Fusobacterium necrophorum*) suddenly makes his appearance in these cancer cases [25]. When different bacteria are present in the premalignant cases compared to the cancer cases or the healthy controls and thus no clear trend is seen, the question of cancer-microbe causality remains: Was the change in microbiome present before the cancer, or vice versa?

This highlights again the importance of knowing the underlying pathways. Many different pathways have been described and suggested, some have become quite clear and well known, for example the effect of *Candida* in the oral microbiome, while many others are still unrevealed, for instance the mechanism of *Prevotella* and how it interacts with other bacteria. These mechanisms that are responsible for the evolution of a healthy tissue to cancer development, and the involvement of specific bacteria in that evolution might be necessary to know and understand before any of these findings can be translated into the clinical practice of every day. Thus further research on these underlying pathways is strongly recommended. And these pathways can be complex. Besides,

there is not always one microbe responsible for one disease since the composition of the microbiomes remains a complex and dynamic entity. For this reason some studies not only compare the bacteria themselves between cases and controls, but they also form ratios or clusters/communities of several different bacteria. This might be a more realistic vision on the relation between the microbiome and cancer development where microbiota do not only interact with the human body but also with each other.
Furthermore, not only the presence of certain specific bacteria in the microbiome of the cases is interesting, but since many bacteria have beneficial and protective effects on the human body also their absence is useful to know. Further research could reveal whether giving these missing microorganisms (or products that will stimulate their growth) to the diseased patients will help to halter the tumorigenesis and maybe cure the patients, for illustration using a lotion that contains *Corynebacterium spp.* for patients with cancer cachexia.

On the other hand, the composition of the microbiome is mostly not the only cause of cancer. Many factors can contribute to barrier breakdowns and cancer development by affecting the microbiome or affecting the human cells or even both. This could make the person more vulnerable for the effects of several bacteria or amplifying their impact. For instance, a semi-Western diet together with an *Atopobium vaginae*-dominant cervical microbiome has shown a synergistic effect on the cervical cancer risk [27]. In other words, as Hosgood et al. describe it: '*Microbiota may influence the body's ability to process and respond to environmental exposures, and environmental conditions can influence the microbiota's composition and function*' [30]. Other examples of these environmental factors are alcohol consumption affecting the oral microbiome and the risk on oral cancer, atopic patients showing different skin microbiomes compared to healthy controls etc. [14, 15, 17, 33]. Altogether, these exposures can change the interaction between the microbiome and the human body. Since these factors also play an important role in the tumorigenesis, it would be useful to define them and their possible impact. Furthermore, these factors might be the hazards that are easy to change or to influence in such a way that it lowers the cancer risk in humans, for instance changing towards smokeless coal for the household heating in China.
Genetics might be a special one of these factors. These days, any person can order a DNA test online revealing their personal risks on several diseases in just a few days. Our genes define our human body and though the microbiota inside our body are not made out of our genes, our human DNA might also have an influence on the composition of the microbiome and its effects. This is illustrated by the variances in microbiome that are present in healthy individuals of different race

and ethnicity [74]. Thus, in diverse populations, bacteria might have diverse effects and an altered strategy for diagnosis and therapy might be needed depending on ethnicity of the patients.
Another current topic that has been extensively associated with cancer is obesity. Although some of the microbiome-cancer studies investigated the dietary patterns of the population, only six studies used in this review took obesity into account. None of the other studies checked the BMI of the population and executed a control or a correction for this possible confounder. Several studies are investigating a link between the gut microbiome and obesity, but what if obesity itself also changes the microbiome elsewhere?

This review investigated several microbiomes in relation to several cancers, but many more possible associations could be present in the human body (See appendix 9). For instance, hepatocellular carcinoma might be related with changes in the bile duct microbiome in a similar way as ECCA. Analogous, many tumors might be associated with changes in the microbiome that inhabits the organ or area where they are developing. This could be due to increased or decreased metabolites, effects on the host cells, activating certain inflammatory responses etc. Thus, there could be an association between the intrauterine microbiome and endometrium cancer, the skin microbiome and skin cancer (for example melanoma), the eye microbiome and intraocular cancer and so on. But also more distant relations might be present. Parallel to the link between oral microbiome and colorectal, lung or gastric cancer, the intrauterine microbiome might be related to ovarian cancer, known as ‘the silent killer’. Hence, when a relation between these two would have been established, the intrauterine microbiome could be used to screen for an ovarian cancer for example. This knowledge could make ‘the silent killer’, and maybe even other silent tumors, more easy to detect in the clinical practice since it would form a less expensive and less invasive strategy that might be lifesaving. At last, even less obvious associations might occur, for example, an association between the urine microbiome and leukemia. Since leukemia often affects the kidneys due to leukemic infiltration and metabolites of the leukemic cells such as phosphate and uric acid, this might be causing changes in the urinary tract and thus in the composition of the urine microbiome. On the one hand, the frequency of renal diseases depends on the type of leukemia but on the other hand they can also be a consequence of nephrotoxic drugs used during the therapy, for example Cyclosporine A [75]. Thus, when the urine microbiome is shown to be related to leukemia, this might form an important tool that could contribute to an early diagnosis of the leukemia patients but also it might be useful for screening those with a high risk for the side effects of certain therapies. Of course, a lot of further investigation is needed to prove these links and the possibility of applying them in the clinical practice.

Of course, studying the microbiome also has its limitations. First of all, the studies used in this review each investigated very different populations. The studies were executed in many different places across the world and even when studies were conducted in the same countries, the ethnicity of the subjects could vary tremendously (mainly in the U.S.A.). Furthermore, the defining of the cases and controls sometimes show small differences that might have large microbiota changes as consequence. For instance, when researching the oral microbiome, some articles investigate cases with head and neck cancer in general, while others use cases with oral cancer and some others investigate OSCC in specific. Since these cases do not have precisely the same types of cancer (squamous cell carcinoma vs. adenocarcinoma for example), there might be differences in the microbiota causing them. Thus, further research of more specific cancer types might be required to increase the comparability between studies. Similar differences in nuances can be seen in the controls. Some articles compare the cases with healthy controls, others compare them with controls with benign pathologies and some others even use control samples of healthy tissue of the cases themselves. Secondly, the number of studies exploring the microbiome is limited, especially the bile duct microbiome, the lung microbiome, the skin microbiome and the urine microbiome are poorly investigated. Furthermore, elder studies using other techniques for identifying the microbiota than 16S rRNA gene analysis (i.e. using subcultures on several agar media) are very restricted in their results. Luckily, the interest and the number of studies concerning the microbiome and its relation to cancer risks is rising, as well as the use of the newer techniques. Thus, the limited number of studies on the one hand and the very diverse populations on the other hand, make it currently really complicated to draw definite conclusions. In addition, most of the studies showed a decent quality, especially for selection and exposure of the Newcastle – Ottawa quality assessment scale. However, the scores for comparability were quite divers: of the thirty-one articles, seven did not even fulfilled the criteria to obtain any star and six articles only received one. Taken together, not only the comparability between studies but also the comparability between cases and controls within a certain study remains a very complex task. Both aspects complicate the revelation of a clear link between specific microbiota and certain cancers.

In general, more research with the newer techniques for identifying the microbiota is recommended to obtain more detailed and correct information about the microbiomes and to make study results more comparable. Also protocols for a uniform sample collection with minor amounts of contaminants would be a great help. As mentioned above, research on the underlying pathways of

the interaction between the microbiome and cancer development remains crucial as well as the other factors that could influence this development. A next step will be searching for clinical implications based on this knowledge. And these options are huge. In a world where diagnostic tools and therapeutic interventions are becoming more and more expensive, new insights and low-cost methods are very valuable. The use of the information that the microbiome can provide us might form a less expensive and less invasive method to detect or cure patients with common and severe disease as cancer and many others. Even when there is no causal link between a bacteria and a certain cancer, those microbiota might still be useful as a therapeutic target or useful as a biomarker for diagnosis, prognosis or for identifying those most at risk for treatment-related complications.

Finally, the complexity of the structure, functions and interactions of the microbiome might not withdraw researchers to explore this crucial part of the human body, but this complexity should be seen as the amount of possibilities that the microbiome has to offer.

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APPENDICES

APPENDIX 1. EMBASE search strategy

#1 Neoplasm
#2 Cancer
#3 Microbiome
#4 #1 or #2 and #3

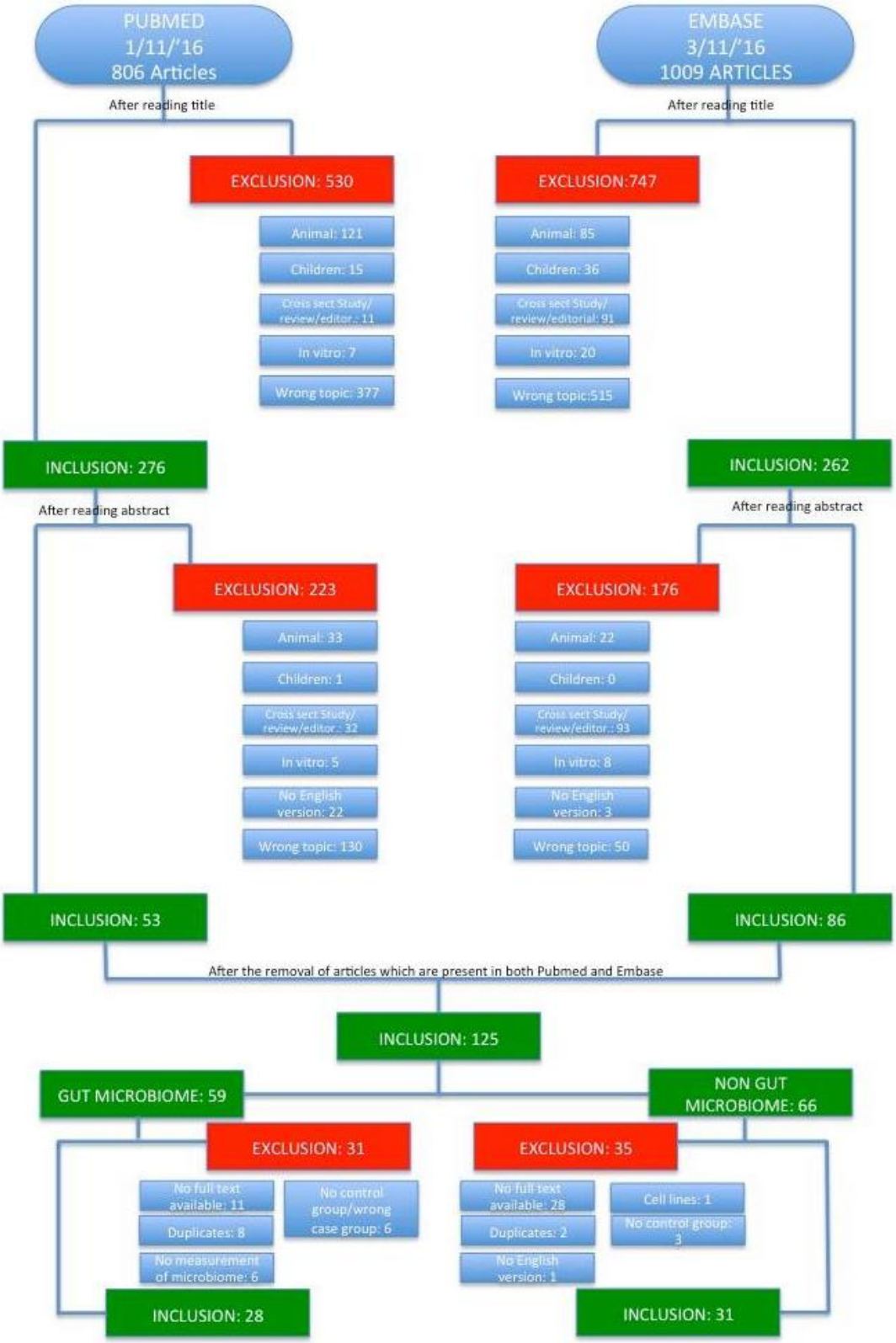
APPENDIX 2. PubMed search strategy

#1 Neoplasms[MeSH Terms]
#2 cancer
#3 microbiome[MeSH Terms]
#4 microflora
#5 review[Publication Type]
#6 infection[Title/Abstract]
#7 infections[Title/Abstract]
#8 (#1 or #2) and (#3 or #4) not #5 not #6 not #7

APPENDIX 3. The Cochrane Library search strategy

#1 Intestinal bacteria
#2 cancer
#3 intestinal microbiome
#4 #2 and #3
#5 neoplasm
#6 neoplasia
#7 microbiome
#8 microflora
#9 microbiota
#10 (#2 or #5 or #6) and (#7 or #8 or#9)

APPENDIX 4. Flowchart



APPENDIX 5. Phyla and bacteria

ACTINOBACTERIA

Atopobium
Bifidobacteriaceae
Corynebacterium spp
Dietziaceae
Gardnerella
Nesterenkonia
Propionibacteirum
Rothia
Stomatococcus

BACTEROIDETES

Bacteroides
Porphyromonas
Prevotella

FIRMICUTES

Clostridium
Dialister
Granulicatella
Lactobacillus
Lactococcus
Lachnospiraceae
Megasphaera
Oribacterium
Shuttleworthia
Staphylococcus
Streptococcus

PROTEOBACTERIA

Aggregatibacter
Actinobacter
Campylobacter
Enterobacteriaceae
Haemophilus
Helicobacter
Klebsiella
Methylophilaceae
Mesorhizobium
Neisseria
Oxalobacteraceae
Pasteurellaceae
Pseudomonadaceae
Sphingomonadaceae
Stenotrophomonas

FUSOBACTERIA

Leptotrichiae (Sneathia spp.)

TENERICUTES

Mycoplasma

ACIDOBACTERIA

PLANCTOMYCETES

APPENDIX 6. The design of the articles

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Aviles-Jimenez, 2016 Case control	Cholangiocarcinoma cases	100 pat. with extrahepatic cholangiocarcinoma (ECCA) and 100 pat. with benign pathology of the common bile duct (BBP).	(ECCA) 66,2 years [range 50-82 years], (BBP) 53,1 years [range 23-83].	Mexico	Case-control matching for sex, age (± 5 years) and place of residence + clinical variables incl. the time of evolution of lithiasis, H. bilis/ H. hepaticus infection, sex, age or body mass index.
Chng, 2016 Case control	Cholangiocarcinoma cases	60 pat. with cholangiocarcinoma (CCA) and adjacent matched normal samples , 5 non-cancer hepatic samples, 2 bile fluid samples and 4 non-cancer gastric mucosa samples (non-CCA). Of the (CCA), 28 pat. are Opisthorchis viverrine associated (OVa), and 32 pat. are not (non-OVa).	(CCA OVa) 57,9 years [range 38-71], (CCA non-OVa) 56,6 years [range 33-69]. For the other samples, mean age is 59,6 years [range 46-82].	Thailand, Singapore or Romenia	Case-control matching for age, gender and anatomical subtype.
Audirac-Chalifour, 2016 Case control	Cervical cancer cases	32 subjects: 8 with cervical cancer and HPV+ (CC), 4 with squamous intraepithelial lesions and HPV+ (SIL) and 20 healthy controls with no cervical lesions (H): 10 HPV- and 10 HPV+.	(CC) 43 years [±SD 11], (SIL) 40 years [±SD 14], (H) 34 years [±SD 8].	Mexico	Excluded: subjects with insufficient reads, subjects not fulfilling next inclusion criteria: patient´s recruitment on the same day of menstrual period, the non-use of douches and no sexual activity in previous days of the sampling, records of used medication in the last 30 days previous to sampling and molecular HPV+ diagnosis. Corrections for age, parity, contraceptive method and HPV-genotype.
Oh, 2015 Case control	Cervical cancer cases (CIN)	120 women: 70 with cervical intraepithelial neoplasia (CIN) 55 CIN1, 15 CIN2 or CIN3, and 50 healthy controls (H).	42 subjects younger than 39 years, 37 between 40 and 49 years, 30 subjects between 50 and 59 years and 12 subjects 60 years or older.	Korea	Inlcuded: currently sexually active or seeking birth control, not pregnant, intact uterus, no current referral for hysterectomy, no history of treatment for CIN within the previous 18 months. Excluded: history of gynecological cancers, insufficient data on the questionnaire, inadequate blood for evaluation, chronic disease, drug dependency, or psychological problems. Corrections for age, marital status, menopause, smoking status, oral anticonceptive use and histological grade but not significant.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Seo, 2016 Case control	Cervical cancer cases (CIN)	65 pat. with cervical intraepithelial neoplasia (CIN), from who 50 pat. With CIN1 and 15 with CIN2 or CIN3, and 72 healthy controls (H).	43,6 years [±SD 11,2].	South Korea	Included: sexually active, using birth control methods, not pregnant, an intact uterus, no illnesses requiring referral to hysterectomy, received no CIN treatment within the previous 18 months. Excluded: histories of gynecologic cancers, chronic diseases, drug dependencies, or psychological problems, insufficient data on the questionnaire or in specimens. Corrections for daily intake of nutrients, age, BMI, marital status, menopausal status, smoking status, alcohol drinking status, oncogenic HPV infection, monthly family income, parity and oral contraceptive use.
Fang, 2016 Case control	Chronic endometritis cases with endometrial polyps	20 women with endometrial polyps: 10 with chronic endometritis (EP/CE) and 10 without (EP), and 10 healthy controls (H).	(EP/CE) 35,2 years [±SD 1,83], (EP) 34,4 years [±SD 2,44] and (H) 30,9 years [±SD 1,56].	China	Excluded: intrauterine lesions, uterine myoma, endometriosis, ovarian tumor and hydrosalpinx, abnormal sex hormone level, abnormal leucorrhea, vaginitis or PID. Corrections for age, BMI, gravidity, parity, age of menarche, menstrual duration, menstrual average cycle.
Amir, 2014 Case control	Barret esophagus cases	34 pat. with heartburn: 13 with oesophagitis (OE), 6 with Barret's esophagus (BE) and 15 with normal-appearing esophagus mucosa (H).	(H) 44,46 years [range 18-66], (OE) 54,53 years [range 28-67], (BE) 63,16 years [range 49-80].	Australia	Excluded: subjects taking antibiotics or acid suppressive therapy during 2 months prior to endoscopy.
Mannell, 1983 Case control	Esophageal cancer cases	50 pat. with esophagus carcinoma (EC) and 51 healthy controls (H).	Not mentioned.	South Africa	/
Nasrollahzadeh, 2015 Case control	Esophageal cancer cases	91 subjects: 19 pat. with esophageal squamous cell carcinoma (ESCC), 18 pat with esophageal sqoamous dysplasia (ED), 17 pat with mid-esophageal espophagitis as the diseased controls (DC) and 37 healthy controls (HC).	(ESCC) and (ESD) 64,5 years [±SD 11,8], (DC) 63,6 years [±SD 14,0] and (HC) 62,1 [±SD 16,3].	Iran	Included: cases diagnosed with clinical Stage I–II ESCC + all patients diagnosed with ESD during study. Control groups were randomly selected from endoscopy clinic patients with the same referral pattern as cases, incl. healthy controls with endoscopically and histologically normal esophagus and diseased controls with histologic esophagitis in mid-esophageal biopsies. Excluded: samples < 1000 reads. Case-control matching for age and sex.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Aviles-Jiminez, 2014 Case control	Gastric cancer cases	15 pat.: 5 pat. with non-atrophic gastritis (NAG), 5 pat. with intestinal metaplasia (IM) and 5 pat. with an intestinal-type of gastric cancer (GC).	(NAG) 44 years [range 32-76], (IM) 67 years [range 60-71], (GC) 70,6 years [range 52-81].	Mexico	Excluded: pat. with immunodeficiencies, diabetes or other chronic diseases, pat. who received certain medication during the last three months, or pat. who previously received therapy for H. pylori eradication. Corrections for sex and age.
Seo, 2014 Case control	Gastric cancer cases	16 pat. with gastric cancer from who two samples each were taken: one sample of the gastric cancer (GC), one of the adjacent normal gastric mucosa (H).	67,18 years [range 37-75] for the 11 pat whos samples were used for statistical analyses.	South Korea	/
Inouye, 1989 Case control	Gastric cancer cases	103 pat. with several complaints (P) and 20 healthy controls (H).	(P) 50.8 years [range 18-79]. (H) 49 years [range 26-70].	Japan	Excluded: 58 specimen that consisted of only superficial or crushed fragments (of the total 318 biopsy specimen). Correction for age and gender.
Gong, 2013 Case control	Laryngeal cancer cases	29 pat. with laryngeal squamous cell carcinoma (LSCC), 31 controls with vocal cord polyps (H).	In the (LSCC) group, 11 patients were younger than or exactly 60 years while 18 patients were strictly older than 60. In (H), 131 patients were younger than or exactly 60 years while 18 patients were strictly older than 60 years.	China	Excluded: patients with a history of antibiotic use in the previous 3 months or active bacterial or viral infections in other parts of the body, controls not free of cancer and controls with evidence of epithelial dysplasia. No corrections, but each patient is his or her own control.
Carpagnano, 2014 Case control	Lung cancer cases	43 pat. with non-small cell lung cancer (NSCLC) and 21 healthy controls (H).	(NSCLC) 68,4 years [±SD 9,2], (H) 64,1 years [±SD 13,1].	Italy	Corrections for sex, age, histotype, stage, smoking habit, pack years, time since quitting smoking in subjects with fungal colonization.
Hosgood, 2014 Case control	Lung cancer cases	8 never smoking female lung cancer pat. (LC) and 8 never smoking female healthy controls (H).	The age range of (LC) and (H) was 45-72 years.	China	Included: cases aged 18–79 years at time of diagnosis, controls never been diagnosed with lung cancer. Controls were selected from never smoking female patients aged 18–79 years old, they were required to have admission diagnoses diseases and conditions that were unrelated to the study's primary hypotheses (but >20% of controls did not have any one condition). Subjects for this analysis were restricted to those residing in the Laibin and Reshui communities of Xuanwei. Case-control matching by age and hospital. Only never smokers were included.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Brian Schmidt, 2014 Case control	Oral cancer cases	For study 1 (discovery cohort): 5 pat. with oral cancer (OC1) and 5 healthy controls (H1). For study 2 (confirmation cohort): 10 pat. with oral cancer (OC2), 1 pat. with carcinoma in situ (CIS), 8 pat. with pre-cancer stages (PRE) and 20 healthy controls (H2).	(OC1) 69,2 years [range 62-84], (H1) no age mentioned . (OC2) 59 years [range 39-78], in (CIS) the pat. was 84 years, (PRE) 68,4 years [range 49-79] and (H2) 30 years [range 30-30].	U.S.A.	Excluded: The cancer pat. from study 1 were excluded from study 2. No corrections, but each patient is his or her own control.
Guerrero-Preston, 2016 Cohort study	Head and neck cancer cases	17 pat. with head and neck squamous cell carcinoma (HNSCC), 25 healthy controls (H). 11 pat. with an oropharyngeal squamous cell carcinom (OPSCC): 7 were HPV+ and 4 HPV-. 6 pat with an oral cavity squamous cell carcinom (OCSCC), all HPV-.	(OPSCC) mean age of 62 years, (OCSCC) mean age of 66. Mean age of controls is not mentioned.	U.S.A.	/
Berkovitz, 2016 Case control	Oral cancer cases	60 pat.: 20 pat. (14 m. and 6 fem.) with oral squamous cell carcinoma (OSCC), 40 healthy controls (22 m. and 18 fem.)	(OSCC) 62 years [range 44-86], (H) 67 years [range 49-82].	Hungary	Excluded: controls not free of oral pathology.
Henrich, 2014 Case control	Oral cancer cases	2 pat. with Fanconi Anaemia and oral squamous cell cancer (FAC)+ (FAC2), 2 pat. with Fanconi Anaemia and benign oral lesion (FAB) + (FAB2), and 5 healthy controls (H).	(FAC) was 41 years, (FAC2) was 27 years, (FAB) was 27 years, (FAB2) was 33 years and (H) 45,8 years [range 32-43].	Germany	/
Homann, 2000 Case control	Oral cancer cases	326 volunteers: 26 pat. with a malignant tumor of the oral cavity (T), 64 alcoholics (A), 24 pat. seeking a dental examination or treatment (DE), 90 unemployed volunteers (UN) and 114 healthy volunteers (H).	93 of the participants younger than 41 years, 162 were between 41-58 years and 74 older than 58.	Finland	Excluded: ex-smokers with a cessation of less than 5 years, treatment with oral antiseptic or antibiotics in the past month, food or fluid intake, smoking or toothbrushing in the past 90 min., recent alchol intake or measurable amount of alcohol in the saliva by head space GC. Corrections for age, smoking, alcohol, tooth brushing, tooth loss, eating between meals, periodontitis, frequency of dentist visits, mouthwash use, dentures and self-reported dry mouth and burning mouth.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Marttila, 2013 Case control	Oral cancer cases	30 pat. with oral squamous cell carcinoma (OSCC), 30 pat. with oral lichenoid disease (OLD) and 30 healhy controls (H).	(OSCC) 65,6 years [range 39-85], (OLD) 54 years [range 24-74] and (H) 30,4 years [range 19-56].	Finland	Excluded: patients with antimicrobial therapy within the past 7 days, those diagnosed with human immunodeficiency virus or hepatitis virus infection. Corrections for impact of drinking and smoking on acetaldehyde production.
Jokelainen, 1996 Case control	Oral cancer, laryngeal cancer and pharyngeal cancer cases	25 pat. with oral cavity, laryngeal or pharyngeal cancer (CA) and 28 healthy controls (H).	(CA) 61 years [range 39-96], (H) 57 years [range 27-75].	Finland	The dependency between acetaldehyde formation and degree of smoking and alcohol consumption was tested.
Chen, 2015 Case control	Esophageal cancer cases	87 pat. with esophageal squamous cell carcinoma (ESCC), 63 subjects with dysplasia (DYS) and 85 healthy controls (H).	(ESCC) 64,8 years [±SD 8,0], (DYS) 65,5 years [±SD 7,6], (H) 66 years [±SD 7,3].	China	Excluded: cases collected during Novembre of 2010 and March of 2011 to avoid confounders (i.e. ambient temperatures and different dietary habits during different seasons), cases with no histopathological confirmation, no complete questionnaire or no saliva samples. Case-control matching for sex and age. Adjustment for education, smoking, alcohol drinking, family history of ESCC, MFT, times of tooth brushing per day, daily consumption of pickled vegetables and daily consumption of fresh fruits.
Fan, 2016 Prospective nested case control	Pancreatic cancer cases	CPS II cohort: 170 cases with primary pancreatic adenocarcinoma (PAD1), 170 matched controls (H1). PLCO: 191 cases with primary pacreatic adenocarcinoma (PAD2), 201 matched controls (H2).	(PAD1) 73,7 years [±SD 5,7], (H1) 73,7 years [±SD 5,7]. (PAD2) 63,8 years [±SD 5,2], (H2) 63,8 years [±SD 5,4].	U.S.A.	Excluded: cases with a history of cancer prior to pancreatic adencocarcinoma (except non-melanoma skin cancer), controls with cancer prior to selection. Case-control matching for age, sex, race and calendar year. Corrections for pancreatic cancer status, race, BMI, smoking status, alcohol consumption status and history of diabetes.
Farrell, 2011 Case control	Pancreatic cancer cases	For discovery phase: 10 pat. with pancreatic cancer (PC1) and 10 matched controls (H1). For independent validation phase: 28 pat. with pancreatic cancer (PC2), 28 matched controls (H2) 27 pat. with chronic pancreatitis (CP).	(PC1) 66,5 years [±SD 8,9], (H1) 66,4 years [±SD 10,5]. (PC2) 69,9 years [±SD 11,6], (H2) 65,1 years [±SD 10,1] and (CP) 57,8 years [±SD 11,0].	U.S.A.	Excluded: cases with evidence of locally advanced pancreas cancer due to arterial involvement or direct extension into adjacent organs, metastatic pancreatic cancer, chemotherapy or radiation therapy prior to saliva collection and a diagnosis of other malignancies within 5 years from the time of saliva collection. Case-control matching for age, gender and ethnicity. Corrections for smoking and drinking history.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Torres, 2015 Case control	Pancreatic cancer cases	108 pat.: 8 with pancreatic cancer (PC), 78 with other diseases (OD) and 22 healthy controls (H).	(PC) 71,1 years, other ages are not (clearly) mentioned.	U.S.A.	Excluded: participants undergoing active chemotherapy or radiation therapy or use of antibiotics two weeks prior to saliva collection or with invasive surgery in the past year. Included: healthy controls with no documented chronic digestive or non-digestive disease and a 5-year resolution of any previously documented digestive or non-digestive disease.
Han, 2014 Case control	Colorectal cancer cases	47 pat. with colorectal cancer (22 with rectal cancer, 25 with colon cancer) (CRC), 45 healthy controls (H). (CRC) was divided into a tick group, with 9 cases, and a thin group with 5 cases.	(CRC) 53,24 years [±SD 9,70], (H) 51,57 years [±SD 8,01], calculated for the prior bigger study group containing 45 pat. and 47 controls. Thick group: 53,78 years [±SD 14,43], thin group: 48,60 years [±SD 8,56], and the 7 healthy controls: 52,14 years [±SD 10,63].	China	Excluded: controls with gastrointestinal diseases, oral diseases, malignant tumor and cancer related symptoms in the last 2 years. Corrections for age, weight, smoking, hypertension and diabetes. Chemotherapy and surgical treatment are possible reasons for a thicker tongue coating.
Han, 2016 Case control	Colorectal cancer, lung cancer and gastric cancer cases	386 pat.: 90 pat. with colorectal cancer (CRC), 96 pat. with lung cancer (LC), 100 pat. with gastric cancer (GC) and 100 healthy controls (H).	(CRC) 55,45 years [±SD 11,55], (LC) 55,14 years [±SD 9,80], (GC) 56,20 years [±SD 10,24] and (H) 53,57 years [±SD 8,32].	China	Excluded: controls with digestive diseases, respiratory diseases, oral disease, malignant tumor and cancer related symptoms in the last two years. Corrections for age, sex, BMI, smoking status, hypertension and diabetes.
Hu, 2015 Case control	Gastric cancer cases	For tongue images, 74 pat. with gastric cancer (GC) and 72 healthy controls (H). For the samples, 34 pat. with gastric cancer, from who 16 had thin coatings (GCtn) and 18 had thick tongue coatings (GCtk), and 17 healthy controls (Hs).	(GC) 57,46 years [±SD 8,43], (H) 54,55 years [±SD 9,63].	China	Excluded: controls with stomach discomfort over the past three years, malignant tumours, oral diseases or gastric diseases; subjects that had used any antibiotics within the past two months. Corrections for chemotherapeutics and surgery, BMI, diabetes, hypertension, smoking and drinking.
Li, 2014 Case control	Cancer cachexia cases	70 pat. with cancer cachexie (CC) and 34 healthy controls (H).	(CC) range 39-82 years, (H) range 45-81 years.	China	Excluded: patients with pre-cachexia, refractory cachexia, or cachexia due to a disease other than cancer; healthy controls with no good health or with chronic metabolic diseases or current skin infections, who received any antibiotics until one month prior to the study. Correction for sex.

RESEARCH ARTICLE	DEFINITION OF THE CASES	SAMPLE SIZE	MEAN AGE OF PARTICPANTS	ORIGIN OF SUBJECTS	EXCLUDED AND CONFOUNDERS
Adlercreutz, 1982 Case control	Breast cancer cases	28 femal postmenopausal pat.: 10 omnivorous (O), 10 vegetarian (V) and 8 healthy w. with surgically removed breast cancer (H).	(O) 56.6 years [±SD 3,1], (V) 58.3 years[±SD 3,2], (H) 58.0 years [±SD 1,9]	U.S.A.	Excluded: subjects with major diseases, subjects treated with certain medication and subjects consuming large amounts of alcohol. Enterolactone excretion to fibre intake ratio were tested.

APPENDIX 7. The results of the articles

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Aviles-Jimenez, 2016 Case control	Epithelial cells of the bile duct	Bile duct microbiome	DNA extraction and 16S rRNA gene analysis of the V4 region + PCR.	All groups: mainly Proteobacteria. (ECCA) ↑ Fusobacteria, Acidobacteria and Planctomycetes, ↑ *Methylophilaceae, Fusobacterium, Prevotella, Helicobacter* and *Campylobacter,* ↓ *Nesterenkonia, Rothia* and *Mesorhizobium.*	Significant separation between (ECCA) and (BBP) was observed.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Chng, 2016 Case control	Tissue samples of the liver, of the bile duct, bile fluid samples and gastric mucosa samples.	Bile duct microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V6 region.	(CCA) dominated by Dietziaceae, Pseudomonadaceae, Oxalobacteraceae. (CCA) normal tissue ↑ Enterobacteriaceae, Lachnospiraceae, Sphingomonadaceae and Bifidobacteriaceae. (CCA) cancer tissue ↑ *Stenothrophomonas.*	Gastric tissue microbiome was clearly distinguishable from the bile duct. (CCA) tumor tissue ~ adjacent normal tissue. Significant differences with the controls.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Audirac-Chalifour, 2016 Case control	Cervical scraping swabs and fresh cell biopsies.	Cervical microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V4 region.	(CC) characterised by presence of *Sneathia spp.* and *Fusobacterium spp.* and absence of organisms from the Bifidobacteriaceae family. (CC) ↓ *Lactobacillus crispatus*, *Lactobacillus iners* and *Gardnerella vaginalis*, ↑ *Fusobacterium necrophorum.* (SIL) dominated by *Fusobacterium spp, Sneathia spp., Shuttleworhia satelles* and *Megasphaera spp.*	Higher alpha diversity in the (SIL) group and (CC) group than in the (H) + notably different beta diversity in every stage of cervical cancer.	Selection: ★★★★ Comparability: ★★ Exposure: ★
Oh, 2015 Case control	Cervical swab	Cervical microbiome	DNA extraction and 16S rRNA gene analysis of the V1-V3 region.	All groups: dominated by Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Tenericutes, Fusobacteria, and candidate division TM7. Predominance of *A. vaginae, L. iners and G. vaginalis* and paucity of *L. crispatus* = risky microbial pattern. Synergistic effect of risky microbial pattern with high risk HPV infection on CIN risk. Different *A. vaginae* and *L. crispatus* ratio between (H) and (CIN).	(CIN): higher numbers of OTU.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Seo, 2016 Case control	Cervical swab	Cervical microbiome	DNA extraction and 16S rRNA gene analysis of the V1-V3 region.	*Lactobacillus iners*-dominant microbial type B + *A. vaginae*-dominant microbial type C: ↑ risk of CIN. Synergistic effect between semi-Western diet and microbial type C. No synergistic effect between semi-Western diet and microbial type B.	Semi-Western diet: ↑ risk of CIN + synergistically ↑ risk with the dominance of *A. vaginae.*	Selection: ★★★★ Comparability: ★★ Exposure: ★★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Fang, 2016 Case control	Vaginal and endometrial samples	Intrauterine microbiome	DNA extraction and 16S rRNA gene analysis of the V4 region.	All vaginal samples: mainly Firmicutes and Actinobacteria, Lactobacillus, Gardnerella and Streptococcus. All intrauterine: mainly Proteobacteria, Firmicutes and Actinobacteria. Intrauterine of (EP) and (EP/CE): dominated by *Lactobacillus, Enterobacter, Pseudomonas, Gardnerella* and *Desulfosporosinus.* Intrauterine of (H): dominated by *Enterobacter, Pseudomonas, Lactobacillus, Desulfosporosinus, Ralstonia* and *Gardnerella.* Intrauterine of (EP/CE) ↑ Firmicutes and ↓ Proteobacteria. (EP) and (EP/CE): intrauterine ↑ *Lactobacillus, Bifidobacterium, Gardnerella, Streptococcus* and *Alteromonas,* ↓ *Pseudomonas.* (EP/ CE): ↓ *Enterobacter* and *Sphingomonas,* ↑ *Prevotella.*	The intrauterine microbiome significantly different of the vaginal microbiome. (EP/CE) higher beta diversity than (H) and (EP).	Selection: ★★ Comparability: ★★ Exposure: ★★
Amir, 2014 Case control	Gastric fluid and esophageal biopsies	Gastric microbiome and esofagus	DNA extraction and 16S rRNA gene analysis of the V6 and V7 region.	Esophageal biopsies of all groups: mainly Proteobacteria and Firmicutes. No clear trend separating normal and abnormal esophageal tissues. Gastric fluid of all groups: mainly Proteobacteria, Firmicutes and Bacteroidetes dominated in the gastric fluid of all groups. (BE) ↑Enterobacteriaceae and Methylobacteriaceae, ↓ Pasteurellaceae and Porphymonodaceae.	No significant differences between the esophageal biopsies of (H), (OE) and (BE). Significant differences between the gastric fluid of the patients versus the controls with heartburn.	Selection: ★★★★ Comparability: Exposure: ★★
Mannell, 1983 Case control	Esphageal aspirates	Esophageal microbiome	Incubated and then aerobic and anaerobic subculturing onto selective agar media. Indentified by their morphology and biochemical reactivity.	(H) and (EC): mainly *Streptococcus viridans, Haemophilus influenza and Neisseria catarrhalis*, *Klebsiella pneumoniae* and *Streptococcus group B*.	No significant difference in the number and type of bacterial species between (H) and (EC).	Selection: ★★★ Comparability: Exposure: ★
Nasrollahzadeh, 2015 Case control	Gastric tissue	Gastric microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V4 region.	All groups: mainly Firmicutes, Bacteroidetes and Proteobacteria. (EC) ↑ Clostridiales and Erysipelotrichales, ↓ Helicobacteraceae.	Significant differences in gastric mucosa of (ESCC) and (ESD) compared to (H).	Selection: ★★★ Comparability: ★ Exposure: ★★
Aviles-Jiminez, 2014 Case control	Gastric samples	Gastric microbiome	DNA extraction and 16S rRNA gene analysis (no region mentioned).	Gastric tissue of all groups: mainly Proteobacteria and Firmicutes. (GC) ↑*Lactobacillus coleohominis* and Lachnospiraceae, ↓ 2 TM7, 2 *Porphyromonas* and *Neisseria.*	Differences between (GC) and (NAG), but not between (IM) and (GC) or (IM) and (NAG). From (NAG) to (IM) to (GC), the microbiota diversity showed a trend to disminish.	Selection: ★★★★ Comparability: ★ Exposure: ★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Seo, 2014 Case control	Gastric tumor tissue and adjacent normal mucosa	Gastric microbiome	DNA extraction and high-throughput RNA sequencing (no further details mentioned).	11/16 samples dominated by H. pylori. (GC) ↓ *H. pylori, Propionibacteirum spp., Staphylococcus spp.*, and *Corynebacterium spp*. ↑ *Clostridium spp.* and *Prevotella spp.*	Significant differences between the (GC) and (H) tissue.	Selection: ★★ Comparability: Exposure: ★★
Inouye, 1989 Case control	Gastric tissue and fluid	Gastric microbiome	Incubated and then aerobic and anaerobic subculturing onto selective agar media. Identified by colonial morphology and gram staining, sometimes electron microscopy.	85% of overall patients: *C. pylori* present. Ulcer pat. ↑ detection rate, idem for (GC) but not significant. ↓ detection rate of *C. pylori* in gastric juice of pat. than in the mucosa. *C. pylori* present in all pH levels. ↑ total bacterial counts in gastric contents of the pat.	*C. pylori* was detected in 85% of overall patients. ↑ detection rates of *C. pylori* in ulcer pat.	Selection: ★★★ Comparability: ★ Exposure: ★★
Gong, 2013 Case control	Tissue samples of the larynx	Laryngeal microbiome	DNA extraction and 16S rRNA gene analysis of the V1-V3 region.	All samples: mainly Firmicutes, Fusobacteria, Bacteroidetes, Proteobacteria, and Actinobacteria (in that order of frequency) and at genera level *Streptococcus, Fusobacterium, Prevotella, Neisseria* and *Gemella.* (LSCC) ↑ *Fusobacterium, Prevotella* and *Gemella* and ↓ *Streptoccocus* and *Rothia. Prevotella* and Solobacterium were significantly more prevalent in T3-T4 tumors than T1-T2.	Significantly different microbiomes between (LSCC) and controls.	Selection: ★★★★ Comparability: Exposure: ★
Carpagnano, 2014 Case control	Exhaled breath condensate (EBC) and bronchial brushing.	Lung microbiome	Subculturing of the colonies and incubated using 3 different agars.	12 of the (NSCLC): colonized with fungi. 0 of the (H) colonized. Mostly *Aspergillus niger* (5), by *Aspergillus ochraceus* (3) and *Penicillum spp.* (4).	Fungal colonization by *Aspergillus niger*, *Aspergillus ochraceus* and *Penicillium ssp*. in the EBC of (NSCLC) and not of the (H).	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Hosgood, 2014 Case control	Buccal and sputum samples	Lung microbiome	DNA extraction and 16S rRNA gene analysis of the V1-V2 region.	All buccal samples: mainly Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Fusobacteria. Sputum of (LC): ↑ *Granulicatella, Abiothrophia* and *Streptococcus.* No difference in the lung microbiota between the controls from Laibin and of Reshui, but a difference in the cancer pat. from those two villages. Reshui: ↑ Proteobacteria, ↑ *Neisseria,* ↓ *Bacilli* and *Streptococcus.*	Clear differences between the sputum samples of (LC) and (H).	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Brian Schmidt, 2014 Case control	Oral swab	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V4 region.	All samples: mainly Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria. (OC2) ↑ Fusobacteria and Bacteroidetes, ↓ Firmicutes and Actinobacteria,↓ *Streptoccocus* and *Rothia,* ↑ *Fusobacterium* and *Prevotella.* (PRE) ↑ Bacteroidetes and ↓ *Streptoccocus.*	Significant differences between the groups and within the same patient (oral lesions vs. anatomically matched samples).	Selection: ★★★ Comparability: ★★ Exposure: ★★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Guerrero-Preston, 2016 Cohort study	Saliva and tumor samples	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V5 region.	All samples: mainly Firmicutes, Bacteroidetes, Protebacteria, Actinobacteria and Fusobacteria (in order of frequency in (H) group). (HNSCC) ↑ Firmicutes, ↓ Bacteroidetes and Proteobacteria, ↑ *Streptoccocus* and *Lactobacillus,* ↓ *Agreggatibacter, Lautropia, Haemophillus, Neisseria, Prevotella,* Gemellaceae and *Leptotricha.* (OCSCC) ↑ *Neisseria* and ↓ *Citrobacter* than (OPSCC).	(HNSCC): ↓ diversity. (OCSCC): ↑ diversity than (OPSCC).	Selection: ★★★ Comparability: ★ Exposure: ★★
Berkovitz, 2016 Case control	Oral swab	Oral microbiome	Yeast colonization first by agar plates and incubation, then by macro- and microscopic morphology, catalase test and CHROMagar Candida plates, and finally MALDI-TOF analysis.	All samples: predominant fungal genus was *Candida.* (OSCC) ↑ frequency of oral yeast colonization, more yeast cells and ↑ fungal burden.	(OSCC): ↑ diversity of fungi. No significant differences in lipase and protease activity.	Selection: ★★★★ Comparability: Exposure: ★★
Henrich, 2014 Case control	Oral swab	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V1-V2 region, as well as a Candida-specific qPCR.	All (FAC) samples: mainly Bacteroidetes, Firmicutes, Proteobacteria and Tenericutes. All (FAB) and (H): mainly Actinobacteria, Bacteriodetes, Firmicutes and Proteobacteria. (FAC) ↑ Mycoplasmataceae (M. salivarium), Pseudomonadaceae, *P. salivae* and *Prevotella spp*., ↓ *Streptococcus, Rothia mucilaginosa*. All (FAC): C*andida* positive, all other samples Canidida negative.	(FAC) ↓ diversity, microbiota is getting less diverse the more the samples are moving into a tumourous state.	Selection: ★ Comparability: Exposure: ★★
Homann, 2000 Case control	Saliva	Oral microbiome	Head space gas chromatography (GC) + several specific and non-specific agar edia.	High acetaldehyde producers (especially smokers and heavy drinkers): ↑ count of aerobes like *Streptococcus salivarius, Streptococcus viridans, Corynebacterium sp., Stomatococcus sp*., and yeasts (higher concentration + more frequently), also ↑ count of anaerobes. No bacterial species was significantly more frequent among the low producers.	Significant differences between high and low producers. Smoking and heavy alcohol intake are strong predictors of microbial acetaldehyde production.	Selection: ★★★ Comparability: ★★ Exposure: ★★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Marttila, 2013 Case control	Microbial sample of the oral mucosa	Oral microbiome	Gas chromatography + several agar media.	(OSCC) ↑ numbers of microbes, especially aerobic bacteria. Lesions site of the (OSCC): ↑ amount of anaerobic bacteria. Lesion sites of (OSCC) and (OLD): ↑ frequency and density of candidal colonization + significantly more frequently mutagenic amounts of acetaldehyde. Cultures producing mutagenic concentrations of acetaldehyde: ↑ *Candida* colonization.	The majority (68%) of the cultures from all groups: mutagenic levels of acetaldehyde. No correlation between acetaldehyde levels and total amount of cultivable microbes in any patient group or sample site. Smokers: ↑ mean acetaldehyde production. Non-smokers: ↑ diversity.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Jokelainen, 1996 Case control	Saliva	Oral microbiome	Head space gas chromatography.	(CA) ↑ acetaldehyde forming capacity. (CA) ↑ consumption of alcohol and cigarettes, ↓ dental status. No link between acetaldehyde producing capacity and ethanol consumption or degree of smoking.	(CA) ↑ acetaldehyde forming capacity.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Chen, 2015 Case control	Saliva	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V4 region.	All samples: mainly Bacteroidetes, Firmicutes, Protebacteria, Fusobacteria, and Actinobacteria (in order of frequency). (ESCC): ↑ *Prevotella, Streptococcus* and *Porphyromonas,* ↓ *Megasphaera, Aggregatibacter, Atopobium, Lautropia, Actinobacillus, Bulleidia, Catonella, Filifactor, Corynebacterium, TG5, Acholeplasma, Moryella, Butyrivibrio, Dialister, Peptococcus,* and *Cardiobacterim.* (ESCC) compared to (DYS) ↓*Lautropia, Bulleidia, Catonella, Corynebacterium, Moryella, Peptococcus* and *Cardiobacterium.*	Significant difference in OTU diversity and richness. (ESCC): ↓ microbial diversity. ↓ *Streptococcus* ~ ↑ TNM stage, ↑ *Lactobacillus* ~ ↑ TNM stages.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Fan, 2016 Prospective nested case control	Oral mouthwash	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V4 region.	*P.gingivalis*: associated with ↑ risk of panreatic cancer (OR 1,6) for low relative abundance and high relative abundance, thus showing a dose-response relationship. *A. actinomycetemcomitans*: associated with ↑ risk of pancreatic cancer (OR 2,20), more in ever-drinkers (OR 3,03) than in never-drinkers (OR 0,47). Fusobacteria and its genus *Leptotrichia:* associated with ↓ pancreatic cancer risk (OR 0,94).	Carriage of the periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitan*s, and ↓relative abundance of Fusobacteria and its genus *Leptotrichia,* are associated with subsequent risk of pancreatic cancer, unlikely due to smoking or other confounders.	Selection: ★★★★ Comparability: ★★ Exposure: ★★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Farrell, 2011 Case control	Saliva	Oral microbiome	DNA extraction and 16S rRNA gene analysis using universal primers + HOMIM array was used for profiling.	All samples: mainly Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria. (PC1): ↑ 31 species/clusters and ↓ 25. Potential biomarker candidates: *Streptococcus, Prevotella, Campylobacter, Granulicatella, Atopobium* and *Neisseria.* (PC2): ↓ *Streptococcus* and *Neisseria,* ↑ *Granulicatella.* (CP): ↓ *Streptococcus* and ↑ *Granulicatella,* compared to (PC2).	Significant differences between (PC) and (H). Using *Streptococcus* and *Neisseria* as biomarker: 96.4% sensitivity and 82.1% specificity for distinguishing patients with pancreatic cancer from healthy subjects. Using *Streptococcus* and *Granulicatella:* 85.7% sensitivity and 55.6% specificity.	Selection: ★★★ Comparability: ★★ Exposure: ★★
Torres, 2015 Case control	Saliva	Oral microbiome	DNA extraction and 16S rRNA gene analysis using a 'universal' bacterial primer 515F + qPCR.	All samples: mainly Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Fusobacteria. (PC): ↑ *Leptotrichia,* ↓ *Porphyromonas* and *Neisseria.* No difference in *Streptococcus* or *Granulicatella* between (PC) and (H). (PC): ↑ abundance ratio of *Neisseria* to *Porphyromonas.*	Significant differences in microbiome between (PC) and (H). No differences among the 3 main groups in beta diversity or alpha diversity. The *Leptotrichia* to *Porphyromonas* ratio as a potential diagnostic biomarker for pancreas cancer.	Selection: ★★★ Comparability: Exposure: ★★
Han, 2014 Case control	Tongue coating samples and images.	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V2-V4 region.	Thick (CRC): ↓ OTU's, ↑ *Prevotella, Leptotrichia* and *Actinomyces,* ↓ *Gemella,* compared to thin (CRC) and (H). Thin (CRC): ↓ *Veilonella.* compared to thick (CRC) and (H). General (CRC): ↑ *Streptoccocus* and ↓ *Haemophilius* than in (H).	Significant thicker tongue coating (CRC). Different bacteria depending the thickness of tongue coating.	Selection: ★★★ Comparability: ★★ Exposure: ★★
Han, 2016 Case control	Tongue coating samples and images.	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V2-V4 region.	Cancer group: ↓ *Neisseria, Haemophilus, Fusobacterium* and *Porphyromonas* (i.e. *Fusobacterium periodonticum, Haemophilus parainfluenzae, Peptostreptococcaceae bacterium, Prevotella aurantiaca, Prevotella salivae* and a *TM7).*	Significant ↑ mirror-like tongues and thicker tongue coating in the cancer group. Significant bacterial changes.	Selection: ★★★★ Comparability: ★★ Exposure: ★★
Hu, 2015 Case control	Tongue coating samples and images	Oral microbiome	DNA extraction and 16S rRNA gene analysis of the V2-V4 region.	All samples: mainly Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Fusobacteria, and TM7. (GC): ↑ Actinobacteria and ↓ Proteobacteria, ↓ *Fusobacterium, Neisseria, Haemophilus* and *Porphyromonas.* (GCtn): mainly *Prevotella, Veillonella, Leptotrichia, Lactococcus*, and *Streptococcus.* (GCtk): mainly *Prevotella, Streptococcus, Actinomyces, Veillonella*, and *Leptotrichia,* ↑ *Actinomyces* and *Streptococcus* compared to the others.	Significant difference in the thickness of tong coating. (GCtk) significantly ↓ microbial community diversity.	Selection: ★★★★ Comparability: ★★ Exposure: ★★

RESEARCH ARTICLE	ANALYSED SPECIMEN	ANALYSED MICROBIOME	MEASUREMENT OF THE MICROBIOME	MICROBAL COMPOSITION ALTERATION	OUTCOME	QUALITY OF THE ARTICLE
Li, 2014 Case control	Skin swab of the axillary fossae	Skin microbiome	DNA extraction and 16S rRNA gene analysis of the V3-V4 region.	All samples: mainly Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes, *Staphylococcus spp.* and *Corynebacterium spp*. (CC): ↓ number of OTU's, ↑ Firmicutes, Proteobacteria and Bacteroidetes, ↓ Actinobacteria (only significant one). (CC): ↑ *Staphylococcus spp.* and *Staphyloccocus epidermis*, ↓ *Corynebacterium spp. (*only significant one).	(CC): ↓ α diversity, ↑ intra-group similarity.	Selection: ★★★★ Comparability: ★ Exposure: ★★
Adlercreutz, 1982 Case control	Urine	Urine microbiome	Capillary gas chromatographic procedure (GC) and mass spectrometry.	(H) group: ↓ enterolactone excretion and enterodiol ecxretion and ↓ enterolactone excretion to fiber intake ratio.	Fibre intake ~ enterolactone and enterodiol secretion, not with equol excretion.	Selection: ★★★★ Comparability: ★ Exposure: ★★

APPENDIX 8. Results from the review of Dias-Jácome et al. [49]

Table II. Microbiota changes from healthy individuals to atrophic gastritis, intestinal metaplasia and gastric cancer stages

	AG	*IM*	*GC*	*Control group*	*References*
Proteobacteria					
Neisseria spp	↘	↘	↘	No	(22)
Haemophilus spp	↘	↘	↘	No	(22)
Bergeriella denitrificans	↘	↘	↘	No	(22)
Klebsiella pneumoniae			↑	Non-ulcer dyspepsia, peptic ulcer disease	(19)
Acinetobacter baumanii			↑	Non-ulcer dyspepsia, peptic ulcer disease	(19)
Epsilonproteobacteria	-	-	↓	No	(20)
Helicobacteriaceae	-	-	↓	No	(20)
Firmicutes					
Lachnospiraceae	↗	↗	↗	No	(22)
Bacilli	-	-	↑	No	(20)
Lactobacillus coleohominis	↗	↗	↗	No	(22)
Streptococacceae	-	-	↑	No	(20)
Streptococcus sinensis	↘	↘	↘	No	(22)
Bacteroidetes					
Porphyromonas spp	↘	↘	↘	No	(22)
Prevotella pallens	↘	↘	↘	No	(22)
Actinobacteria					
Actinomycetales	↑			Omeprazole patients	(25)
Other bacteria					
TM7 group	↘	↘	↘	No	(22)
Bulleidia spp	↘	↘	↘	No	(22)

-: No significant changes; ↑: An increase; ↓: A decrease; ↗: An increasing trend along the sequential stages of gastric carcinogenesis; ↘: A decreasing trend during the sequential stages of gastric carcinogenesis. Note that the arrows only describe which bacteria are increased or decreased in each stage of carcinogenesis, but do not quantitate the changes of specific bacteria. For more detailed information, please read the text or consult the references indicated in the table. AG: Atrophic gastritis; IM: Intestinal metaplasia; GC: Gastric cancer.

APPENDIX 9. Possible link between microbiomes and cancers

MICROBIOME	LINK WITH THE FOLLOWING CANCER(S)	INVESTIGATED
Bile duct microbiome	Cholangiocarcinoma	Yes
	Hepatocellullar cancer	No
Cervical microbiome	Cervical cancer	Yes
	Ovarian cancer	No
Intrauterine microbiome	Chronic endometritis	Yes
	Endometrium cancer	No
	Ovarian cancer	No
Vaginal microbiome	Vaginal cancer	No
	Endometrium cancer	No
	Ovarian cancer	No
Esophagus microbiome	Esophagus cancer	Yes
Gastric microbiome	Gastric cancer	Yes
	Esophagus cancer	Yes
Esophagus and gastric microbiome	Barret esophagus	Yes
Nasal and sinus microbiome	Nasopharyngeal cancer	No
	Skin cancer	No
Laryngeal microbiome	Laryngeal cancer	Yes
Lung microbiome	Lung cancer	Yes
Oral microbiome	Oral cancer	Yes
	Head and neck cancer	Yes
	Oral, pharyngeal and laryngeal cancer	Yes
	Esophagus cancer	Yes

Oral microbiome	Pancreatic cancer	Yes
	Gastric cancer	Yes
	Colorectal cancer	Yes
	Colorectal, lung and gastric cancer	Yes
Skin microbiome	Cancer cachexia	Yes
	Skin cancer (e.g. melanoma)	No
	Adrenal cancer	No
	Thyroid cancer	No
Ear microbiome	Skin cancer (e.g. basal-cell carcinoma)	No
	Head and neck cancer	No
Ocular microbiome	Intraocular cancer (e.g. retinoblastoma)	No
	Skin cancer	No
Urine microbiome	Breast cancer	Yes
	Cancers of the urinary tract	No
	Leukemia	No