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## Interest of studying the saliva metabolome, transcriptome and microbiome in screening for pancreatic cancer

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### Abstract

**Introduction:** Pancreatic cancer is a public health problem because its mortality rate is close to its incidence rate. If it were possible to detect this cancer before the onset of symptoms, 5-year survival could reach 75%. Numerous studies have attempted to accelerate the diagnosis to improve survival. Saliva presents interesting characteristics as a fluid for screening and diagnosis. Its many components provide a promising source of constitutive biomarkers with a specific signature of the disease.

The aim of this work was to determine the interest of studying the metabolome, the transcriptome and the microbiome of saliva in screening for pancreatic cancer.

**Materials and Methods:** A review of the literature was conducted using the PubMed search engine. The last search was conducted in July 2017.

**Results:** Nine references, all original studies, published between 2010 and 2017 were included.

**Discussion:** Different combinations of metabolites, RNA and bacteria were found. Analysis of the saliva transcriptome and metabolome seems to be the most promising avenue.

**Conclusion:** The identification of an early salivary signature of pancreatic cancer is still in its infancy and the results obtained here must be confirmed in larger prospective multicentre studies.

**Keywords:** biomarkers, saliva, screening, pancreatic neoplasm

## Introduction

Worldwide, about 200,000 people every year develop pancreatic cancer and 98% of these people die from this disease[1]. Median survival is 6 months [2]. When the diagnosis is made at an early stage, 5-year survival is greater than 20% and drops to 5% when the diagnosis is made at an advanced stage [3,4]. Pancreatic cancer is thus a public health problem because of its mortality rate which is close to its incidence rate [5]. In 2030, pancreatic cancer could become the second leading cause of death from cancer in the United States [6].

The symptoms that reveal this cancer are non-specific: epigastric abdominal pain and weight loss [6]. The clinical manifestations often occur at an advanced loco-regional or even metastatic stage [1,3,4]. At the loco-regional stage, only 15 to 20% of cancers are resectable at the time of the diagnosis [5,7,8]. Frequently, the treatment can thus be regarded as palliative radiochemotherapy [3,8].

If it were possible to detect the disease before the onset of symptoms, 5-year survival could reach 75% [3]. The disease could take 5 years to progress from the first tumour cells to the metastatic stage [3]. This window of opportunity could be propitious to early detection and perhaps curative treatment of the disease [3]. Several methods exist to detect pancreatic cancer early, but they are generally invasive and given the small number of patients concerned, they have neither the sensitivity nor specificity necessary to set up more generalised screening [3,6]. Currently, only the serum tumour antigen 19-9 [CA19-9] has shown any value, but only for the follow-up of patients undergoing treatment [3]. The discovery of non-invasive biomarkers would thus bring hope [3].

Saliva, a biological fluid that contributes to the lubrication of the oral cavity for speech and digestion, protects against micro-organisms and plays a role in social life, could be used as a non-invasive means of detection [1]. Saliva is composed of nucleic acids, electrolytes, proteins, hormones, medications and micro-organisms from local and systemic sources [2,9]. Saliva glands are highly vascularised [1]. This hypervascularisation allows the passage of numerous molecules from the blood to the saliva [1].

This could allow the analysis of saliva with regard to:

- The metabolome, which is the complete set of intracellular metabolites [1].
- The transcriptome, which corresponds to the sum total of all RNA molecules expressed by the genome at a precise moment in response to a given environment [10]. Studies often analysed messenger RNA (mRNA), microRNA (miRNA) and long non-coding RNA (lnc-RNA) [2,5,8–11].
- The microbiome, which is the genome coded by the microbiota [4]. Its composition varies depending on the genotype of the host, the host's physiological status, way of life and environment, the concomitant presence of micro-organisms, and the anatomical site [4].

Taking saliva samples is simple, non-invasive, inexpensive, acceptable and reproducible [1,2].

The aim of this work was to determine the interest of studying the metabolome, the transcriptome and the microbiome of saliva in screening for pancreatic cancer.

## **Materials and methods**

The search engine PubMed was used to search for articles written in English on the subject.

The first search was based on the following equations:

- Equation: [« pancreatic neoplasm » OR « pancreatic cancer » OR « pancreatic tumour »] AND [«saliva»].
- Equation: [« pancreatic neoplasm » OR « pancreatic cancer » OR « pancreatic tumour »] AND [« oral bacteria » OR « oral microbiota » OR « oral microbiome »].

This first search was completed by a second search using the followed MeSH combination of terms:

- Equation MeSH: [« pancreatic neoplasms » [Mesh]] AND [« saliva [Mesh]].
- Equation MeSH: [« pancreatic neoplasms » [Mesh]] AND [[« bacteria »[Mesh] AND « mouth »[Mesh]]] OR [« microbiota » [Mesh] AND « mouth» [Mesh]]].

The pertinence of the articles was evaluated from the title and/or the abstract before being selected. Only articles for which at least the abstract was available were retained. For duplicate publications, the most recent or the most pertinent publications were kept. No articles were excluded because of the level of evidence.

The search collected articles published up to 7th July 2017.

## **Results**

Of the 442 articles found using the equations, 34 met the inclusion criteria, were deemed pertinent and were thus selected for further evaluation. The selection of articles is shown in the flow chart (Figure 1).

Altogether, nine articles, all original studies and published between 2010 and 2017 were finally included. There were five case-control studies, three pilot studies and one transversal study. Six of the nine studies were carried out in the United States. The size of the studies ranged from 17 to 732 people and the detection methods varied [1,2,4,5,7–11]. The details of these studies, which analysed the discriminatory power of the saliva metabolome, transcriptome and microbiome profile to detect pancreatic cancer is reported in Table 1.

## **Discussion**

This study showed that screening saliva to detect pancreatic cancer could be based on the saliva metabolome, transcriptome and microbiome.

### **Saliva metabolome**

Metabolomics, that is to say analysis of the metabolome, has become a novel and powerful tool to better understand cell function [1].

A retrospective single-centre study showed that by coupling capillary electrophoresis with mass spectrophotometry to analyse saliva, it was possible to identify a specific saliva metabolite profile in patients with pancreatic cancer [1]. Of the eight metabolites found [1], five were essential amino acids (leucine, isoleucine, tryptophan, valine and phenylalanine) and the three others were non-essential amino acids (glutamic acid, glutamine and aspartic acid) [1]. However, the number of cases (n=18) was clearly insufficient to draw any conclusions.

### **Saliva transcriptome**

These studies concerning the specificity of the transcriptome in pancreatic cancer analysed messenger RNA (mRNA), microRNA (miRNA) and long non-coding RNA (lnc-RNA) [2,5,8–10].

#### **messenger RNA**

mRNA is present in the saliva supernatant [10]. It is relatively stable and could be a source of biomarkers that are discriminatory for pancreatic diseases [10]. Certain combinations of mRNA could serve as biomarkers for pancreatic cancer. For example, the association of four saliva mRNA found by Zhang et al may make it possible to distinguish between patients with pancreatic cancer and those without cancer (chronic pancreatitis and healthy subjects) (Se = 90%) [10]. These mRNA are: KRAS, MBD3L2, ACRV1, DPM1 [10]. More recently, Liu et al. identified 29 new saliva mRNA, from 516 genes related to pancreatic cancer (5.6%). The combination of these 29 genes presented better sensitivity (Se = 92%) than the four mRNA above to identify subjects with pancreatic cancer [8].

#### **micro-RNA**

miRNA are regulators of gene expression and present high stability in comparison with proteins or mRNA [2]. It is thus possible to quantify these miRNA in small quantities of material (fluid or tissue) or in material that has deteriorated [2]. Recent data show that miRNA profiles may be discriminatory concerning the presence of cancerous pancreatic tissue, the cancer prognosis and the response to treatment [2].

Combinations of miRNA (for example, four miRNA) could identify cancers of the pancreas with sensitivity ranging from 62.5% to 85.7% and specificity ranging from 70% to 100% [2,9]. The four miRNA mentioned are: hsa-miR-21, hsa-miR-23a, hsa-miR-23b and miR-29c. More particularly hsa-miR-23a and hsa-miR-23b, which are overexpressed in the

saliva of patients with pancreatic precursor lesions, are indicators of poor survival [2]. They appear to be expressed early in pancreatic carcinogenesis and may be able to regulate cellular autophagy associated with the radioresistance of pancreatic tumour cells [2]. Targeting these miRNA appears to lead to tumour regression in animal models and *in vivo* [2].

### **long non-coding RNA**

lnc-RNA are transcripts of more than 200 nucleotides which activate several biological processes such as the transcription, translation and expression of genes etc. [5]. They exhibit great tissue specificity, thus explaining their interest in the search for cancer biomarkers in bodily fluids like saliva [5]. Two saliva lnc-RNA, HOTAIR and PVT1, are significantly increased in pancreatic cancer patients compared with healthy subjects or patients with benign pancreatic tumours [5]. Levels of these lnc-RNA fall after surgical resection [5]. Salivary HOTAIR and PVT1 could have the potential to become novel non-invasive biomarkers for the detection of pancreatic cancer as their sensitivity and specificity can be as high as 97% [5]. The HOTAIR and PVT1 genes, which are located on chromosomes 12 and 8, respectively, have pro-oncogenic activity and correlate with a poor prognosis. They are known to play a role in several cancers notably breast cancer [5].

The study of the saliva transcriptome is promising. However, currently, the mechanisms underlying the specific genetic alterations related to tumour development are still unclear. The origin of the specific RNA related to pancreatic cancer and detected in the bloodstream is still unknown: from the lysis of tumour cells or active secretion by tumour cells [5]. Even though dozens of molecular biomarkers have been identified in the saliva to detect different diseases, the reasons for their presence and the relationship between the systemic diseases and the biomarkers in the saliva have not yet been established precisely [5]. Zhang et al. put forward the hypothesis that tumours produced mediators (hormones, cytokines) that could regulate the activities and the expression patterns of genes, DNA, RNA and proteins in distal organs, such as the saliva glands through the vascular system [10].

### **Saliva microbiome**

Because the microbiome is so varied, it is difficult to interpret. Recently, statistical associations have been found between different bacterial populations and pancreatic cancer [4,7,11]. It appears that this is related to a modification to all or part of the oral flora rather than the implication of a single group of bacteria [4,7,11]. For example, the increased risk of pancreatic cancer seems to be related to the presence of certain germs (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*), and a decreased risk in the presence of other germs (*Fusobacterium* genus and the *Leptotrichia* species) in the saliva [11].

As for the transcriptome, several studies have used associations of markers to obtain more robust results. The principal bacterial combinations that could play a role are *N. elongata* and *S. mitis*, *G. adiacens* and *S. mitis*, *Porphyromonas* and *Leptotrichia*. It could thus be possible to distinguish between patients with pancreatic cancer and healthy controls by determining the relative proportions of genera: high *Leptotrichia* to *Porphyromonas* ratio, and lower abundances of *Neisseria elongata* and *Streptococcus mitis* in pancreatic cancer patients

than in controls (Se =96.4%, Sp = 82.1%) [4,7]. In the same way, the combination of *Granulicatella adiacens* and *Streptococcus mitis* was able to distinguish between pancreatic cancer and chronic pancreatitis with sensitivity of 85.7% and specificity of 55.6% [7]. No validated bacterial biomarkers were significantly modified in the microbial profile of patients with lung cancer [7]. This comparison of diseases suggests that the validated salivary microbial biomarkers could be specific to pancreatic cancer [7].

The microbiota can vary so much (diet, smoking, alcohol consumption, hygiene, stress, ongoing treatment, dental infection, etc.) that several questions arise:

- Does the cancer modify the oral microbiota?
- Or do the modifications in the microbiome result from progression of the cancer?

Changes in the diversity of the salivary microbiome could be a multifactorial systemic response to pancreatic cancer [4]. Pancreatic cancer affects the immune system and could lead to the excessive proliferation of bacteria with the displacement of oral bacterial balance towards invasive pathogenic periodontal agents [4]. The proliferation of pathogenic bacteria could also contribute to cancer progression by decreasing immune defences and/or through systemic inflammation [4]. If this is the case, it suggests the presence of a vicious circle: pancreatic cancer would thus lead to the proliferation of pathogens, which would aggravate the disease by acting on the immune system. In contrast, Farrell et al. suggested causality rather than reactivity in the association between variations in the oral microbiome and the pancreatic disease [7].

For these reasons, the oral microbiome is the most difficult tool to use to evaluate pancreatic cancer.

## **Conclusions and perspectives**

The identification of an early salivary signature for pancreatic cancer involves several elements:

- The transcriptome, which seems to be the most promising tool; the identification of better combinations would be a major step forward.
- Saliva metabolic profiles: to identify one or several specific profiles. The ideal solution would be to determine a limited number of metabolites. These profiles could be more discriminatory if they were associated with blood profiles.
- The microbiome, which is probably the most difficult tool to understand and analyse.

Alterations of salivary DNA methylation could be an interesting track in this field.

As saliva is outside the body, it cannot reflect components of the blood. The two fluids, even though they are interactive, have very different functions.

Establishing a saliva profile, which could be an efficient screening strategy for pancreatic cancer, is still in its infancy and the results obtained so far must be confirmed in larger multicentre prospective studies.

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## Table and Figure

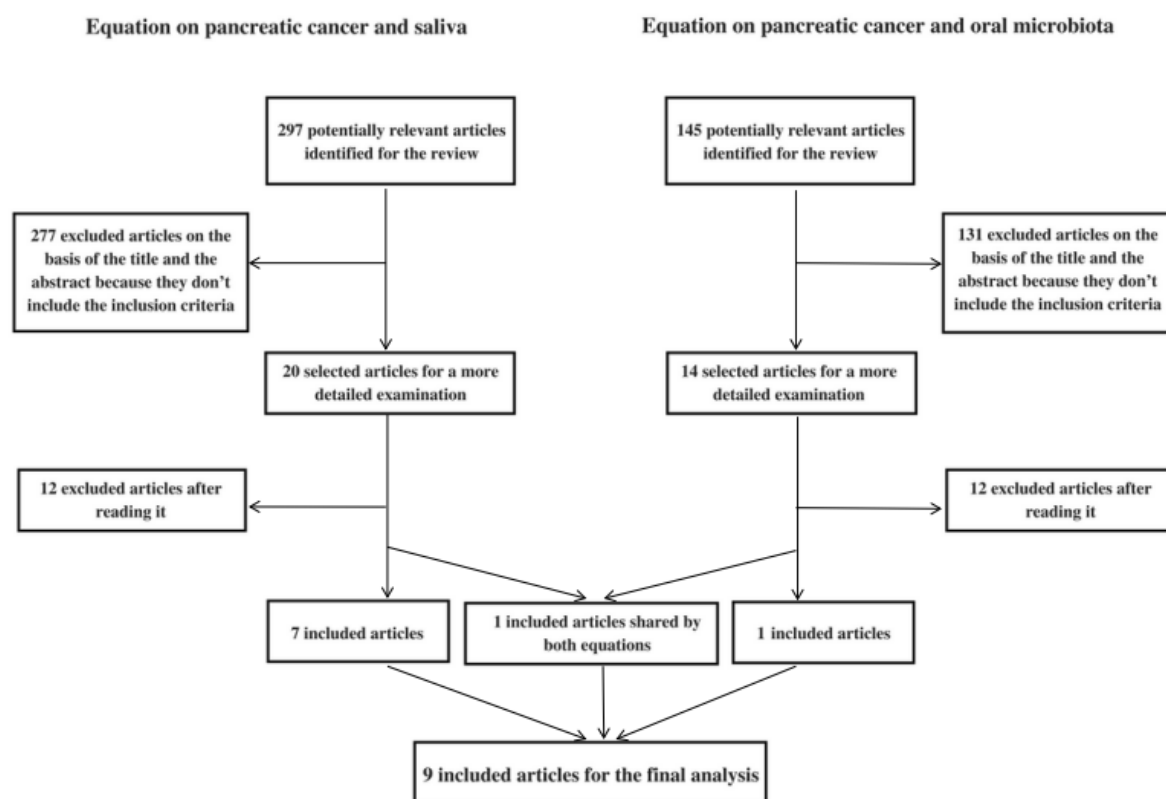


Figure 1 Flow chart: Review of the literature of studies on pancreatic cancer and saliva/the oral microbiome

Table 1: Saliva and pancreatic cancer

	References	Study design	N° case s	N° Controls	Detection method	Results and conclusions of authors
M E T A B O L O M E	Sugimoto et al.[1] 2010 United States	Case- control	18	87	Capillary electrophoresis and mass spectrophotomet ry	The presence of <b>8 specific metabolites in</b> saliva (leucine and isoleucine, tryptophan, valine, glutamic acid, phenylalanine, glutamine, aspartic acid) was revealed in PC compared with healthy subjects. These metabolites could be promising biomarkers for medical screening.
	Zhang et al. [10] 2010 United States	Pilot	30	30 chronic pancreatitis 30 healthy	Microarray and qPCR	The combination of <b>4 biomarkers of mRNA</b> KRAS, MBD3L2, ACRV1, DPM1 distinguished between patients with PC and subjects without cancer (AUC = 0.971, 95% CI= 0.911-0.994, p<0.001, Se = 90%, Sp = 95%). The saliva biomarkers could be discriminatory for the non-invasive detection of resectable PC.
	Liu et al. [8] 2017 United States	Case- control	12	12	Microarray and qPCR	The characterisation of 516 new genes related to pancreatic cancer brought to light <b>29 novel mRNA</b> in the saliva that could allow the detection of PC with a precision of 92%.
	Humeau et al.[2] 2015 France	Pilot	7	4 pancreatitis 2 IPMN 4 non-related digestive diseases	qPCR	The significant <b>increase in 4 miRNA</b> : hsa-miR-21 (Se = 71.4%), hsa-miR-23a (Se = 85.7%), hsa-miR-23b (Se= 85.7%) and miR-29c (Se = 57%) in the saliva discriminated between patients with PC and a control group presenting an unrelated digestive disease with Sp =100%. hsa-miR-23a and hsa-miR23b were overexpressed in the saliva of patients with precursor PC lesions. Saliva miRNA could be used as a biomarker for the early diagnosis of patients with non-resectable PC.
T R A N S C R I P T O M E	Xie et al.[9] 2015 Chine	Case- control	40	20 BPT 40 healthy	Microarray and qPCR	The combination of <b>2 saliva miRNA</b> (mi-3679-5p and miR-940) could make it possible to distinguish between resectable PC and healthy controls (Se = 72.5, Sp = 70.0%), and patients with BPT (Se= 62.5%, Sp = 80.0%) and patients without cancer (healthy + BPT) (Se = 70.0%, Sp = 70.0%). The evaluation of saliva miRNA could be a novel method for the early detection of PC.
	Xie et al. [5] 2016 Chine	Pilot	55	20 BPT 55 healthy	qPCR	The <b>2 saliva lnc-RNA</b> , HOTAIR and PVT1, discriminated between patients with PC and healthy controls and patients with BPT with sensitivity and specificity ranging from 60 to 97%. Saliva HOTAIR and PVT1 could have potential as novel non-invasive biomarkers for the detection of PC.
M I C R O B I O M E	Farrell et al. [7] 2012 United States	Case- control	28	27 chronic pancreatitis 28 healthy	Microarray and qPCR	The combination of <i>N. elongata</i> and <i>S.mitis</i> distinguished between PC and healthy controls with AUC = 0.90, 95%CI = 0.78-0.96, p< 0.0001. The combination of <i>G. adiacens</i> and <i>S. mitis</i> distinguished between PC and healthy subjects with AUC = 0.68, 95% CI= 0.57-0.78, p=0.0063. The associations <i>N.elongata</i> / <i>S.mitis</i> and <i>G.adiacens</i> / <i>S.mitis</i> could serve as diagnostic biomarkers with a Se = 95% and Sp = 82%, Se = 86% and Sp = 83%, respectively. This could provide evidence that the saliva microbiome is a informative source for the discovery of non-invasive biomarkers of systemic diseases
	Torres et al. [4] 2015 United States	Transversal	8	78 other diseases 22 healthy	Sequencing of the rRNA 16S and qPCR	The <i>Leptotrichia/Porphyromonas</i> (L/P) ratio was higher in PC patients than in healthy subjects, (p=0.001), while <i>Neisseria</i> (p=0.07) and <i>Aggregatibacter</i> (p=0.09) were less abundant in the former. The L/P ratio could be a reliable biomarker for the diagnosis of PC. The profiles of bacteria abundance in the saliva could be useful biomarkers for PC.
	Fan et al. [11] 2016 United States	Case- control	361	371	Sequencing of the rRNA 16S	<i>P. gingivalis</i> (OR = 1.60, 95% CI= 1.15-2.22) and <i>A. actinomycetemcomitans</i> (OR = 2.20, 95% CI = 1.16-2.18) may increase the risk of PC. <i>Fusobacterium</i> (OR= 0.94, 95%CI= 0.89-0.99) and <i>Leptorichia</i> (OR= 0.87, 95%CI = 0.79-0.95) may decrease the risk of PC. This is evidence that the oral microbiome could play a role in the etiology of PC.

AUC: area under the curve, mRNA: messenger ribonucleic acid, miRNA: micro-ribonucleic acid, rRNA: ribosomal ribonucleic acid, PC: pancreatic cancer, CI: confidence interval, OR: odds ratio, p: threshold of significance, qPCR: quantitative polymerase chain reaction, RR: relative risk, Se: sensitivity, Sp: specificity, IPMN: intraductal papillary mucinous neoplasm of the pancreas, BPT: benign pancreatic tumour