Pancreatology 17 (2017) 867–874

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

The potential role of gut microbiota in pancreatic disease: A systematic review

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ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form
4 September 2017
Accepted 5 September 2017
Available online 6 September 2017

Keywords:
Chronic pancreatitis
Microbiome
Microbiota
Pancreatic diseases
Pancreatic ductal cancer

ABSTRACT

Background: Several studies have suggested a link between microbiota imbalance and some gastrointestinal, inflammatory and neoplastic diseases. However, the role in pancreatic diseases remain unclear. To evaluate the available evidence for pancreatic diseases, we undertook a systematic review.
Methods: OVID Medline (1946–2017), EMBASE (1980–2017) and the Cochrane Central Register of Controlled Trials (CENTRAL Issue 3, 2017) were searched for studies on microbiota in pancreatic disease. We also searched the reference lists of retrieved papers, and conference proceedings. We excluded animal studies, reviews, and case reports.
Results: A total of 2833 articles were retrieved. After screening and applying the exclusion criteria, 10 studies were included. Three studies showed lower levels of *Bifidobacterium* or *Lactobacillus* and higher levels of *Enterobacteriaceae* in chronic pancreatitis. Two of these studies were uncontrolled, and the third (controlled) study which compared patients with endocrine and exocrine insufficiency, reported that *Bacteroidetes* levels were lower in those patients without diabetes, while *Bifidobacteria* levels were higher in those without exocrine insufficiency. Only one study investigated acute pancreatitis, showing higher levels of *Enterococcus* and lower levels of *Bifidobacterium* versus healthy participants. There was an overall association between pancreatic cancer and lower levels of *Neisseria elongate*, *Streptococcus mitis* and higher levels of *Porphyromonas gingivalis* and *Granulicatella adiacens.*
Conclusions: Current evidence suggests a possible link between microbiota imbalance and pancreatic cancer. Regarding acute and chronic pancreatitis, data are scarce, dysbiosis appears to be present in both conditions. However, further investigation is required to confirm these findings and to explore therapeutic possibilities.

Background

The microbial community in the human gut plays a role in the balance between health and disease. The pool of microbes inhabiting the body is known as 'microbiota' and their collective genomes as 'microbiome'. The human intestine is colonised by 100 trillion microorganisms and over 1000 different resident bacterial species [1,2].

Gastrointestinal (GI) microbiota has recently emerged as an important factor in human physiology, both under homeostatic and pathological conditions. Characterisation of gut microbiota may identify gut-related abnormalities and play an important role in investigating functional linkages to health status. Microbiota imbalance (also known as dysbiosis or dysbacteriosis) has been linked to dysregulation of immune effector cells and activation of inflammatory cytokines, playing a role in several inflammatory-mediated diseases. Some of the GI tract disorders with associated dysbiosis include coeliac disease [3], irritable bowel syndrome (IBS) [4], and inflammatory bowel disease (IBD) [5]. Some studies have

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http://dx.doi.org/10.1016/j.pan.2017.09.002
1424-3903/

also suggested a link between dysbiosis and gastric, oesophageal and colorectal cancer, as well as obesity [2]. In general, data for benign or malignant pancreatic diseases remain scarce.

Pancreatic diseases generally result in a considerable metabolic imbalance [1]. Chronic pancreatitis (CP) is an inflammatory disease of the pancreas characterised by irreversible morphological changes, typically causing chronic pain and/or exocrine dysfunction, and/or endocrine dysfunction [6]. The necrosis-fibrosis hypothesis suggests that the initial damage is caused by an initial acute inflammatory process, progressing to chronic irreversible damage as a result of repeated acute attacks. Although alcohol consumption has long been considered the predominant risk factor in the development of CP, only 3% of the alcoholic population develop this disease [7,8]. Therefore, other additional triggers or initiating factors may play a significant role.

Small intestinal bacterial overgrowth (SIBO) occurs in 3–92% of patients with CP [9], and it is associated with chronic intestinal symptoms, such as abdominal discomfort, bloating, diarrhoea, and malabsorption [10]. This condition may be associated with imbalance of colonic microbiota in CP patients, however, this has not yet been investigated. Pancreatic Ductal Cancer (PDC) is one of the most aggressive malignancies. For the majority of patients it remains a lethal disease and it is the 4th leading cause of cancer death in Europe. Most patients have advanced disease at presentation, which contributes to poor outcomes. This is exacerbated by other factors, including its aggressive biology, resistance to conventional and targeted therapeutic agents, and lack of biomarkers for early detection. Some of the known risk factors are smoking, obesity, diet, genetics and CP [11]. The association between CP and PDC suggests that inflammation may be involved in the initiation and/or promotion of the mutagenesis process [12,13].

Fluctuations in the composition of gut microbiota are associated with the development of several disorders, while microbial stability is associated with health [11,14]. Several factors such as diet, age, environment, or antibiotics have a significant influence on gut microbiota. Therefore, studies on this topic may lead to novel therapeutic options such as probiotics, targeted antibiotics, dietary modification, and faecal microbiota transplantation (FMT). FMT has already been showed to be effective in treating recurrent/resistant *Clostridium Difficile* infection [15] and is showing promise as a potential treatment for IBD [5]. Therefore, proving a causal association between microbiota imbalance and pancreatic diseases could potentially lead to the development of therapeutic or prevention tools in diseases like CP or PDC. Gut microbiota is normally measured by oral, bowel, or feacal samples using DNA-based analysis, as well as by specific antibodies against known pathogens [11,16]. To date, no systematic review on the link between microbiota and pancreatic diseases has been published.

Methods

A systematic electronic literature search was conducted to identify studies on microbiota in pancreatic disease. Two independent reviewers (RM and YB) performed the search. The search strategy was designed by two medical librarians (AM and JM) in March 2017 using a combination of MeSH and textwords for OVID Medline, adapted for use in CENTRAL and EMBASE. OVID Medline (1946–2017), EMBASE (1980–2017), and the Cochrane Central Register of Controlled Trials (CENTRAL Issue 3, 2017) were searched. The search strategy (Appendix 1) adopted two approaches, the first was to identify articles indexed by subject headings and keywords relating to pancreatic diseases and limited by the topical subheading 'microbiology'. The second approach was to search for studies on pancreatic disease and named types of bacteria. The two approaches were combined and duplicates were removed. Animal studies were excluded using the search hedge developed by the Cochrane Collaboration. Study type restrictions were not applied in the search strategy, although editorials, letters, comments, case reports, and reviews were excluded during screening, while case-control, cohort, randomised controlled trials, and observational studies were included. (NOS) was used to assess quality. Studies investigating *Helicobacter pylori* in pancreatic disease were also excluded due to the fact that several meta-analysis on the subject have been published, and this topic fell outside our objectives. No time or language restrictions were applied. The reference lists of all potentially relevant studies were also searched by two researchers (RM and YB) to identify further relevant studies. Online lists of relevant conference proceedings were also searched. The data were sorted through using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) method [17] (Fig. 1). Studies published in the Russian language were translated by one of the authors (YV), fluent in Russian.

Results

Summary of studies included

Following the search strategy, a total of 2833 articles were initially retrieved. Abstracts were reviewed manually, and relevant articles were screened from the selected papers, yielding 45 papers. After applying the exclusion criteria, 9 studies were deemed suitable for inclusion. Hand searching of reference lists of relevant studies retrieved one further study. Therefore, 10 studies were included in the qualitative analysis, of which 8 were full journal articles and 2 were publications from conference proceedings. The results are summarised in Tables 1 and 2. Of the 10 studies selected, five originated from the United States, two from Russia, one from China, one from India and one from Israel. The majority (9 of 10 studies) were published within the last 5 years. Three studies recruited patients with CP, five studies recruited patients with PDC, and one study recruited patients with AP (one study included both CP and PDC patients). The study on AP patients, one of the studies on PDC and three of the studies on CP patients analysed faecal samples. One study recruiting both CP and PDC patients analysed salivary samples. Four of the studies solely on PDC patients analysed salivary samples too, while the other study on PDC analysed blood samples. Two studies (both on PDC patients) arose from large population-based cohorts. Most of the studies assessed microbiota by the sequencing of 16 S mRNA genes. Qualitative analysis of included articles was assessed independently by two of the authors (RM and DBO'C) using the Newcastle-Ottawa Scale (NOS) (score range 0–9) for non-randomised studies (case-control, cohort and prevalence studies) [18,19]. A study scoring 6 or higher was deemed to be of sufficient quality. There was a high level of agreement between the two reviewers (Kappa = 0.9). The median score was 7 (range 6–8) for the included studies (Table 1).

Studies on patients with acute pancreatitis

We identified only one study evaluating gut dysbiosis in patients with acute pancreatitis (AP) [20]. This multicentre study included 108 patients (44 severe AP, 32 mild AP and 32 healthy participants matched for age, sex and body mass index), with ages ranging between 25 and 65 years. Those taking antibiotics or probiotics in the four weeks prior to sample collection, or consuming yoghurt in the two weeks prior to sample collection, were excluded. Faecal samples were collected within one week of AP presentation. There was no significant difference in the total number of faecal bacteria when the three groups were compared. However, *Enterobacteriaceae* and *Enterococcus* populations were higher in all patients with

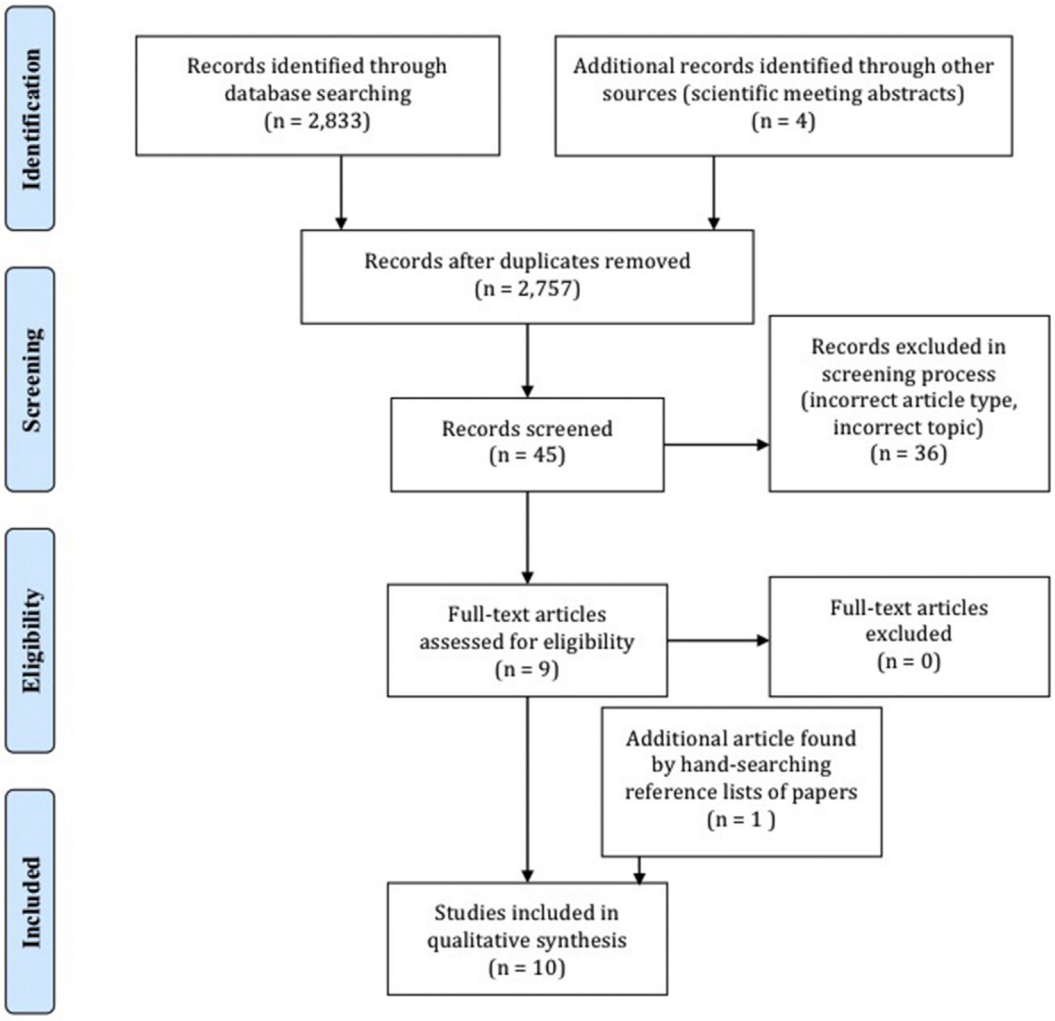

Fig. 1. Preferred reporting items for systematic reviews and meta-analyses.

Table 1
Publication type, study type, and quality score.

Disease	Author, year, country	Publication type	Study type	NOS
CP	Sai Manasa 2017, India	JA	Controlled	8
	Gorovits 2013, Russia	JA (Russian language)	Observational	6
	Farrell 2012, USA	JA	Controlled	8
	Savitskaya 2002, Russia	JA (Russian language)	Observational	6
AP	Tan, 2015 China	JA	Controlled	8
PDC	Michaud 2016, 10 European countries	JA	Prospective cohort (EPIC study)	8
	Fan 2016, USA	JA	Nested case-control (CPS II and PLCO prospective cohorts)	8
	Torres 2015, USA	JA	Controlled	8
	Half 2015, Israel	CPr	Pilot	6
	Lin 2013, USA	CPr	Pilot	7
	Farrell 2012, USA	JA	Controlled	8

NOS, Newcastle-Ottawa Quality Assessment Scale; CP, chronic pancreatitis; AP, acute pancreatitis; PDC, pancreatic ductal cancer; JA, journal article; CPr, conference proceedings; ATBC, Alpha-Tocopherol Beta-Carotene Cancer; EPIC, European Prospective Investigation into Cancer and Nutrition; CPS II, American Cancer Society's Cancer Prevention Study II; PLCO, National Cancer Institute's Prostate Lung Colorectal and Ovarian Cancer Screening Trial.

AP compared to healthy participants. There was no difference between mild and severe AP groups. *Bifidobacterium* was also lower in all AP patients compared to healthy participants. Those with severe AP had higher endotoxin and cytokine levels than either patients with mild AP, or healthy participants.

Studies on patients with chronic pancreatitis

We found four studies that evaluated the link between CP and microbiota. One of the studies, also assessed PDC patients and three of them focused solely on CP patients. The most recent study included 30 CP patients; 14 with and 16 without type 3c diabetes

Table 2
Study descriptives and results.

Disease	Author, year	Patient type	Controls	Sample	Results
CP	Sai Manasa 2017	CP n=30 16 no T3cDM 14 with T3cDM	Healthy participants n=10 (family members)	Faecal	*Bacteroidetes* higher in CP with T3cDM vs CP no T3cDM *Faecalibacterium* lower in CP with T3cDM vs CP no T3cDM Bifidobacterium lower in CP /T3c DM patients with PEI vs without PEI
	Gorovits 2013	CP n=96 Alcohol-induced n=31; biliary-induced n=65	No (comparing with literature references)	Faecal and GLC analysis	*Bifidobacterium* and *Lactobacillus* lower in CP *Enterobacter, Proteus, Kleibsella* and *Morganella* higher in CP
	Farrell 2012	CP n=27 PDC n=38	Healthy participants n=38 (matched for age, gender and ethnicity)	Salivary samples (3-phase study, discovery, verification and independent biomarker validation). HOMIM	*G adiacens* higher in PDC vs CP patients, *S mitis* lower in PDC patients vs healthy participants
	Savitskaya 2002	CP n=60	No (comparing with literature references)	Faecal	*Lactobacillus* lower in CP Bifidobacterium no significant differences *E coli, E faecalis and E faecium* higher in CP
AP	Tan, 2015	Severe AP n=44 Mild AP n=32	Healthy participants n=32 (matched for age, sex and BMI)	Faecal samples within 1 week of presentation (16S mRNA and qPCR)	No difference in total number of faecal bacteria between 3 groups *Enterobacteriaceae* and *Enterococcus* populations higher in severe AP and mild AP groups vs healthy participants (no difference between mild and severe AP groups) *Bifidobacterium* lower in severe AP and mild AP vs healthy participants
PDC	Michaud 2016	PDC n=405	Healthy (non-cancer, still alive) participants n=410 (matched for sex and age at blood draw)	Prediagnosis blood samples to measure antibodies against 25 oral bacteria	High antibody levels of *P gingivalis* ATTC 53978 more common in PDC patients than controls; the highest concentration of *P gingivalis* was associated with a 2-fold increase in PDC risk
	Fan 2016	PDC n=361	Healthy participants n=371 (matched for age, sex, race, calendar of oral wash collection)	Salivary samples (16S mRNA)	Higher *P gingivalis* and *Aggregatibacter* in PDC Lower *Leptotrichia* and *Fusobacteria* in PDC
	Torres 2015	PDC n=8 Other diseases n=78	Healthy participants n=22	Salivary samples (16S mRNA)	*Leptotrichia* higher and *P gingivalis* lower in PDC *Bacteroides* higher (not significant) in PDC *N. elongata* and *Aggregatibacter* lower (not significant) in PDC No difference in *S. mitis* and *G adiacens.*
	Half 2015	PC n=15	Healthy participants n=15	Fecal samples (16S mRNA)	*Bacteroides* and *Verrucomicrobia* increased twofold in PDC *Sutterella, Veillonella, Bacteroides, Odoribacter* and *Akkermansia* also higher in PDC *Firmicutes* and *Actinobacteria* lower in PDC
	Lin 2013	PDC n=13 Pancreatitis n=3	Healthy participants n=12	Salivary samples (16S mRNA)	*Bacteroides* higher in PDC and pancreatitis. Corynebacterium and *Aggregatibacter* lower in PDC.
	Farrell 2012	CP n=27 PDC n=38	Healthy participants n=38 (matched for age, gender and ethnicity) (CP patients were also a control)	Salivary samples (3-phase study, discovery, verification and independent biomarker validation). HOMIM.	Species within 6 genera different between PDC and healthy participants (*Streptococcus, Prevotella, Campylobacter, Granulicatella, Aptopobium, Neisseria*) *N elongata* and *S mitis* lower in PDC patients vs healthy participants *G adiacens* higher in PDC vs CP patients, *S mitis* lower in PDC patients vs healthy participants

CP, chronic pancreatitis; AP, acute pancreatitis; AIP, autoimmune pancreatitis; PDC, pancreatic ductal cancer; BMI, body mass index; M, male; F, female; GLC, gas-liquid chromatography; HOMIM, Human Oral Microbe Identification Microarray; GI, gastrointestinal; qPCR, Quantitative PCR for predominant fecal bacteria; CagA, Cytotoxin-associated gen A-negative H pylori strains; T3cDM, Type 3c diabetes; PEI, Pancreatic exocrine insufficiency; *E coli, Escherichia coli; H Pylori, Helicobacter pylori; E faecalis, Enterococcus faecalis; E faecium, Enterococcus faecium; C albicans, Candida albicans; N elongata, Neisseria elongata; G adiacens, Granulicatella adiacens; P gingivalis, Porphyromonas gingivalis.*

(T3cDM) [21], and 10 unaffected family members. Most of the participants (29/40) were male. The mean age of the controls was higher than in the CP groups with and without T3cDM (42 years versus 35 years and 31 respectively). There were no differences in dietary intake between the three groups, although a higher proportion of T3cDM patients were severely undernourished compared to patients without T3cDM. Bacterial DNA was extracted from fecal samples using 16 S mRNA. Patients with both CP and T3cDM had higher levels of *Bacteroidetes* and lower levels of *Faecalibacterium* compared to those without T3cDM. Patients with T3cDM and pancreatic exocrine insufficiency (PEI) had lower amounts of *Bifidobacterium* compared to those without PEI.

The second CP study, published in Russian, evaluated the microbiota of patients with CP [22]. They conducted both faecal bacteria testing and gas-liquid chromatography (GLC). This uncontrolled observational study included 96 CP patients (31 alcohol-induced and 65 biliary-induced) between the ages 20 and 60 years. Using GLC [23], lower levels of *Bifidobacterium* and *Lactobacillus* and higher levels of *Enterobacter, Proteus, Kleibsella* and *Morganella* were found, compared to literature reference ranges.

Another study from the United States recruited both CP and PDC patients [13]. In total, they recruited 103 participants: 38 PDC

patients, 27 CP patients, and 38 healthy controls matched for age, gender and ethnicity. Microbial composition was determined in salivary samples, extracting bacterial DNA by using Human Oral Microbe Identification Microarray and quantitative real time polymerase chain reaction (PCR) [24]. The CP group had lower levels of *Granulicatella adiacens* compared to those with PDC.

The fourth CP study (also in the Russian language) [25] analysed microbiota during CP exacerbation. Sixty CP patients were recruited, with ages ranging from 18 to 79 years. There was no control arm. Patients with CP had lower levels of *Lactobacillus*, and higher levels of *Escherichia coli, Enterococcus faecalis,* and *Enterococcus faecium* compared to healthy people from literature reference ranges. There was no difference in the amount of *Bifidobacterium* in CP patients compared to normal literature references.

Studies on patients with pancreatic cancer

We found six studies that assessed the potential role of microbiota imbalance in PDC. One study [26] described the results of a prospective cohort included in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. One of the goals of this study was to identify reliable biomarkers of early PDC. Blood samples of 385,000 men and women were collected and participants were followed up over 9 years. Some 405 PDC patients were compared to 416 healthy participants (non-cancer and still alive), and groups were matched for sex, age, fasting state, and recruitment center at blood collection. Antibodies against 25 oral bacteria were determined. There was significantly higher antibody levels of periodontal pathogen *Porphyromonas gingivalis* ATTC 53978 found in PDC patients than in controls, and the highest concentration of *Porphyromonas gingivalis* was associated with a twofold increase in PDC risk. Higher levels of antibodies to commensal oral bacteria was linked to a lower risk of PDC. There was also an inverse association between *Streptococcus mitis* and PDC.

Another study presented data from two large United States prospective cohorts: the American Cancer Society's Cancer Prevention Study (CPS) II and the National Cancer Institute Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial [27]. The CPS II cohort included more than 184,000 participants aged 50–74 years. Oral wash samples were collected during 2000–2002, and new PDC cases diagnosed during follow-up between oral wash collection and 2008 were analysed. The PLCO cohort is a large population-based randomised study of men and women between the ages of 55 and 74 years who were recruited between 1993 and 2001 and followed for cancer incidence. Participants were randomised to either a screening or a control arm. Participants from the two cohorts who developed PDC were compared to controls matched by cohort, age (5-year), sex, race (white, other) and calendar year of oral wash collection. In total, 361 cases of PDC and 170 controls were eligible. Bacterial DNA was extracted from mouth wash samples and 16 S mRNA gene amplification and sequencing was used. Both *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were associated with an increased risk of PDC, while *Fusobacteria* and *Leptotrichia* were associated with decreased risk of PDC.

Another study determined the microbial salivary profile of patients with PDC, and included 108 people (8 PDC patients, 78 non-PDC patients undergoing endoscopy, and 22 unmatched healthy participants) [28]. The average age of the PDC patients, was 71.1 years, while age was unreported in the other groups. Bacterial DNA was extracted from salivary samples using 16 S mRNA. The PDC group had significantly higher levels of *Leptotrichi*a, as well as lower levels of *Porphyromonas gingivalis*. *Neisseria elongate* and *Aggregatibacter actinomycetemcomitans* (although these were not significant). *Bacteroides* was also higher in PDC patients, although this was not significant either. They did not find differences in *Streptococcus mitis* or in *Granulicatella adiacens*.

A study from Israel [29], available only as a conference proceeding, evaluated a potential link between faecal microbiota and PDC. They compared stool samples from 15 newly diagnosed PDC cases (prior to treatment) with stool samples from 15 unmatched healthy controls who were scheduled for screening colonoscopy. Bacterial DNA was analysed from faecal samples using 16 S mRNA gene amplicon sequencing. A twofold higher median relative concentration of *Bacteroides* and *Verrucomicrobia* was found in PDC compared to controls. *Sutterella, Veillonella, Bacteroides, Odoribacter* and *Akkermansia* were also higher in PDC patients relative to controls. *Firmicutes* and *Actinobacteria* were lower in PDC compared to controls.

A further study, aimed to determine if the oral microbiome was linked to PDC and pancreatitis [30]. They analysed ribosomal 16 S mRNA genes from oral wash in 13 patients with PDC, three patients with pancreatitis, and 12 unmatched healthy controls. *Bacteroides* was significantly more abundant in both PDC and pancreatitis patients, compared to controls. *Corynebacterium* was less abundant in PDC and pancreatitis patients compared to controls.

The study by Farrell et al. [13] was previously described as they also included patients with CP. However, this study predominantly evaluated oral salivary microbiota PDC, and recruited both CP and healthy participants as controls. This was a three-phase study, the first part being a discovery phase, followed by a verification phase, and finally an independent biomarker validation phase. They found significant differences in the microflora profiles of PDC compared to healthy participants, specifically *Streptococcus, Prevotella, Campylobacter, Granulicatella, Aptopobium,* and *Neisseria.* Two microbial biomarkers were identified and validated: *Neisseria elongata* and *Streptococcus mitis* were found to be significantly lower in PDC patients compared to healthy participants. *Granulicatella adiacens* was higher in PDC compared to CP patients.

Discussion

This is the first systematic review of the literature to collate evidence on the role of microbiota imbalance in pancreatic disease. In summary, a limited body of evidence suggests that there may be some associations, however, some of the studies show contradictory results.

For patients with CP, there were insufficient controlled studies to draw firm conclusions. Two articles that were unavailable in English suggested a link between dysbiosis and CP [22,25], however, they were uncontrolled studies. The third study [21] suggested that patients with endocrine and exocrine insufficiency may have undesirable alterations in microbiota compared to those with intact pancreatic function. Oral microbiome alterations have previously been reported in diabetes, although determining if this association is causal or consequential requires better delineation [1]. Nevertheless, there are no other published studies linking microbiota imbalance to PEI or T3cDM in CP patients to corroborate the findings of this study. There are certainly possible mechanisms for dysbiosis in CP. CP patients with PEI often develop SIBO, which is defined as an increase in the number of bacteria in the small bowel and/or alteration in the type. This small bowel overgrowth results in excessive fermentation and inflammation, causing steatorrhea, B12 deficiency, protein-losing enteropathy, flatulence, abdominal discomfort, bloating, and undernutrition. Some factors that increase the risk of developing SIBO in CP are malabsorption, diabetic neuropathy, use of drugs that affect motility, use of proton pump inhibitors, alcohol intake, and prior history of surgical procedures [9,10,31]. PEI is often confused with SIBO due to some common

symptoms. Patients with PEI or with acute pancreatitis may have accelerated gastric emptying and small bowel dysmotility, which results in a decreased transit time compared to healthy participants. However, the common use of morphine in some of these patients (a known inhibitor of coordinated myoenteric activity) may cause a marked reduction in propulsion. Furthermore, rupture of the gut barrier and the systemic inflammatory response secondary to cytokine release in both AP and CP, increases bacterial translocation. Both abnormal motility and bacterial translocation are also factors linked to bacterial overgrowth (SIBO) that occur in CP patients [32–34]. It is reasonable to posit that CP patients with overgrowth of bacteria into the small bowel may also have colonic microbiota imbalance, as malabsorption secondary to PEI has a significant impact on the nutrient absorption, and therefore on the availability of nutrients for intestinal microorganisms, and hence microbial composition of the gut. Studies in humans appear to be somewhat replicated by animal studies. For example a study by Hu et al. [35], showed a lower diversity and richness in the gut microbiota of CP mice, with a relative lack of *Firmicutes* and higher levels of *Bacteroidetes*.

Lower levels of *bifidobacterium* in CP was a consistent finding in our review. We cannot conclude that the presence of bifidobacteria are beneficial in CP, as we do not know if the link is reactive or causal. However, a possible beneficial role for *bifidobacterium* has been described in other disorders like atopic disease, coeliac disease, colorectal cancer, obesity, cystic fibrosis (CF), IBS and IBD [1]. For example, both the onset and the perpetuation of IBD seem to be secondary to deregulated immune response against commensal gut bacteria, in which local tolerance mechanisms towards commensal microbes seem to be impaired [1,5,36–38]. Furthermore, in the only study on AP, *bifidobacterium* was also found to be lower in patients [20]. We know that repeat AP episodes commonly precede CP, therefore this finding would be compatible with the hypothesis of a causal association between microbiota imbalance and CP rather than a reactive response in CP patients. However, the administration of enteral and/or parenteral nutrition, may significantly alter gut microbial composition, and thus this could explain the differences found in AP compared to healthy controls [11,14]. Nevertheless, the consistent findings of low levels of 'beneficial' bifidobacteria in pancreatitis (as with other conditions) raises compelling questions about potential therapeutic interventions [39].

Bifidobacteria are anaerobic bacteria and are members of the dominant microbiota naturally present in the gut. Both bifidobacteria and lactobacilli are considered to be health-enhancing bacteria. Probiotic preparations containing either *lactobacillus* alone or in combination with *bifidobacterium* are effective in the prophylaxis of severe necrotising enterocolitis in preterm infants as well as in the prevention of *Clostridium difficile* associated diarrhoea [40]. There are several mechanisms that explain the health beneficial effects of bifidobacteria: they reduce transit time (relieving diarrhoea and malabsorption), and produce short chain fatty acids (SCFA) and lactic acid. Non-digestible dietary carbohydrates enter the colon and are fermented by colonic bacteria to SCFA, lactate, and gases such as CO_2, H_2, and methane. SCFA reduce luminal pH, and this in itself, inhibits pathogenic microorganisms and increases the absorption of some nutrients. SCFA also have a trophic effect on the intestinal epithelium. Bifidobacteria are saccharolytic, and therefore play an important role in carbohydrate fermentation in the colon. Bifidobacteria produce lactate that may be transformed into butyrate, which reduces the rate of transformed cell growth, thus leading to cell reversion from neoplastic to non-neoplastic. In addition to fermentation products, *in vitro*, bifidobacteria are able to synthesise B vitamins. Bifidobacteria also stabilise the intestinal mucosa, normalising intestinal permeability and improving gut immunology, leading to the prevention of the overgrowth of pathogenic microorganisms [2,40–44].

Although the two papers in the Russian language were limited by a lack of a controls group [22] [25], notably both showed higher levels of Enterobacteriaceae. A relative abundance of Enterobacteriaceae could promote a systemic inflammatory response thus contributing to the development of CP. Research on IBD and CF have shown that these diseases are associated with high levels of Enterobacteriaceae [38]. There has been significant interest in the role of *Escherichia coli* particularly in patients with ileal crohn's disease (CD), and biopsies of patients with this disorder have revealed invasion of the mucosa by *Escherichia coli* [5,45]. The involvement of Enterobacteriaceae in the development of pancreatic diseases is likely to be immune-mediated. The commensal bacteria are environmental factors capable of inducing autoimmunity. Yanagisawa et al. [46], showed that *Escherichia coli* was able to induce AIP-like pathological alterations in the pancreas of normal mice. Furthermore, this study also showed higher antibody titres against *Escherichia coli* in patients with AIP compared to disease-free people. Therefore, the findings of Tan et al. [20], were consistent with these data in CP and in other diseases.

There are no intervention studies in CP patients investigating the potential to modify gut microbiota to improve outcomes. In a mouse model, Ren et al. [47] investigated the administration of seleno-lentinan. They reported that this selenium-based product increased the proportion of beneficial bacteria and suggested that seleno-lentinan prevented CP development by elevating antioxidant status and modulating gut microbiota. With only four studies investigating dysbiosis in CP there is, as yet, insufficient evidence. However, ultimately there may be the potential to design studies investigating the effect of specific probiotic and prebiotic products on gut microbiota, gastro-intestinal symptoms, inflammation, and disease progression in patients with CP.

Periodontal disease is defined as gingivitis and periodontitis and is very common, affecting around 90% of general population. The significance of periodontal disease in PDC is unclear. The main microorganism involved in this oral disorder is *Porphyromonas gingivalis* [26,48]. Microbial-mediated pro-carcinogenetic mechanisms of *Porphyromonas gingivalis* are secondary to two particularities: Firstly these bacteria show, both *in vitro* and *in vivo*, an ability to evade the host immune activation, increasing systemic inflammation. Secondly it also increases nitrosamine exposure. Commensal bacteria hypothetically inhibit the growth of pathogenic ones. It is thought that periodontal disease is linked to carcinogenesis due to an abnormal inflammatory response, rather than by having a direct mutagenic effect [24,26,49]. Two large cohort studies demonstrated a link between *Porphyromonas gingivalis* and PDC [26,27], however a third study [28] reported contradictory results. These contradictory findings may be due to smaller numbers and the lack of matched controls in the latter study. Therefore a positive association seems feasible due to the fact that it is consistent in the two larger and better-designed studies. Higher levels of *Bacteroides* in PDC were a common finding in three studies, although two of these studies were conference proceedings. Other microorganisms involved in the association between oral health and PDC are *Neisseria elongate*, *Streptococcus mitis,* and *Granulicatella adiacens.* Lower levels of both *Neisseria elongate*, and *Streptococcus mitis* were reported in two studies on PDC patients [13,26] while *Granulicatella adiacens,* levels were high. *Streptococcus mitis* has been shown to have a protective role against carcinogenic pathogens, which may allow for the overgrowth of *Granulicatella adiacens* [1,2,48,49]. Based on these findings, bacterial profiling has been suggested as a biomarker for PDC [1]. Farrell et al. [13,24], identified salivary biomarkers with high specificity and sensitivity for the detection of PDC. The main limitation of this

study was its cross-sectional nature.

This systematic review aimed to describe the studies investigating dysbiosis in pancreatic disease, but specificallyspecificallay excluded studies on *Helicobacter pylori*. To date, at least five meta-analyses on this topic have already been published [50–54]. The most recent systematic review and meta-analysis [53] of eight studies (including 2757 participants) found that patients positive for *Helicobacter pylori* had a significantly higher risk of developing PDC. However reports were inconsistent, with several meta-analyses finding an association between *Helicobacter pylori* and PDC [52,54], but others reporting no association [50,51]. Notably, while Wang et al. [50] found no significant association in western countries, however they reported a decreased risk of PDC in eastern countries, specifically in patients positive for *Helicobacter pylori and* cytotoxin-associated gene A (CagA). They suggested that the protective association in eastern countries may be part of the long history of co-existence of this microorganism with that population. The review by Schulte et al. [54] also found a protective effect of CagA-positive *Helicobacter pylori* colonisation. They hypothesised that this protective association was based on the fact that CagA strains are highly likely to lead to gastric hyperacidity and lower pancreatic stimulation by secretin, which could result in decreased turnover of pancreatic ductal cells, and subsequently lesser potential for damage to DNA. *Helicobacter pylori* infection may contribute to the development of PDC via complex interactions with the ABO genotype, dietary and smoking habits, hyperacidity, and *N*-nitrosamine exposure of the host [55]. However, no study has isolated *Helicobacter pylori* DNA in any pancreatic sample. As is evidenced by the recent proliferation of meta-analyses on the topic, research has focused more on *Helicobacter pylori* in PDC than on dysbiosis in general.

The main limitation of this review is the lack of studies available, particularly in AP and CP, making it difficult to draw robust conclusions. Despite this, the study quality was high. Another limiting factor in interpreting the results concerns the difficulty in comparing studies across different geographical zones. For example, diet will vary considerably between countries. The composition of the macronutrients is significantly different between Asian, Europeans, and Americans, and this will have a considerable impact on gut microbial composition. Overall, a 'western-style' diet, high in saturated fat and carbohydrates and low in fibre, is positively correlated to *Bacteroides* enterotype, while the Asian diet is commonly lower in fat which may promote a predominance of beneficial *Bifidobacteria* and a greater microbial diversity [11,56,57].

Finding a clear association between alterations in gut microbiota and pancreatic disease would be of significant clinical importance, as it would provide valuable and much-needed options for clinical intervention. Data are as yet limited, but if future studies continue to replicate these findings with clearer evidence of dysbiosis in pancreatic disease, then the next step would be to investigate if dysbiosis is causative or reactive. Further studies are in progress. For example, a group in the United States is investigating whether or not CP and diabetes alter pancreatic, oral, and faecal bacteria leading to PDC [58]. Microbial abnormalities may be explored as early biomarkers for PDC and therefore may potentially have a significant impact on the long-term survival of this lethal disease. Strategies which alter the microbial population of the gut (such as probiotics, antibiotics, dietary changes, targeting of microbe biochemical pathways, and FMT) could be utilised for both pancreatic disease treatment and prevention, and therefore this represents an underexplored research avenue for pancreatic disease. FMT has been shown to be a highly successful treatment in severe and recurrent *Clostridium difficile* infection [15], and there is a growing interest in this therapy in IBD [1,5,11,39,41].

Conclusions

In conclusion, data to support a role of microbiota imbalance in pancreatic disease are scarce, but limited studies support a potentially important association. Current evidence suggests a possible link between specific microbiota abnormalities and PDC. Specifically, *Porphyromonas gingivalis* and *Granulicatella adiacens* appear to be risk factors while *Streptococcus mitis* appears to be a protecting factor in PDC. Regarding pancreatitis, there are few studies, however the available data suggest a plausible association including lower levels of *bifidobacterium* and a higher levels of *enterobacteria*. This is an emerging topic of increasing interest, with 9 of 10 studies being published in the last five years. Overall, further research is needed to confirm the potential role of the microbiota in pancreatic disorders, which may open a new avenue for biomarkers and targeted therapies.

Acknowledgments

The authors wish to thank Professor Harvey Risch (Epidemiology Department, Yale School of Public Health) and Professor Matthias Löhr (Gastroenterology & Hepatology Department, Karolinska Institutet) for providing additional information and clarification about their respective studies. The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pan.2017.09.002.

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