Characterization of micronutrients and macronutritional assessment parameters in chronic pancreatitis outpatients and association with exocrine pancreatic insufficiency

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Resumé

Baggrund og formål:
Kronisk pancreatitis (KP) er en fibro-inflammatorisk sygdom med risiko for udvikling af eksokrin pancreatisk insufficiens (EPI). Dette kan resultere i underernæring, herunder mangel på mikro- og makro-ernæringsparametre. Formålet med dette studie er at belyse forekomst af mangel på mikro- og makro-ernæringsparametre hos patienter med KP. I delstudie 1 sammenfattes en meta-analyse, der angiver eksisterende forskning i henhold til prævalensen af mangel på mikro- og makronæringsstoffer hos patienter med KP. I delstudie 2 undersøges prævalensen af mangel på mikro- og makro-ernæringsparametre i et tværsnitsstudie med en dansk population af patienter med KP, og associationen mellem ernæringsstatus og eksokrin pankreatisk funktion med eller uden enzymbehandling, samt associationen mellem mikro- og makronæringsstoffer belyses.
Metode:
I delstudie 1 (metaanalysen) indsamles originale artikler, som estimerede prævalensen af mikro- og/eller makro-ernæringsparametre. Estimerede prævalenser og heterogenitet udregnes for udvalgte næringsstoffer. I delstudie 2 (tværsnitsstudiet) inkluderes patienter med KP fra Aalborg Universitetshospital (AAUH) i perioden mellem januar 2012 til maj 2017. Alle parametre blev indsamlet via klinisk rutine undersøgelse, blodprøver, bioimpedans samt specifikke funktionsevne tests. Prævalensen af mangel på mikro- og makro-ernæringsparametre estimeres, og associationen mellem ernæringsstatus og eksokrin pankreas funktion med eller uden enzymbehandling, samt associationen mellem mikro- og makro-ernæringsparametre udregnes.
Resultater:
I delstudie 1 blev 19 studier inkluderet. De estimerede prævalenser var følgende: 21.0% for vitamin A mangel, 31.0% for vitamin E mangel, 50.0% for vitamin D mangel, 68.0% for vitamin D insufficiens, 11.0% for BMI <18.5 kg/m^2 og 19.0% for BMI <20.0 kg/m^2 med betydelig heterogenitet for alle analyser.
I delstudie 2 blev 137 patienter med KP inkluderet. De seks hyppigste mangler var følgende: 29.2% for albumin mangel, 26.3% for vitamin D insufficiens, 21.9% for vitamin D mangel, 19.7% for zink mangel, 16.8% for magnesium mangel, og 17.4% havde sarkopeni. En association mellem ernæringsstatus og eksokrin pankreas funktion med eller uden enzymbehandling blev fundet for vitamin A ($p = 0.008$), vitamin E ($p = 0.000$), vitamin B12 ($p = 0.027$), magnesium ($p = 0.038$) og sarkopeni ($p = 0.032$). Yderligere blev BMI fundet associeret til zink ($p = 0.002$), og sarkopeni til vitamin E ($p = 0.019$), vitamin B12 ($p = 0.049$) og zink ($p = 0.003$). Phase angel var associeret til vitamin E ($p = 0.014$), magnesium ($p = 0.041$) og zink ($p = 0.088$).

Konklusion:
I delstudie 1 blev der fundet en stor heterogenitet mellem studierne for de fleste af de eksisterende estimater for mangel på mikro- og makro-ernæringsparametre hos patienter med CP. I delstudie 2 med den danske befolkning var prævalensen af mangel på mikro- og makro-ernæringsparametre lavere sammenlignet med estimaterne fra den eksisterende litteratur. Der var ingen klar association mellem ernæringsstatus og eksokrin pankreatisk funktion med eller uden enzymbehandling for de fleste ernærings parametre. Desuden var associationen mellem mikro- og makro-ernæringsparametre generelt ringe, men der blev fundet en stærk korrelation mellem zink og alle makro-ernæringsparametrene, og yderligere forskning indenfor dette område er nødvendigt.

Abstract

Background and aims:
Chronic pancreatitis (CP) is a fibro-inflammatory disease with risk of developing exocrine pancreatic insufficiency (EPI) resulting in malnutrition. Substudy 1 (meta-analysis) aims to outline the pooled prevalence of micronutritional deficiencies and macronutritional abnormalities among CP outpatients. Substudy 2 (cross-sectional study) investigates prevalence of the micronutritional deficiencies and macronutritional abnormalities in a cross-sectional study of a Danish population of CP outpatients, and examines the association between nutritional status, EPI and/or PERT, and the association between micronutrients and macronutritional assessment parameters in CP outpatients.
Methods:
In substudy 1, original articles reporting prevalence estimates on deficiencies were collected. Pooled prevalences and heterogeneity of selected nutrients were calculated. For substudy 2, outpatients with CP from AAUH were enrolled between January 2012 through May 2017. Assessments were collected through routine clinical examination, blood samples, bioelectrical impedance, and specific functional tests. Prevalences of deficiencies were estimated, and the association between nutritional status, EPI and/or PERT, and association between micronutrients and macronutritional assessment parameters were calculated.
Results:
For substudy 1, pooled prevalences were: 21.0% for vitamin A deficiency, 31.0% for vitamin E deficiency, 50.0% for vitamin D deficiency, 68.0% for vitamin D insufficiency, 11.0% for BMI <18.5 kg/m^2, and 19.0% for BMI <20.0 kg/m^2 with noteworthy heterogeneity.
In substudy 2, 137 patients with CP were enrolled. Most frequent deficiencies were: 29.2% for albumin deficiency, 26.3% for vitamin D insufficiency, 21.9% for vitamin D deficiency, 19.7% for zinc deficiency, 16.8% for magnesium deficiency, and 17.4% had sarcopenia. An association to EPI and/or PERT were found for vitamin A ($p = 0.008$), vitamin E ($p = 0.000$), vitamin B12 ($p=0.027$), magnesium ($p = 0.038$), and sarcopenia ($p = 0.032$). BMI was associated with zinc ($p = 0.002$). Sarcopenia was associated with vitamin E ($p = 0.019$), vitamin B12 ($p = 0.049$), and zinc ($p = 0.003$). Phase angel was associated with vitamin E ($p = 0.014$), magnesium ($p = 0.041$), and zinc ($p = 0.088$)
Conclusion:
For substudy 1, a large heterogeneity was shown between studies for most of the existing estimates on malnutrition in patients with CP.
For substudy 2, the prevalence of malnutrition seem lower in Danish outpatients with CP compared to the estimates provided from the existing literature. There are no clear association between malnutrition and the presence of EPI and use of PERT for most nutritional parameters. Also the

association between micronutrients and macronutritional assessment parameter are generally poor, but a strong correlation between zinc and macronutritional assessment parameters was found, and requires more investigation.

Introduction

Chronic pancreatitis (CP) is a fibro-inflammatory disease characterized by destruction of exocrine and endocrine tissue of the pancreas. Aetiological risk factors associated with CP are wide and complex, but the most common aetiological factor is alcohol consumption that often goes along with smoking, which has also been shown as an independent risk factor[1-4]. The underlying pathogenesis of CP still remains uncertain, but there are several theories. The most widely used theory is episodes of acute pancreatitis with inflammation caused by aetiological risk factors such as alcohol and tobacco. Continued exposure of these trigger recurrent episodes of acute pancreatitis resulting in fibrosis, and consequently leads to CP[2]. Early stage disease can be difficult to visualize on imaging, but the most frequent morphological changes calcifications and/or dilatation of the ducts[5,6].

The most common clinical presentation of CP forms the triad of symptoms: upper abdominal pain, weight loss due to steatorrhea, and diabetes. Reasons of these symptoms can be explained by: inflammation and fibrosis, the exocrine pancreatic insufficiency (EPI) accompanied by maldigestion, and endocrine insufficiency leading to diabetes mellitus[1,2,7].

EPI is defined as lack of a sufficient amount of enzymes in duodenum to maintain a normal digestion of fats, proteins and carbohydrates. Maldigestion of these nutrients leads to malabsorption and malnutrition[3]. However, confounding factors leading to malnutrition can also be pain, alcoholism, and diabetes[8]. Symptoms of maldigestion caused by EPI are weight loss, diarrhoea, flatulence, abdominal distension, and cramps. Although, symptoms only arise when the digestive capacity of the pancreas are exceeded by the amount of ingested food, and patients tend to minimize their intake to avoid symptoms. Therapy for EPI may include healthy diet, nutritional supplementation, and pancreatic enzyme replacement therapy (PERT) if needed. Moreover, it is clinically recommended to monitor macronutritional abnormalities as changes in body mass index (BMI), sarcopenia, phase angle, and albumin of the patients[3]. Changes in BMI are easy to monitor, but sarcopenia has been shown to be a neglected clinical complication in CP patients[9]. As sarcopenia is associated with poor clinical outcome, low quality of life and death[9,10], more focus on the presence of sarcopenia in CP patients should be prioritised.

In addition to the above-mentioned macronutritional abnormalities, patients are in risk of further micronutritional deficiencies. These include fat-soluble vitamins (A, D, E), vitamin B12, and minerals (Mg, Zn, Ca)[1,3,6,7,11,12]. Micronutritional deficiencies can lead to health problems such as osteoporosis, immune dysfunction, neurologic deficits and night blindness, and guidelines suggest monitoring these along with BMI and sarcopenia. However, the rate of deficiencies tend to vary in between studies, and more investigation in the field is needed[3,13].

Therefore, substudy 1 aims to perform a meta-analysis outlining the current research on the prevalence of deficiencies in micronutrients and macronutritional assessment parameters among CP outpatients. The hypotheses are heterogeneity in deficiencies across studies, and a tendency of

deficiencies in CP patients. In addition, substudy 2 (cross-sectional study) investigates outpatients in Aalborg University Hospital, Denmark with the following aims: 1) to characterize the prevalence of micronutritional deficiencies and macronutritional abnormalities of CP outpatients, 2) to investigate the association between nutritional status, EPI and/or PERT, 3) to investigate the association between micronutrients and macronutritional assessment parameters. Finally, the results may contribute to assess which micronutrients and macronutritional assessment parameters that are important to monitor.

Methods

Substudy 1: Meta-analysis

Searches in Pubmed and Embase were made. The Problem-Intervention-Comparison- Outcome search model (PICO) was used to determine which search terms to apply[14]. The three search terms used were CP, EPI and nutrition. Language and publication type were added as limiting filters. Full searches are shown in appendix 1. Further articles were found by chain search. Initially, the selection of articles was based on titles and abstracts, and later reviewed by full text reading. Inclusion criteria were: 1) Age ≥18, 2) diagnosis of CP, 3) focus on EPI, and 4) focus on nutritional status. Exclusion criteria were: 1) animal experiments, and 2) case reports or small (≤10 patients) original studies, 3) articles with pancreatic disorders other than CP or malabsorption caused by other gastrointestinal diseases.

To perform substudy 1, original articles from the above-mentioned literature search were selected. The inclusion criterion was articles reporting prevalence estimates on deficiencies of micronutrients and/or macronutritional assessment parameters. For each study, the following data were extracted: year of publication, country of origin, study design, number of patients, mean age, gender, aetiology, EPI, PERT, endocrine status, methods used to establish diagnosis of CP and EPI, and rate of micronutritional deficiencies and/or macronutritional abnormalities. Study quality was assessed using the Newcastle-Ottawa scale[15]. The study quality score was considered as: low quality <4/10 score and high quality ≥5/10 score[16]. All data from each study were independently extracted by the two reviewers (HJJ and ASM).

Statistical analysis

Random-effects meta-analysis were performed for micronutrients and macronutritional assessment parameters when prevalence estimates of deficiencies was available from 3 or more studies. Results are shown as forest plots. To evaluate whether studies were consistent, test for heterogeneity using the quantity I^2 was conducted. I^2 is the percentage of variation between studies that is caused by heterogeneity rather than by chance. An I^2 value ≤25% were considered as homogeneity, and an I^2 value ≥75% were considered as noteworthy heterogeneity[17]. Finally, for nutritional parameters where 2 or fewer studies were available, the raw prevalence estimates were tabulated.

Substudy 2: Cross-sectional study of CP patients

Study design and setting

This was a cross-sectional study of CP outpatients, conducted at Centre for Pancreatic Diseases, Department of Gastroenterology and Hepatology, AAUH Denmark. Patients were enrolled between January 2012 through May 2017.

All assessments were obtained as part of a routine clinical examination, through review of medical records, biochemistry, and specific functional tests for sarcopenia. The diagnosis of definitive or probable CP was established according to M-ANNHEIM classification system[18]. Inclusion criteria were: i) age 18-75 years, ii) diagnosis of definitive or probable CP. Exclusion criteria were: i) missing data of age and sex, ii) missing data of faecal elastase, iii) missing data of biochemistry (vitamin A, D, E and B12, calcium, magnesium, zinc, albumin), iiii) missing data of BMI, sarcopenia, and phase angel.

Collection of data and storage was approved by the Danish Data Protection Agency, Northern Denmark Region (ID 2008-58-0028).

Patient and disease characteristics

The following patient and disease characteristics were registered in a database: demographics (age and sex), aetiology of CP, presence of EPI and diabetes mellitus, and status of PERT. EPI was determined as a faecal Pancreatic-Elastase 1 concentration <100 µg/g[19] using an enzyme-linked immunosorbent assay (Schebo Pancreatic Elastase Stool test). For association analysis patients were divided into the following subgroups according to the presence of EPI and use of PERT: normal, EPI without PERT, and EPI with PERT.

Micro- and macronutritional assessment parameters

Micronutrients

The parameters of interest were vitamin A, D, E and B12, calcium, magnesium, and zinc. All parameters were routinely determined by analysis of blood samples obtained in the outpatient clinic at AAUH using a Cobas 8000. Micronutritional deficiencies were defined as follows: vitamin A <1.05 µmol/l, E vitamin <12 µmol/l, calcium <2.20 mmol/l, magnesium <0.70 mmol/l, and zinc 10 µmol/l. For vitamin D the level for deficiency was <25 nmol/l, and insufficiency value was <50 nmol/l. For vitamin B12 two different deficiency values were used due to no clear consensus on which reference to use: <125 pmol/l and <200 pmol/l[20].

Macronutritional assessment parameters

The following assessment parameters were recorded to characterize the patient's macronutritional state: albumin, anthropometrics, bioelectrical impedance, and specific functional tests for sarcopenia. Albumin was run on a biochemistry analyser as micronutritional blood samples. The normal range for albumin was as follows: 18-40 years: 36-48 g/l, 40-70 years: 36-45 g/l, >70 years: 34-45 g/l[20]. Anthropometrics included weight, height and BMI. According to the World Health Organization, BMI was calculated as weight/height^2, and defined as underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2) and overweight (≥ 25.0 kg/m^2)[21].

Bioelectrical impedance: the test was used to determine skeletal muscle mass. A multi-frequency analyser (BioScan 920-II) measured the resistance through the body at an alternating electric current (0.8 mA). Patients were instructed to fast for 4 h prior to examination, to abstain for physical activity for 8 h, and to urinate and lay down 10 minutes before the assessment. With patient lying in a supine position with 45 degrees between the legs and 30 degrees between the arms and the torso, four electrodes were placed on the dorsal side of the hand, wrist, foot and ankle on the patient's right side. The assessment parameter was phase angle, which described the relationship between the capacitance (the capacitative reactance) and the resistance[22,23]. Bioelectrical impedance was also used to calculate skeletal muscle mass index (SMI). SMI is calculated as absolute muscle mass (kg) divided by the square of the height (m^2). Sarcopenic SMI cut-offs are ≤10.76 kg/m^2 for males and ≤6.76 kg/m^2 for females[10].

Hand grip strength (HGS): to determine muscle strength, a hydraulic hand dynamometer was used. Patients were instructed to be seated, holding the dynamometer in second position with shoulder adducted, elbow bend 90 degrees and forearm in neutral position[24]. Sarcopenic HGS cut-offs are 30 kg for males and 20 kg for females[10].

Time-up-and-go test (TUG): TUG determines muscle function, and is measured as the time it takes the patient to stand up from a chair, walk three meters, turn around and sit down. Sarcopenic TUG cut-offs are divided in following groups: <70 years: TUG< 9.0 s, 70-80 years: TUG< 10.2 s, > 80 years: TUG< 12.7 s[25].

Definition of sarcopenia: the diagnosis of sarcopenia demands presence of specific criteria according to the European Working Group on Sarcopenia in Older People (EWGSOP). These include a combination of: 1) low muscle mass, 2) low muscle strength, and 3) low physical performance. The criteria were measured by: 1) skeletal muscle mass index (SMI), 2) handgrip strength (HGS), and 3) time-up-and-go (TUG)[10]. Definition of sarcopenia is abnormal values of SMI along with HGS, and/or TUG. Presarcopenia is defined by abnormal SMI, but normal HGS and TUG (figure 1).

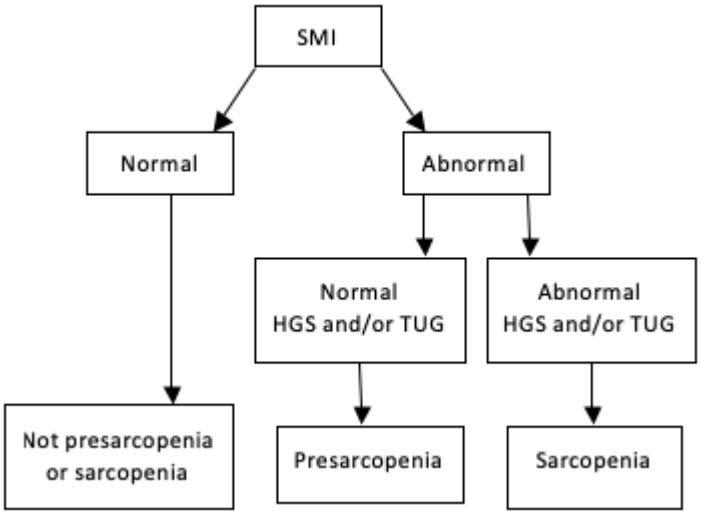

Figure 1: algorithm for presarcopenia and sarcopenia in patients with CP.
SMI = skeletal muscle mass index, HGS = handgrip strength, TUG = time-up-and-go test

Statistical analysis

The statistical analysis was performed using STATA version 16.0. Data are reported as means (SD) or numbers (%) unless otherwise indicated. QQ-plots were used to inspect if continuous variables were normally distributed and Bartlett's test to determine if variance was equal between subgroups. Prevalence estimates of micronutritional deficiencies and macronutritional abnormalities were calculated and reported with 95% confidence intervals (CI). To investigate the associations between micro- and macronutritional status, EPI and/or PERT, analysis of variance or Kruskal Wallis test were used. Categorical variables were analysed using a Fisher's exact test. The association between micronutrients and macronutritional assessment parameters were examined by ANOVA, Kruskal Wallis test or Spearman's rank correlation coefficient. A p-value <0.05 was considered as statistical significant, but in case of multiple comparisons a Bonferroni adjustment was performed.

Results

Substudy 1: Meta-analysis

We identified 784 articles through database search on Embase and 250 on Pubmed (figure 2), and additional 30 articles were identified through other sources. The total number of articles without duplicates was 916. One-hundred-six articles fulfilled the inclusion criteria, and 58 articles were used. Of the 106 articles, 47 were original studies, of which 28 were excluded for the meta-analysis because they did not report relevant prevalences estimates on micronutritional deficiencies and macronutritional abnormalities. Hence, 19 studies were included in the final meta-analysis (table 1).

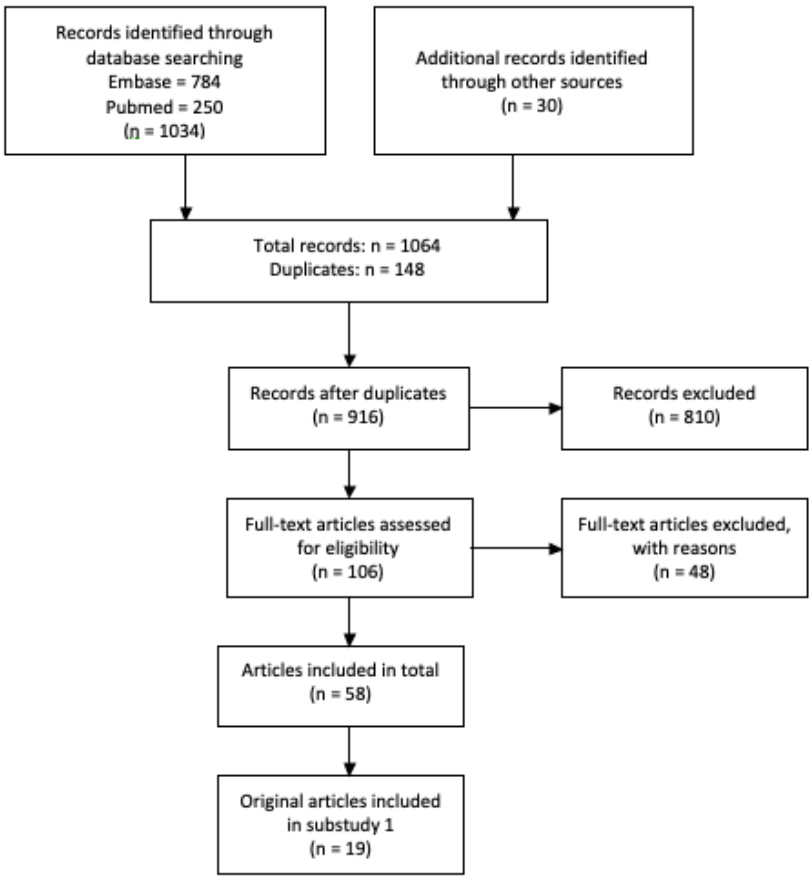

Figure 2: PRISMA diagram of assessment of articles included in meta-analysis and the cross-sectional study.

Author	Country	Study design	Patients	Quality	Mean age	Sex M/F	Etiology of CP	EPI rate
Greer et al 2019[28]	USA	CSS	301	High	50.8 ± 14.1	M: 151/301 (50.2%), F: 150/301 (48.8%)	Alcoholic, non-alcoholic	NR
Vujasinovic et al 2019[39]	Sweden	CS	150	Low	NR	M: 64/150 (42.7%), F: 86/150 (57.3%)	Alcohol, autoimmune, efferent duct factors, smoking, hereditary, idiopathic	80/140 (57.1%)
Stigliano et al 2018[36]	Estonia, Germany, Italy, Poland, Spain, Sweden and the United Kingdom	CSS	211	High	NR	M: 142/211 (67.3%), F: 69/211 (32.7%)	Alcoholic, idiopathic, hereditary, obstructive, other	101/179 (56.4%)
Min et al 2018[29]	USA	CC	91	High	48.6 ± 10.4	M: 34/91 (37.4%), F: 57/91 (62.6%)	Toxic/metabolic, idiopathic, genetic, autoimmune, recurrent acute pancreatitis, obstructive	77/91 (84.6%)
Olesen et al 2017[40]	Denmark	CSS	166	High	58.6 (IQR 23.5–84.9)	M: 117/166 (70.5%), F: 49/166 (29.5%)	Alcoholic, idiopathic, other	128/163 (78.5%)
Olesen et al 2016[41]	Denmark	CS	170	High	61 (IQR 52-67)	M: 118/170 (69.4%), F: 52/170 (30.6%)	Alcoholic, non-alcoholic, other	128/166 (77%)
Pezzilli et al 2015[37]	Italy	CC	30	High	57.0 ± 13.1	M: 15/30 (50.0%), F: 15/30 (50.0%)	NR	17/30 (56.7%)
Haas et al 2015[42]	NR	CS	50	High	45.2 ± 8.4	M: 50/50 (100.0%), F: 0/50 (0.0%)	NR	28/50 (56.0%)
Sikkens et al 2013[30]	The Netherlands	CS	40	High	52 ± 11	M: 23/40 (57.5%), F: 17/40 (42.5%)	Alcoholic, idiopathic, other	28/40 (70.0%)
Lindkvist et al 2012[43]	Spain	CS	114	High	48.1 ± 13.9	M: 97/114 (85.1%), F: 17/114 (14.9%)	Alcoholic, other	38/114 (33.3%)
Regunath et al 2011[44]	India	CSS	54	High	34.5 ± 11.7	M: 47/54 (87.0%), F: 7/54 (13.9%)	Alcoholic, tropical	
Duggan et al 2014[26]	Ireland	CS	62	High	47.9 ± 12.5	M: 45/62 (72.6%), F: 17/62 (27.4%)	Alcoholic	16/46 (34.8%)
Dutta et al 1982[31]	USA	CSS	15	Low	50 ± 3	NR	Alcoholic	15/15 (100%)
Marotta et al 1994[27]	South Africa	CC	44	Low	47 (IQR 29-76)	M: 40/44 (90.9%), F: 4/44 (9.1%)	Alcoholic	23/44 (52.3%)
Prabhakaran et al 2014[32]	India	CS	103	High	38.6 ± 20.6	M: 103/103 (100%), F: 0/103 (0%)	Alcoholic, idiopathic	21/103 (20.4%)
Joshi et al 2011[33]	India	CSS	72	High	31 ± 10	M: 38/72 (52.8%), F: 34/72 (47.2%)	Tropical	38/43 (88.4%)
Dujsikova et al 2008[34]	Czech Republic	CSS	73	Low	46.6 ± 13.2	M: 56/73 (76.8%), F: 17/73 (23.3%)	Alcoholic, idiopathic	NR
Duggan et al 2015[35]	Ireland	CC	29	High	44.3 ± 12.3	M: 12/29 (41.4%), F: 17/29 (58.7%)	Alcoholic, idiopathic, other (autoimmune, genetic)	NR
Morán et al 1997[38]	Argentina	CSS	14	Low	55.6	M: 14/14 (100%), F: 0/14 (100%)	Alcoholic, idiopathic	14/14 (100%)

Table 1: original articles included in meta-analysis.

NR = not reported, CCS = cross-sectional study, CS = cohort study, CC = case control study, IQR = interquartile range, M = male, F = female, CP = chronic pancreatitis, EPI = exocrine pancreatic insufficiency.

Author	Patients on PERT	Endocrine insufficiency rate	Diagnosis of CP	EPI diagnosis	Micronutrient (reference)	Macronutrient (reference)
Greer et al 2019[28]	166/301 (55.1%)	116/301 (38.5%)	Radiological (Cambridge classification) or histological	NR	A (<30.0 µg/dl) D (<10.0 ng/ml) E (<5.7 mg/l)	BMI (<18.0 kg/m^2)
Vujasinovic et al 2019[39]	NR	42/150 (28.0%)	NR	Faecal elastase		BMI (<18.5 kg/m^2)
Stigliano et al 2018[36]	116/211 (55.0%)	77/211(36.5%)	Diagnosis of probable or definite CP according to the M-ANNHEIM criteria	Faecal elastase	D (<20.0 ng/ml)	
Min et al 2018[29]	77/91 (84.6%)	NR	Endoscopic ultrasound criteria and/or secretin stimulation testing	Reported symptoms and/or faecal fat assays and/or secretin stimulation tests	A (NR) D (NR) E (NR)	BMI (<18.5 kg/m^2)
Olesen et al 2017[40]	NR	60/165 (36.4%)	Mayo Clinic criteria (Lüneburg), and CP was defined as a score of 4 points	Faecal elastase, 72 h faecal fat collection, and 13C-mixed triacylglycerol breath test		BMI (<20.0 kg/m^2)
Olesen et al 2016[41]	128/166 (77.1%)	58/168 (34.5%)	Mayo Clinic criteria (Lüneburg), and CP was defined as a score 4 points	Faecal elastase, 72 h faecal fat collection, and 13C mixed triglyceride breath test		BMI (<18.5 kg/m^2)
Pezzilli et al 2015[37]	NR	7/30 (23.3%)	Clinical and radiological	Faecal elastase	D (<20.0 ng/ml)	BMI (<18.5 kg/m^2)
Haas et al 2015[42]	28/50 (56.0%)	NR	Clinical and radiological	Faecal elastase		BMI (<20.0 kg/m^2)
Sikkens et al 2013[30]	19/40 (47.5%)	18/40 (45.0%)	Clinical and radiological, or PEI	Faecal elastase	A (<1.25 µmol/l) D (<38.0 pmol/l) E (<16.5 µmol/l)	
Lindkvist et al 2012[43]	0/114 (0.0%)	23/114 (20.2%)	Radiological	13C mixed triglyceride breath test		BMI (<20.0 kg/m^2)
Regunath et al 2011[44]	NR	16 (29.6%)	Clinical and radiological	Faecal elastase		BMI (<18.5 kg/m^2)
Duggan et al 2014[26]	NR	20/46 (43.5%)	Clinical and radiological (Cambridge classification)	Faecal elastase	A (<1.6 µmol/l) D (<30.0 nmol/l) E (<21.3 µmol/l)	
Dutta et al 1982[31]	15/15 (100%)	NR	NR	24 h faecal fat collection	A (<41.0 µg/dl) D (<18.0 ng/ml) E (<5.3 µg/ml)	
Marotta et al 1994[27]	0* (1 week washout period)	22/44 (50.0%)	Clinical, secretin pancreatic function test, and/or ERCP	72 h faecal fat collection	A (<10.4 µmol/l) E (NR)	
Prabhakaran et al 2014[32]	NR	39/103 (37.9%)	Clinical and radiological	NR	D (<10.0 ng/ml)	
Joshi et al 2011[33]	33/72 (45.9%)	52/72 (72.2%)	Clinical and radiological	Faecal elastase	D (<50.0 nmol/l)	
Dujsikova et al 2008[34]	NR	NR	EUS (3 or more criteria's according to Wiersema)	NR	D (<50.0 nmol/l)	
Duggan et al 2015[35]	NR	NR	Clinical and functional test and/or radiological	NR	D (<30.0 nmol/l)	
Morán et al 1997[38]	14/14 (100%)	6/14 (42.9%)%	Clinical, radiological or histological	Duodenal intubation using secretin test	D (<20.0 ng/ml)	

Table 1 continued

PERT = pancreatic enzymes replacement therapy, BMI = body mass index

Three micronutrients (vitamin A, D and E) and one macronutritional assessment parameter (BMI) met the criterion of more than three studies estimating or giving information necessary to estimate the prevalence of deficiency. Consequently, meta-analysis was performed for these parameters (figure 3-6). The remaining micronutrients and macronutritional assessment parameters are listed in table 2.

Assessment variables	Study	Reference	Prevalence (%)
Albumin	Lindkvist et al 2012[43]	<4.0 g/dl	6.2
	Olesen et al 2016[41]	<36.0 g/l	29.4
Calcium	Morán et al 1997[38]	<8.5 mg/dl	85.7
	Pezzilli et al 2015[39]	<8.4 mg/dl	0.0
Magnesium	Lindkvist et al 2012[43]	<1.55 mg/dl	2.0
Sarcopenia	Olesen et al 2019[9]	EWGSOP	17.0
Vitamin B12	Glasbrenner et al 1991[47]	<190 pg/ml	5.1
	Greer et al 2019[28]	<110 pg/ml	0.3
	Lindkvist et al 2012[43]	<211 pg/ml	0.0
Zinc	Lindkvist et al 2012[43]	<65.0 µg/dl	7.1
	Vujasinovic 2019[39]	<11.0 µmol/l	26.0

Table 2: the remaining micronutrients and macronutrient assessment parameters in the meta-analysis.
EWGSOP = European Working Group on Sarcopenia in Older People

Six studies[26-31] evaluated the prevalence of vitamin A deficiency (figure 3). The pooled prevalence of vitamin A deficiency was 21.0% (95% CI, 10.0-32.0%) with noteworthy heterogeneity between studies (I^2 = 91.24%). Out of the six studies, one study did not report the deficiency cut-offs used. Six studies[26-31] evaluated the prevalence of vitamin E deficiency (figure 4). The pooled prevalence of vitamin E deficiency was 31.0% (95% CI, 15.0-47.0%) with noteworthy heterogeneity between studies (I^2 = 94.94%). Out of the six studies, one study did not report the deficiency cut-offs used. Twelve studies[26,28-38] reported the prevalence of vitamin D deficiency and insufficiency (figure 5). For vitamin D deficiency, the pooled prevalence was 50.0% (95% CI, 29.0-72.0%) with noteworthy heterogeneity (I^2 = 77.06%). For vitamin D insufficiency the pooled prevalence was 68.0% (95% CI, 52.0-83.0%) with noteworthy heterogeneity (I^2 = 89.65%). Out of the 12 studies, one study did not report the deficiency cut-offs used. Nine studies[28,29,37,39-44] evaluated the prevalence of underweight (figure 6). The pooled prevalence of underweight (BMI <18.5 kg/m^2) was 11.0% (95% CI, 6.0-16.0%) with noteworthy heterogeneity (I^2 = 80.26%). The pooled prevalence of underweight (BMI <20.0 kg/m^2) was 19.0% (95% CI, 9.0-29.0%). Heterogeneity was not possible to determine due to a limited number of studies using a BMI cut-off of 20 kg/m^2 (n = 3).

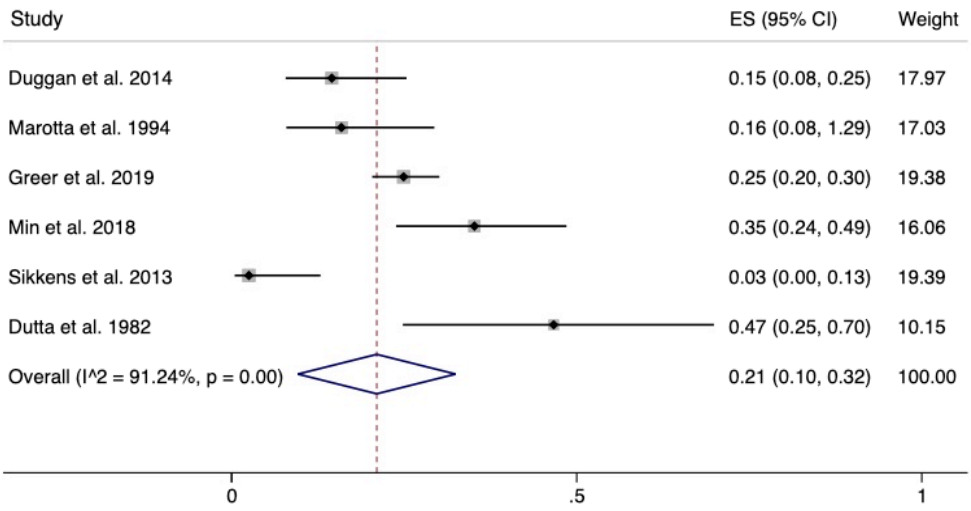

Figure 3: forest plot of the pooled prevalence (ES) with heterogeneity (I^2) of vitamin A deficiency.

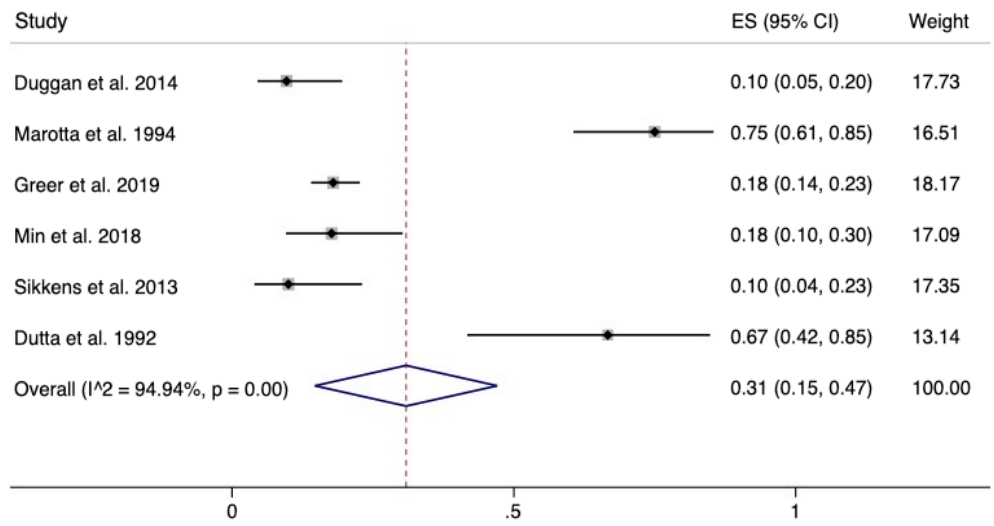

Figure 4: forest plot of the pooled prevalence (ES) with heterogeneity (I^2) of vitamin E deficiency.

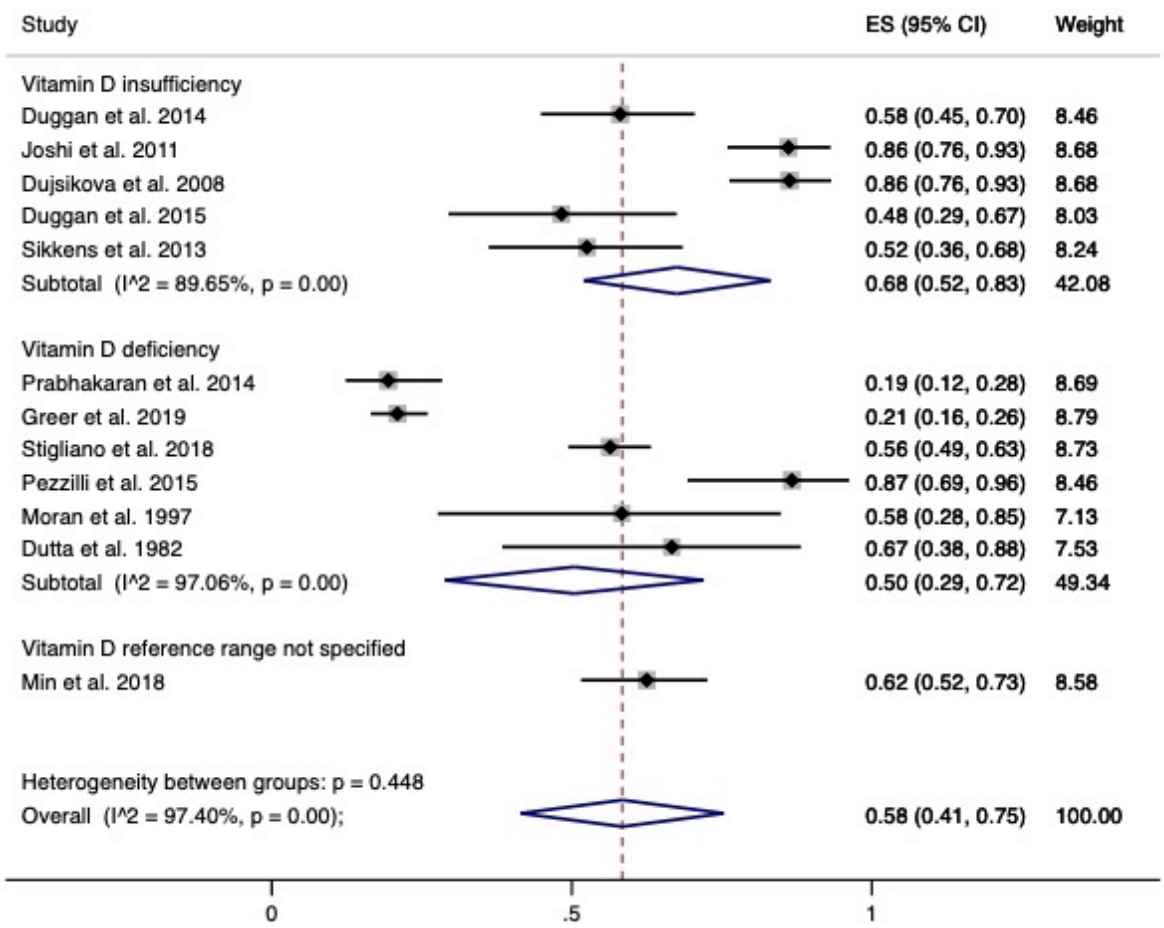

Figure 5: forest plot of the pooled prevalence (ES) with heterogeneity (I^2) of vitamin D deficiency and insufficiency.

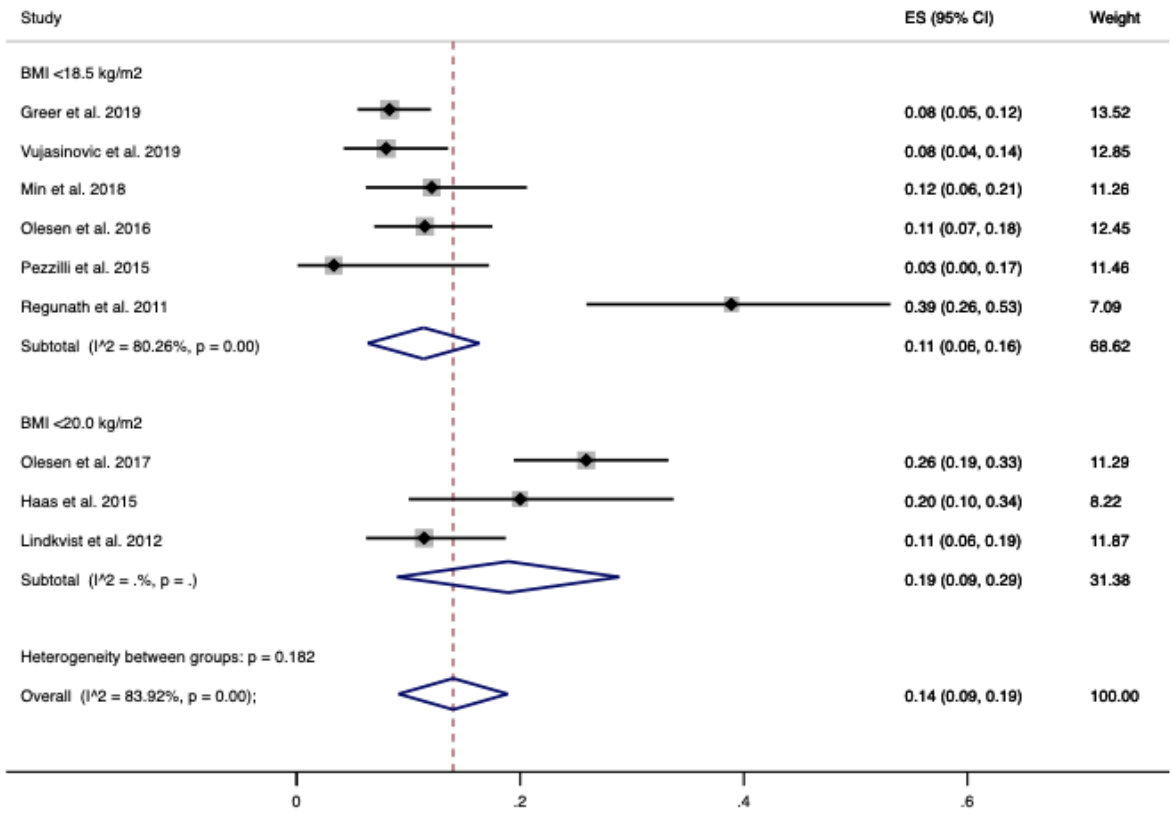

Figure 6: forest plot of the pooled prevalence (ES) with heterogeneity (I^2) of BMI.

Substudy 2: Cross-sectional study of CP outpatients

Study characteristics

In total 233 patients with CP were seen in the outpatient clinic between January 2012 and through May 2017, of whom 137 and 115 patients were included respectively. Reasons for exclusion and division into subgroups are shown in figure 7. Table 3 shows the characterization of the two study populations. For mean age, gender and EPI and/or PERT status, the two study populations were comparable. For both, the aetiology were mostly multifactorial, where the most frequent aetiological risk factors being alcohol and nicotine.

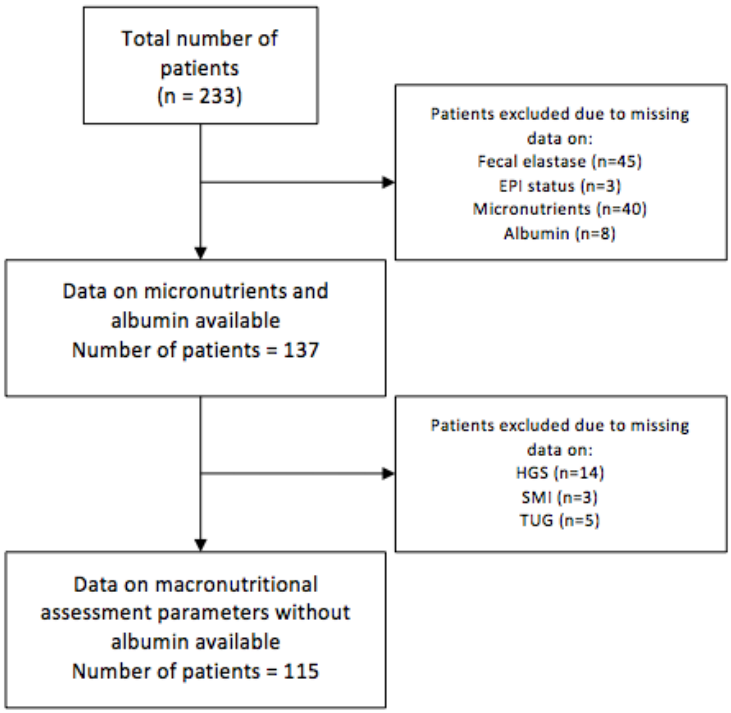

Figure 7: inclusion- and exclusion of patients in this study.
EPI = exocrine pancreatic insufficiency, HGS = handgrip strength, SMI = skeletal muscle mass index, TUG = time-up-and-go.

	137 patients	115 patients
Age (years)	57.82 ± 1.15	57.36 ± 1.29
Sex, n (%)	M: 97 (70.80)	M: 82 (71.30)
Duration of CP, years (95% CI)	4.11 (3.12-5.10)	4.32 (3.18-5.46)
Aetiology, n (%)*		
Alcohol	66 (49.25)	55 (49.11)
Nicotine	102 (75.0)	89 (78.07)
Nutrition	0 (0)	0 (0)
Hereditary	36 (26.87)	31 (27.68)
Efferent duct	15 (11.19)	14 (12.0)
Immunology	2 (1.49)	0 (0)
Miscellaneous	10 (7.46)	7 (6.25)
Unknown	2 (1.45)	2 (1.74)
EPI, n (%)	84 (61.31)	71 (61.74)
PERT, n (%)	51 (37.23)	42 (36.52)
Diabetes, n (%)	50 (37.31)	38 (33.63)

Table 3: study characteristics.
M = male, CP = chronic pancreatitis, EPI = exocrine pancreatic insufficiency, PERT = pancreatic enzymes replacement therapy.
* The same patient can have multiple risk factors in the M-ANNHEIM classification.

Prevalence of the micronutritional deficiencies and macronutritional abnormalities

The prevalence of micronutritional deficiencies and macronutritional abnormalities are shown in table 4 and figure 8. The most frequent micronutritional deficiencies were vitamin D insufficiency (26.3%), followed by vitamin D deficiency (21.9%), zinc deficiency (19.7%), and magnesium deficiency (16.8%). In addition, the most frequent macronutritional abnormalities were albumin deficiency (29.2%) followed by presence of sarcopenia (17.4%).

Assessment	Prevalence (%)	95% CI	Number of patients
Vitamin A	10.22	5.70-16.55	137
Vitamin E	7.30	3.56-13.01	137
Vitamin D insufficiency	26.28	19.13-34-48	137
Vitamin D deficiency	21.90	15.29-29.76	137
Vitamin B12 <125	0	0	137
Vitamin B12 <200	8.76	4.61-14.80	137
Calcium	0	0	137
Magnesium	16.79	10.95-24.12	137
Zinc	19.71	13.41-27.36	137
Albumin	29.20	21.75-37.57	137
Underweight	9.57	4.87-16.47	115
Sarcopenia	17.39	10.96-25.57	115
Phase angle	7.55*	7.33-7.78	115

Table 4: prevalence of the micronutritional deficiencies- and macronutritional abnormalities.
* Calculated in mean.

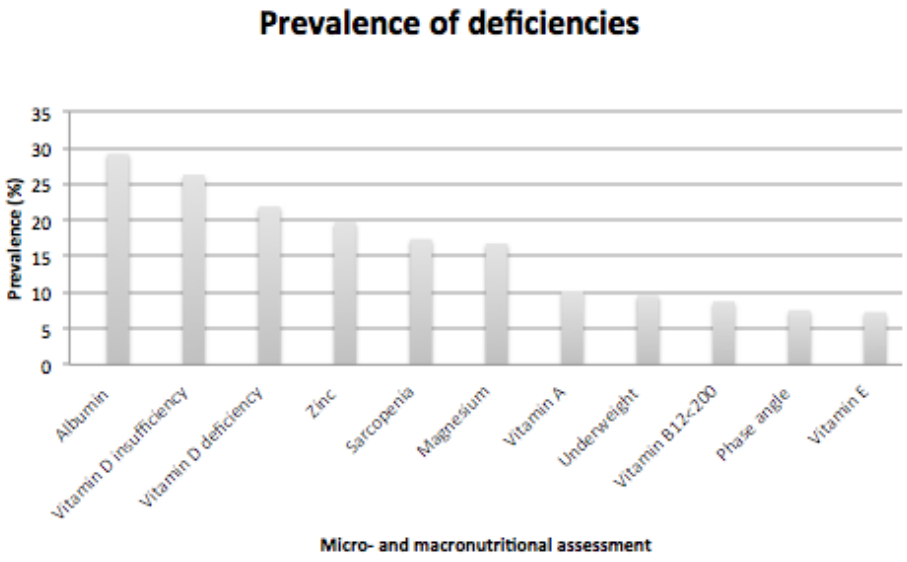

Figure 8: prevalence of the micronutrients deficiencies and macronutritional abnormalities.

Association between nutritional status, EPI and/or PERT

Significant differences between the three subgroups defined by EPI and PERT, were found for vitamin A (p = 0.008), vitamin E (p = 0.000), vitamin B12 (p = 0.027), magnesium (p = 0.038), sarcopenia (p = 0.032), and BMI (p = 0.026). For the statistical significant micronutrients, figure 9-13 visualizes the distribution of data as dot plots. The proportionate distribution of sarcopenia stratified by EPI/PERT subgroups are shown in figure 14. The majority (72.4%) of patients with EPI but not

treated with PERT had presarcopenia. A proportion of 28.6% in the subgroups of EPI with PERT had sarcopenia, which is more than double of the other groups. No significant difference within the three subgroups were found for vitamin D (p = 0.855), calcium (p = 0.108), zinc (p = 0.303), albumin (p = 0.070), and phase angle (p = 0.059). Dot plots for these are shown in appendix 2.

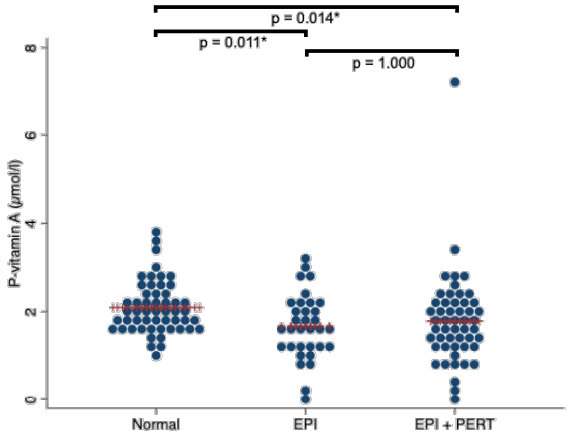

Figure 9: significant association (p<0.05) between vitamin A and EPI and/or PERT.

*** Demonstrates significant difference between normal and EPI without PERT as well as normal and EPI with PERT.**

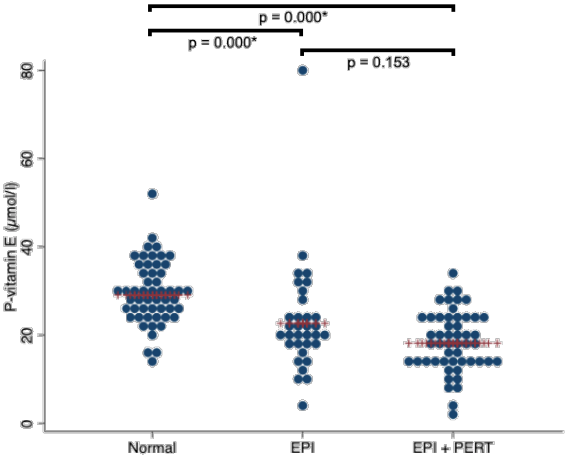

Figure 10: significant association (p<0.05) between vitamin E and EPI and/or PERT.

***Demonstrates significant difference between normal and EPI without PERT as well as normal and EPI with PERT.**

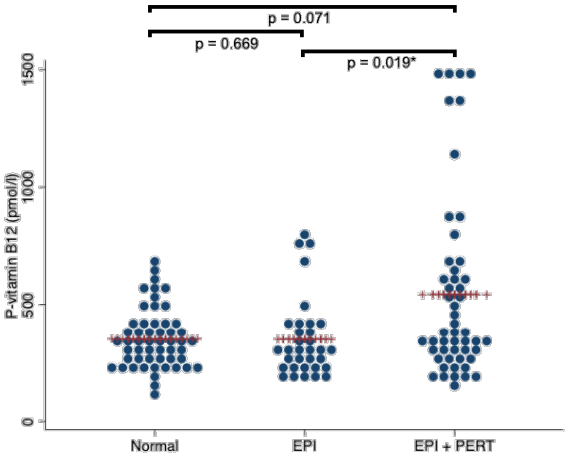

Figure 11: significant association (p<0.05) between vitamin B12 and EPI and/or PERT.

*** Demonstrates significant difference between EPI without PERT and EPI with PERT.**

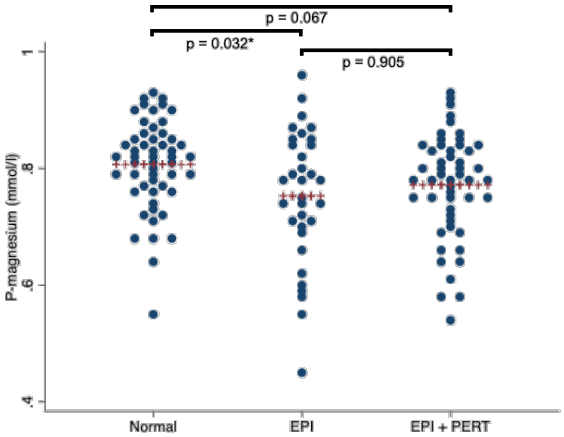

Figure 12: significant association (p<0.05) between magnesium and EPI and/or PERT.

*** Demonstrates significant difference between normal and EPI without PERT.**

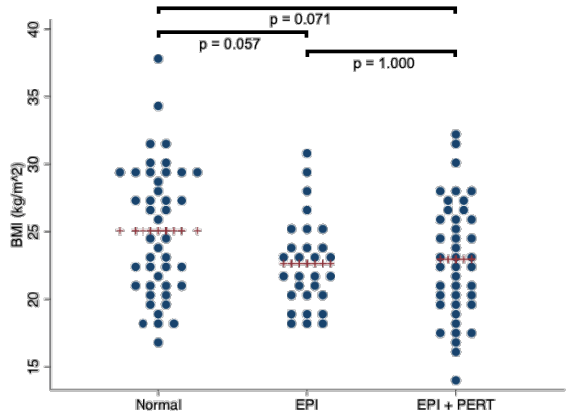

Figure 13: significant association (p<0.05) between BMI and EPI and/or PERT. No significant difference between groups were found (p>0.05)

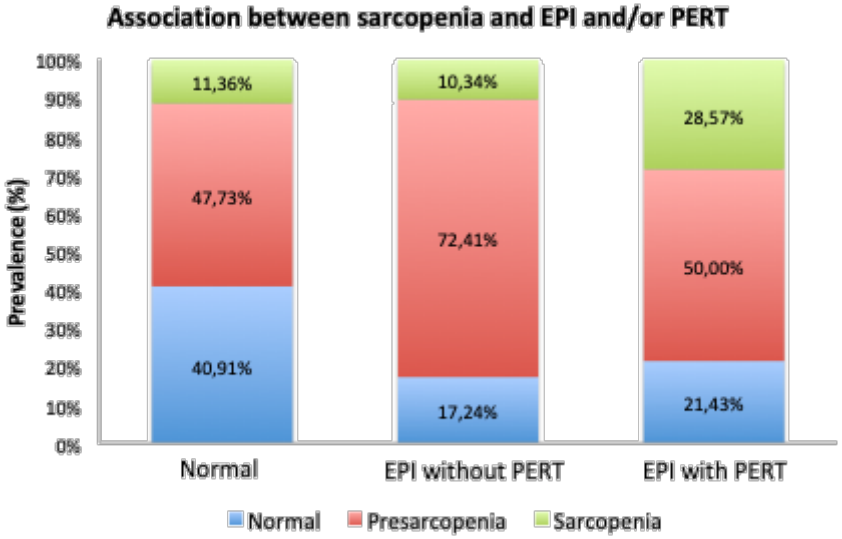

Figure 14: association between sarcopenia and EPI and/or PERT.

Association between micronutrients and macronutritional assessment parameters

The patterns of associations between macronutritional assessment parameters (BMI, sarcopenia and phase angle) and micronutrients are shown in table 5. BMI was associated with zinc (p = 0.002) with lower levels of zinc in underweight persons compared to their normal (p = 0.032) and overweight (p = 0.002) counterparts (figure 15). Sarcopenia was associated with vitamin E (p = 0.019), vitamin B12 (p = 0.049), and zinc (p = 0.003). Lower levels of vitamin E were found in patients with sarcopenia compared to their normal counterparts (p = 0.007) (figure 16). Higher levels of vitamin B12 were found in patients with sarcopenia compared to their presarcopenia counterparts (p = 0.024) (figure 17). Lower levels of zinc were found in patients with presarcopenia (p = 0.043) and sarcopenia (p = 0.003) compared to their normal counterparts (figure 18). Phase angle was associated with vitamin E (p = 0.014, figure 19), magnesium (p = 0.041, figure 20), and zinc (p = 0.088, figure 21). For all three micronutrients, there was a positive correlation. There was no significant association between the remaining micronutrients and macronutritional assessment parameters, which are shown in appendix 3.

	BMI	Sarcopenia	Phase angle
Vitamin A	-	-	-
Vitamin E	-	+	+
Vitamin D	-	-	-
Vitamin B12	-	+	-
Calcium	-	-	-
Magnesium	-	-	+
Zinc	+	+	+

Table 5: association between micronutrients and macronutritional assessment parameters.
+ = significant association, - = no significant association.

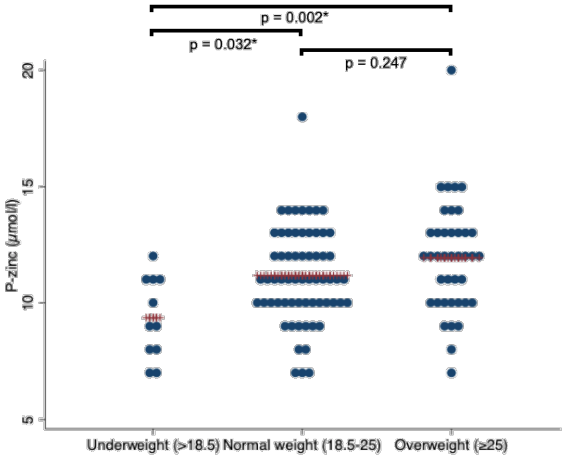

Figure 15: significant association (p<0.05) between zinc and BMI.
*** Demonstrates significant difference between underweight and overweight and underweight and normal.**

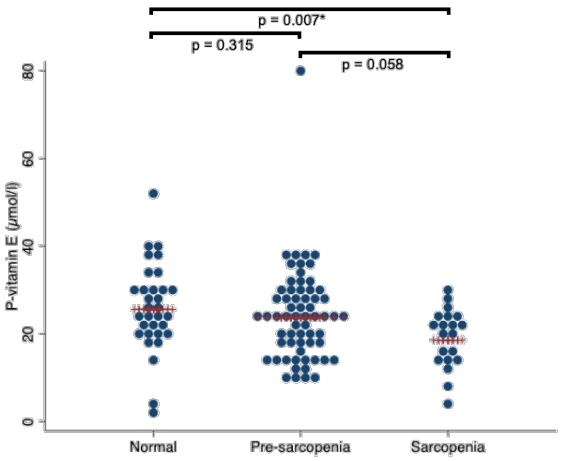

Figure 16: significant association (p<0.05) between vitamin E and sarcopenia.
*** Demonstrates significant difference between normal and sarcopenia.**

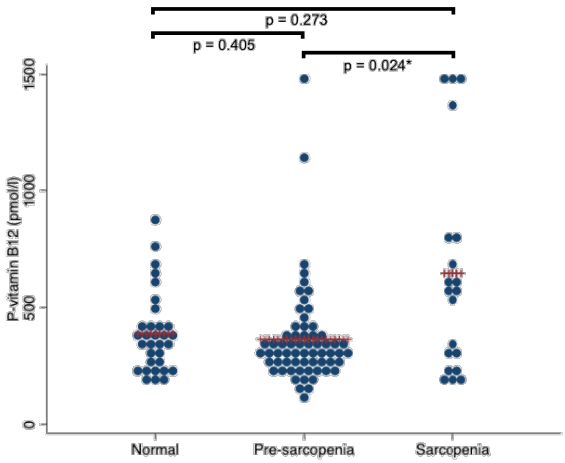

Figure 17: significant association (p<0.05) between vitamin B12 and sarcopenia.
*** Demonstrates significant difference between presarcopenia and sarcopenia.**

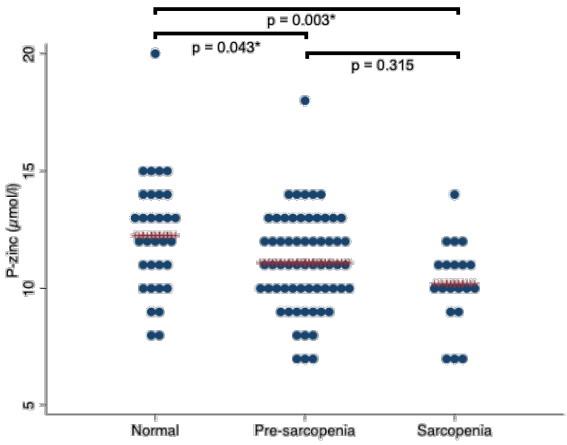

Figure 18: significant association (p<0.05) between zinc and sarcopenia.
*** Demonstrates significant difference between normal and presarcopenia, and normal and sarcopenia.**

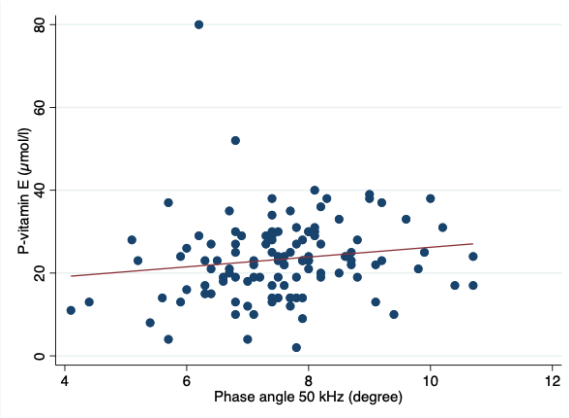

Figure 19: significant association (p<0.05) between vitamin E and phase angle with a positive correlation.

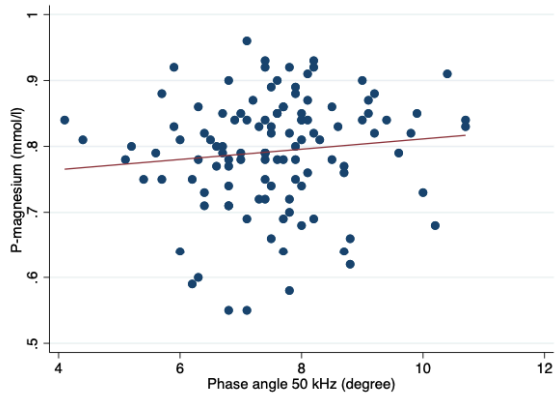

Figure 20: significant association (p<0.05) between magnesium and phase angle with a positive correlation.

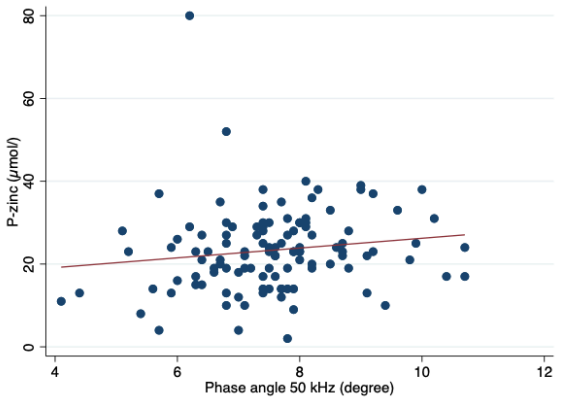

Figure 21: significant association (p<0.05) between zinc and phase angle with a positive correlation.

Discussion

Substudy 1: Meta-analysis

In substudy 1, a high prevalence of micronutritional deficiencies as well as macronutritional abnormalities were observed in patients with CP. However, all prevalence estimates were associated with a noteworthy heterogeneity, and therefore the interpretation must be handled with caution. One systematic review and meta-analysis, Martínes-Moneo et al[8], on this field has yet been published, including 12 studies, and only describing prevalence of deficiency of fat-soluble vitamins in CP patients. The results showed comparable prevalences of deficiency for vitamin A, E and D of 16.8%, 29.2%, and 57.6% respectively, as well as a large heterogeneity in between studies. Reasons for the conformity are that this meta-analysis included 10 of the same studies as Martínes-Moneo et al, which is almost half of our 19 studies. Therefore, the same variation in country of origin and study design were applied in both meta-analyses. However, the small deviation that exists can be due to inclusion of 2189 patients in substudy 1, compared to the 548 patients in the meta-analysis by Martínes-Moneo[8].

In between studies there was a large difference in the prevalence of micronutritional deficiency and/or macronutritional abnormality. Reasons for this disagreement can be due to several things. The assessment and definition of CP and test to determine EPI was done differently. Secondly, country of origin was also very divergent, and different lifestyle and amount of sun exposure has an influence on the nutritional state of the patient population. For example, the prevalence of vitamin D was investigated in both Ireland and India, and the lifestyle and amount of sun exposure varies a lot. Thirdly, the size of patient population varied in between studies, however sample size in the majority of studies were <100 patients. Finally, different settings and study design were used. The risk of bias in between studies varies according to the type of study design, and the meta-analysis included both cross-sectional, case-control, and cohort studies. Risk of bias also varies according to study quality, and studies included in the meta-analysis were classified as both high and low quality. The same goes for the different deficiency cut-offs used, which also could have biased the estimated pooled prevalence and heterogeneity.

Further limitations of substudy 1 are that the setting of studies were collection of patients at tertiary centres and thereby perhaps giving rise to selection bias due to lack of patients with mild CP. To account for previous discussed limitations, it could be beneficial to separate each of the following into 2 groups as done in Martínes-Moneo et al.: i) quality (low vs high), ii) country of origin (Asian vs Western), iii) rate of EPI in CP. A difference in the pooled prevalence between the two groups would contribute to explain whether the factor influence on the heterogeneity.

Substudy 2: Cross-sectional study of CP outpatients

Overall the prevalence of micronutritional deficiencies and macronutritional abnormalities were lower in the AAUH patient cohort compared to most of the studies in substudy 1. This may indicate that Danish patients with CP are less likely to suffer from severe micro- and macronutritional deficiencies compared to other patient populations. There were no clear associations between the presence of EPI and/or treatment with PERT for most nutritional parameters and also association between micro- and macronutritional parameters were limited expect for zinc. This may indicate

that zinc could serve as a useful biomarker for malnutrition in patients with CP but further validation is needed.

Prevalence of the micronutritional deficiencies and macronutritional abnormalities

A surprising finding was that less than one third of AAUH patients compared to half of patients from the meta-analysis had vitamin D deficiency. Since Denmark is a northern country, the prevalence of deficiency for Danish patients was expected to be higher than for other countries with more sun exposure. However, Morán et al[38] had a study population from Argentina with vitamin D deficiency prevalence of 58.0%, and for Pezzilli et al[37] from Italy the prevalence was 87.0%. The fact that countries with higher sun exposure can have higher deficiency of vitamin D than the Danish population questions the impact of sun on the level of vitamin D. Therefore it could be interesting to investigate whether there is a seasonal variation in blood samples in the vitamin D levels in CP patients. A Danish study, Mosekilde et al[45], found a lower level of vitamin D in winter compared to summer in healthy patients. Other factors explaining the difference in deficiencies could be variation in diet, clothing, consumption of vitamins and other supplements, smoking, age, BMI, and traveling[45].

Differences in diet and consumption of vitamins and other supplements in between studies, can also explain why prevalence of deficiency of vitamin A and E differs from what was found in substudy 1, which were 21.0% and 31.0% respectively. Moreover, low levels of vitamin A has been found associated to alcohol consumption[46]. Since alcohol was the most frequent aetiological risk factor (49.11-49.25%) in the Danish population, this raises an expectation of more frequent alcoholic aetiology in the studies of the meta-analysis. In contrast, the rate of alcoholic aetiology for Greer et al[28], Sikkens et al[30] and Duggan et al[26] were 49.8%, 50.0% and 38.7% respectively.

The deficiency rate of vitamin B12 was not high, which is in agreement with findings of other studies (5.1%[47], 0.3%[28], 0%[43]). However, there is no consensus on the used cut-off for deficiency, and results are difficult to compare. The prevalence was expected higher due to various reasons. First, alcoholism often leads to poor dietary habits. Second, pancreatic proteases play a role in the binding of vitamin B12 to intrinsic factor, and lack of proteases would then result in vitamin B12 not being absorbed[47]. The best explanation of this finding could be vitamin B12 injections, and falsely increased B12 due to coexistent alcoholic liver disease[48].

The deficiency rate of calcium of 0% could be consistent with the lower deficiency rate of vitamin D. Two studies, Morán et al[38] and Pezzilli et al[37] found prevalences of 85.7% and 0%, which are very divergent. This could be explained by the large difference in patient population, settings and across countries in between studies. In contrast to previous findings, the deficiency rate of magnesium was higher than what was found in Lindkvist et al[43] with a prevalence of 2.0%. A possible explanation of the higher deficiency rate could be a higher prevalence of steatorrhea caused by EPI in the Danish population. This results in binding of magnesium to faecal fat, and washing out the magnesium[49]. Consequently, it is interesting to establish the relationship between EPI and/or PERT status and magnesium.

Only one previous study, Olesen et al[9], has investigated the prevalence of sarcopenia, which was 17.0%. The result is very similar to this study with a prevalence of sarcopenia at 17.4%, but the two

studies also investigated an overlapping patient population at AAUH. Sarcopenia was found to be associated with reduced survival in Olesen et al[9], and therefore clinical attention to this subject should be paramounted.
The prevalence of underweight was almost comparable to meta-analysis prevalence of 11.0% (BMI <18.5 kg/m^2). This smaller prevalence of underweight of BMI can be related to the fact that the prevalence of micronutrient deficiency of the Danish population also was lower than of the meta-analysis. This may reflect that the Danish population is less sick and have fewer complications compared to patients in the meta-analysis. It is possible that there is different recommendations on when and how to begin treatment with PERT in CP patients across countries, and the finding may be due to Danish patients being well treated. A hypothesis could be that the most diseased patients in the study population of 115 patients were excluded, because they could not fulfil bioimpedance and/or tests for sarcopenia. In addition, it is noteworthy that the prevalence of underweight is much lower than the prevalence of sarcopenia, since undernutrition is considered as an important risk factor for development of both sarcopenia[50] and/or underweight. This study indicates that it is possible to be normal weight but still have sarcopenia. Therefore, it is interesting which of BMI or sarcopenia that are most sensitive to express undernutrition. Another indicator for malnutrition is phase angle. The mean phase angle was found to be 7.55 degrees. A systematic review and meta-analysis, Mattiello et al[51], have investigated gender and age specific reference values for phase angle in a healthy population. Nevertheless, there was high heterogeneity in between studies, and it is questionable if these reference values are comparable to CP patients.

Association between nutritional status, EPI and/or PERT

No studies have so far investigated the association between vitamin A and E, and EPI and/or PERT status. However, this study found that PERT does not improve deficiency of vitamin A and E, indicating that increased clinical focus on the level of these vitamins in patients with EPI is needed. Clinical consequences of deficiency of these vitamins are night blindness and immune dysfunction for vitamin A, and neurologic deficits for vitamin E[3,13,29].
An explanation of the higher levels of vitamin B12 for EPI with PERT could be the vitamin B12 injections. The result is in contrast to Lindkvist et al[43], who found no association between vitamin B12 and EPI and/or PERT status. Lindkvist et al[43] also found that magnesium was a nutritional predictor of EPI, which is in agreement with the findings of this study, suggesting that PERT improves magnesium absorption. In contrast to this, De la Iglesia-Garcia et al[52], found a difference between normal and EPI with PERT.
Shintakuya et al[53] reported an independent association between EPI and sarcopenia, which is in line with this substudy. To sum up, the agreement of an association of nutritional status to EPI and/or PERT was found to be inconclusive in between studies.

In this study no significant difference within the three subgroups were found for vitamin D, calcium, zinc, albumin, BMI, and phase angle. In agreement to this, Haaber et al[54] shows no association between EPI with PERT and vitamin D, calcium, and BMI compared to normal. Lindkvist et al[43] found no association of EPI without PERT to zinc and BMI compared to normal, however they found low albumin to be associated with EPI without PERT.
In two studies[52,55], the effect of PERT on albumin level is shown ambiguous. De la Iglesia-Garcia et al[52] shows lower albumin level in EPI with PERT patients compared to normal. In contrary, Trolli et al[55] shows higher albumin level in EPI with PERT patients compared to normal. Reason for

inconsistent associations could be the difficulty in controlling for confounding factors to malnutrition, such as vitamin supplementations, diabetes, pain, and alcoholism[8].

Consequently, the studies show varying associations to EPI and/or treatment with PERT. However, in this substudy no clear associations between the presence of EPI and/or treatment with PERT for most nutritional parameters was found. In general, the clinical recommendation is to continue monitoring of all micronutrients and macronutritional assessment parameters in all patients, but increased attention should be paid to vitamin A and E, magnesium, and Sarcopenia in patients with EPI. Despite the fact that treatment with PERT improves the level of magnesium, some of the patients with PERT are still in lack of magnesium. So as not to miss these patients, magnesium should be measured as the remaining nutrients in patients treated with PERT.

Association between micronutrients and macronutritional assessment parameters

To our knowledge, this is the first study to evaluate the association between various micronutrients and macronutritional assessment parameters in CP patients. The macronutritional assessment parameters are difficult to characterize, and consequently not part of the regular setup in outpatient clinic. Therefore, it is the attempt to study if micronutrients could serve as biomarker for the macronutritional status, and thereby contributing to good clinical practice. Three micronutrients were shown associated to macronutritional assessment parameters. Higher levels of vitamin B12 are shown for patients with sarcopenia compared with patients with presarcopenia. A possible explanation for this result might be vitamin B12 injections in patients with sarcopenia; a confounder that has not been accounted for in the study. Future studies therefore have to be aware of this factor. Vitamin E were shown associated to both sarcopenia and phase angle. Patients with sarcopenia had lower levels of vitamin E compared to their normal counterparts. Vitamin E is known to maintain healthy muscle mass by preventing inflammation and oxidative stress, and Khor et al[56] reviewed vitamin E as a protective factor for the development of sarcopenia. The antioxidative effect of vitamin E can also be a plausible explanation for the positive association shown between vitamin E and phase angle. A remarkable result was the association of zinc to all three macronutritional assessment parameters. Since zinc has been described as an important micronutrient in metabolic pathways, and in maintaining cellular structure and function[57], this could be an argument for the found association. No studies have yet investigated the association between zinc and macronutritional assessment parameters in CP patients. However, the same association between zinc and sarcopenia has been investigated in patients with chronic liver disease[58], and zinc supplementations has been found to increase phase angle in children[57].
Even though this study found a strong correlation between zinc and macronutritional assessment parameters, more investigation on whether zinc can be used as a biomarker for macronutritional abnormality in outpatient clinic setup is needed.

Strength and limitations

This study was able to include a large study population, which were well-characterized patients with CP. Another considerable strength is that this study is the first that aims to describe the association between micronutrients and macronutritional assessment parameters. Some limitations of this study should also be considered. First, data of patients were collected at a tertiary centre, and this could lead to that mild cases of CP might not be included, resulting in selection bias. Second, two study population sizes are used due to lack of collected data, which also may cause bias. However,

the characterization of the two study populations sizes are comparable (table 3). Third, a healthy control group would have been an advantage for the interpretation of results. Fourth, the study was not able to account for vitamin and other supplementations, and future studies should control for this lack of information. Finally, measurement error can occur in the collection and analysis of data as blood samples and bioimpedance.

Conclusion

Substudy 1:
A large heterogeneity was shown between studies for most of the existing estimates on malnutrition in patients with CP. This suggests large differences in the prevalence of CP associated malnutrition across countries, patient populations and settings. However, studies were found to be of different scientific quality and there is a great paucity on high quality studies in this field.
Substudy 2:
Overall the prevalence of malnutrition (micro- or macronutritional) seems lower in Danish outpatients with CP compared to the estimates provided from the existing literature. There are no clear association between malnutrition and the presence of EPI and use of PERT for most nutritional parameters. Also the association between micronutrients and macronutritional assessment parameters are generally poor, but a strong correlation between zinc and macronutritional assessment parameters was found. This knowledge has important clinical implications, since the application of zinc as a biomarker for macronutritional abnormality could replace the regular setup in outpatient clinic to examine macronutritional assessment parameters.

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