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Exocrine pancreatic and enterocyte function in patients with advanced pancreatic cancer

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SUMMARY

Background & aims: Exocrine pancreatic function is affected in patients with locally advanced pancreatic cancer (LAPC), clinically leading to steatorrhea. It is unknown whether maldigestion and malabsorption can also be attributed to impaired intestinal enterocyte function. In this exploratory study enterocyte function was assessed in patients with locally advanced pancreatic cancer, treated with Irreversible Electroporation (IRE).
Methods: Enterocyte function was studied by Citrulline Generation Test (CGT). Intestinal absorption capacity of energy and fat was calculated from the differences between nutritional intake (four-days diary) and quantified fecal losses energy and fat in three-days feces collection.
Results: Twelve patients were included before IRE, and 5 patients had follow-up measurements. Fasted citrulline [CIT] and glutamine [GLU] levels were below reference levels of healthy subjects ([CIT] 38 ± 8 μmol/L; [GLU] 561 ± 77 μmol/L) both before ([CIT] 25 ± 9 μmol/L; [GLU] 65 ± 35 μmol/L) and after IRE ([CIT] 19 ± 9 μmol/L; [GLU] 53 ± 26 μmol/L) whereas CGT curves were normal, indicating normal enterocyte function (slope 0.21 ± 0.12 and 0.17 ± 0.07 μmol/L/min; [CIT] increment 63 ± 39 and 80 ± 44% respectively). Severe energy/fat malabsorption was present in 6 out of 12 patients with LAPC (mean loss 349 kcal/d, 13 g fat/d) before and in 4 out of 5 patients (mean loss 509 kcal/d, 32 g fat/d) after IRE respectively.
Conclusions: Enterocyte function was generally within reference limits in patients with advanced pancreatic cancer. Severe malabsorption may be explained by exocrine pancreatic insufficiency.

1. Introduction

Pancreatic cancer is a highly aggressive cancer accounting for 4% of all cancer deaths worldwide [1,2]. It is often diagnosed at an advanced stage because of the late presence of clinical symptoms resulting in thirty to forty percent of patients presenting with locally advanced pancreatic cancer (LAPC). Due to limited treatment options, the median overall survival of these patients is approximately one year and this has barely improved over the past decades [3,4]. Experimental treatment procedures to improve prognosis and quality of life are being explored; one of these is Irreversible Electroporation (IRE) [3]. IRE is a tumor ablation technique based on the pulsatile application of electric energy delivered between two electrodes. The electric pulses change the existing cellular membrane potential, resulting in nanoscale defects in the lipid bilayer of the membrane, which disrupts cellular homeostasis and leads to apoptosis, [5]. In a recent phase I/II study with IRE in LAPC patients, it has been described that this procedure was well tolerated [3]. However, both major and minor complications may occur and further research is needed to address the efficacy of IRE in LAPC [6]. Approximately half of patients undergoing IRE developed gastro-intestinal adverse side effects. One of the reported side effects was (mucosal) duodenitis [3], which may affect enterocyte function. This may be clinically relevant in a group of patients that

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is already prone to malnutrition, due to a combination of elevated energy expenditure and increased fecal losses of fat and energy as a consequence of exocrine pancreatic insufficiency (EPI) [7]. Exocrine pancreatic insufficiency is characterized by a deficiency of pancreatic enzymes due to obstruction by the tumor, resulting in maldigestion, malabsorption, steatorrhea, and gastrointestinal complaints, which, in turn, all contribute to malnutrition, a critical problem in patients with pancreatic cancer [8]. So far it remains unknown whether the reported duodenitis after IRE, in addition to exocrine pancreatic insufficiency, may induce impaired enterocyte function (EF) and, thus may contribute to malabsorption.

The citrulline generation test (CGT) is a novel, sensitive tool to test intestinal enterocyte function (EF). In previous studies, fasted citrulline has been used as marker for enterocyte function. However, since a catabolic state, commonly seen in LAPC, is associated with a low fasting glutamine (a precursor of citrulline) concentration, a single measurement of fasted citrulline is not apt to adequately measure enterocyte function [9–11]. The CGT, a test based on the physiologic pathway in which glutamine is converted into citrulline after an oral dose of glutamine [10], is suggested as an alternative to study enterocyte function. Neither fasted citrulline levels nor the CGT has been assessed in LAPC patients. Besides, intestinal absorption capacity has never been measured after IRE, a unique and potentially targeted therapy. So far it remains unknown if enterocyte function is affected by IRE, and if it contributes to malabsorption in patients with LAPC. Thus, this study was conducted to unravel enterocyte function, intestinal absorption capacity and exocrine pancreatic insufficiency in patients with locally advanced pancreatic cancer, treated with IRE.

2. Materials and methods

2.1. Study design

This prospective nutrition and absorption study included 15 patients from the PANFIRE pilot study (PANFIRE – Pilot-study: Irreversible Electroporation (IRE) to Treat Locally Advanced Pancreatic Carcinoma, registered at clinicaltrials.gov NCT01939665) [3]. The Medical Ethics Review Committee of the Amsterdam UMC, Vrije Universiteit (the Netherlands) approved the study and patients gave written informed consent for participation before entering the study.

2.2. Patients

Fifteen consecutive patients with LAPC who were eligible for experimental, palliative Irreversible Electroporation procedure and included for the PAN-FIRE trial [3], were referred to a research dietitian 2–4 weeks before and 3 months after IRE to measure nutritional status, enterocyte function and intestinal absorption capacity to assess maldigestion, malabsorption and exocrine pancreatic insufficiency.

2.3. Baseline characteristics

Demographic, anthropometric and medical data (sex, age, weight, height, body mass index (BMI), tumor location and previous treatment) were obtained from medical patient records at time of referral.

2.4. Enterocyte function

Enterocyte function was measured with the Citrulline Generation Test (CGT), according to previously described procedures [9] and based on the assumption that the amino acid glutamine [GLU] is the precursor of citrulline [CIT] and converted in the enterocyte (Fig. 1). This test measures plasma [GLU] and [CIT] levels after an overnight fast and then stimulated plasma [CIT] levels 15, 75 and 90 min after an oral bolus of 20 g of dipeptide alanine–glutamine (Dipeptiven®), using reverse-phase high-performance liquid chromatography (HPLC) [12]. Results were compared with normative values in healthy subjects [9].

2.5. Intestinal absorption capacity

To measure intestinal absorption capacity and fecal losses, patients were instructed to collect all feces over a 3-day period in a pre-weighted 5 L bucket. Use of pancreatic enzyme replacement therapy was discontinued at least one day before and during feces collection. Collected feces were subsequently weighed, homogenized and stored below 4 °C until analysis. In parallel, starting one day before feces collection, patients filled-out a detailed 4-days food diary after instructions by a skilled and experienced dietitian. These records were used to calculate average daily nutritional intake of energy and fat using a computerized food calculation program (based on The National Dutch Food Composition Table 'NEVO 2006') [13].

Feces was analyzed for quantitative contents of fat (g/d; Van de Kamer method), nitrogen (g/d; micro-Kjeldahl method) and energy (kcal/d; bomb calorimetry) [14–16]. Fecal protein content was calculated from the fecal nitrogen content by multiplying nitrogen by 6.25. Finally, the intestinal absorption coefficients of energy, fat and protein were calculated as percentage of energy intake. Severe malabsorption was defined as an intestinal absorption capacity below 75%. In addition, the percentage of fecal energy loss caused by fecal fat loss was calculated using the equation: (fecal fat (g/d) × 9.40/fecal energy (kcal/d)). Results were compared with normative values in healthy subjects [17].

2.6. Statistical procedures

Variables are described as mean and standard deviation (SD) in case of normally distributed data or as median and inter quartile ranges (IQR) in case of not normally distributed data. Paired t-tests were performed for normally distributed variables, and Wilcoxon Signed rank tests for not normally distributed variables, to compare results of LAPC patients pre- and post-IRE. One sample t-tests were performed for normally distributed variables, and Mann Whitney U tests for not normally distributed variables, to compare results of LAPC patients before IRE with results of healthy

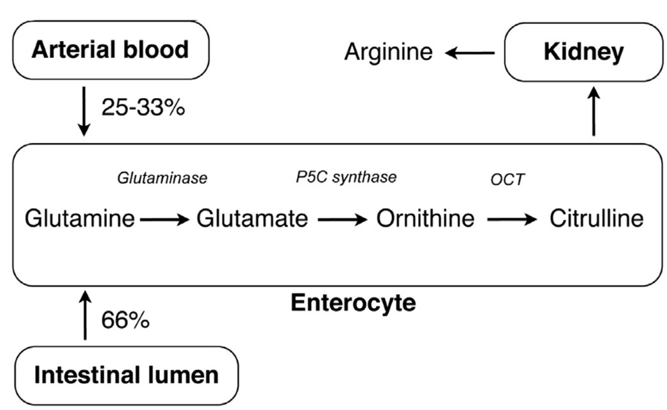

Fig. 1. Metabolization of arterial and enteral glutamine into citrulline by the enterocyte. P5C, pyrroline-5-carboxylate; OAT, ornithine aminotransferase; OCT, ornithine transcarbamylase [9].

controls. A p-value below 0.05 was considered statistically significant. All data were analyzed in Statistical Package for Social Sciences (SPSS) version 22.

3. Results

Fifteen LAPC patients were eligible for IRE in the PANFIRE study. Figure 2 displays the study flow chart for the enterocyte function and intestinal absorption capacity tests with inclusion and exclusion criteria of LAPC patients before and after IRE.

3.1. Baseline characteristics

Baseline patient characteristics of the 12 included patients with LAPC before IRE are shown in Table 1.

Eleven out of 12 patients who were included had undergone previous treatment: chemotherapy ($n = 2$), percutaneous transhepatic biliary drainage or plastic endoprosthesis ($n = 2$), chemotherapy and percutaneous transhepatic biliary drainage ($n = 2$), gastrojejunostomy ($n = 1$), hepaticojejunostomy ($n = 2$), chemotherapy and hepaticojejunostomy ($n = 1$) and gastrojejunostomy and hepaticojejunostomy ($n = 1$).

3.2. Enterocyte function

Table 2 and Fig. 3 show the results of the enterocyte function tested by citrulline generation test (CGT) before and after IRE. Data of 12 patients were available for the pre-IRE measurements and data of 5 patients post-IRE. Seven patients were lost to follow-up due to progressive disease ($n = 5$) or death ($n = 2$).

Fasted [CIT] did not differ before and after IRE ($p = 0.69$), nor did the stimulated [CIT] peak value ($p = 0.50$) or percentage [CIT] increment ($p = 0.72$). Accordingly, fasted [GLU] and [CIT] slopes of the CGT curves were not different before and after IRE ($p = 0.29$ and $p = 0.48$ respectively).

However, fasted [CIT], [CIT] peak and fasted [GLU] before IRE differed significantly from values of healthy controls ($p < 0.001$, $p < 0.001$ and 0.002 respectively). The [CIT] increment and [CIT] slope of the curve before and after IRE were not different from the increment and slope of curve of reference healthy controls.

Table 1
Patient characteristics of included patients.

Patient characteristics	$N = 12$
Sex (male/female)	6/6
Age (years), mean ± SD (range)	61 ± 9 (51–78)
BMI (kg/m^2), mean ± SD (range)	24.5 ± 2.5 (19.5–28.7)
Weight loss past 6 months (%), mean ± SD (range)	13 ± 6 (6–20)
Pancreas tumor location: head/tail/uncinate process, (n)	6/1/5
Previous medical treatment (yes/no)	11[a]/1

[a] Chemotherapy ($n = 2$), percutaneous transhepatic biliary drainage or plastic endoprosthesis ($n = 2$), chemotherapy and percutaneous transhepatic biliary drainage ($n = 2$), gastrojejunostomy ($n = 1$), hepaticojejunostomy ($n = 2$), chemotherapy and hepaticojejunostomy ($n = 1$) and gastrojejunostomy and hepaticojejunostomy ($n = 1$).

3.3. Intestinal absorption capacity

Table 3 shows intestinal energy and fat absorption capacity. Fecal energy and fecal fat losses were already higher than reference values for healthy subjects, and losses increased after IRE ($p = 0.0017$ for energy and $p = 0.036$ for fat, compared to pre-IRE).

4. Discussion

Patients with locally advanced pancreatic cancer appeared to have a normal enterocyte function measured by citrulline generation test (CGT), but fasted glutamine and citrulline levels in these patients were remarkably low. Intestinal absorption capacity was severely impaired in half of the patients at study initiation and in 80% after IRE.

Enterocyte function may be affected by IRE since duodenitis has been reported as an adverse event of experimental IRE treatment in patients with LAPC. We studied whether this phenomenon contributed to malabsorption which in turn may contribute to concurrent malnutrition being observed in more than half of the studied population [7]. We found that exocrine pancreatic insufficiency (resulting in increased fecal fat and energy losses), but not impaired enterocyte function, was critically important since enterocyte function remained intact as shown by CGT. Fecal energy losses was mainly due to steatorrhea which is consistent with e.g. exocrine pancreatic insufficiency.

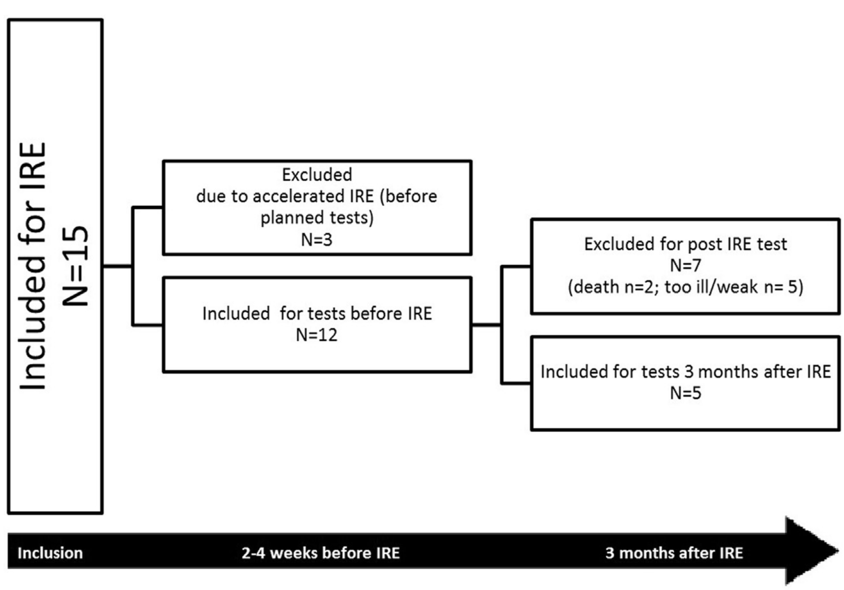

Fig. 2. Flow-chart of in- and exclusion of LAPC patients for the tests of enterocyte function and intestinal absorption capacity.

Table 2
Results of the citrulline generation test (CGT) to measure enterocyte function in patients with locally advanced pancreatic cancer before and 3 months after Irreversible Electroporation (IRE) in comparison with healthy subjects.

	Before IRE ($n = 12$) mean ± SD	3 months after IRE ($n = 5$) mean ± SD	References healthy subjects ($n = 19$)[a] mean ± SD
Baseline [GLU] (μmol/L)	65 ± 35	53 ± 26	561 ± 77
Baseline [CIT] (μmol/L)	25 ± 9	19 ± 9	38 ± 8
Peak [CIT] (μmol/L)	39 ± 15	33 ± 13	55 ± 10
Increment [CIT] (%)	63 ± 39	80 ± 44	44 ± 13
Slope (μmol/L/min)	0.21 ± 0.12	0.17 ± 0.07	0.22±0.08

IRE = Irreversible Electroporation, GLU = glutamine, CIT = Citrulline.
[a] 8 males/11 females, age (mean ± SD): 44 ± 13 years, BMI (mean ± SD): 23.6 ± 1.8 kg/m^2 (9).

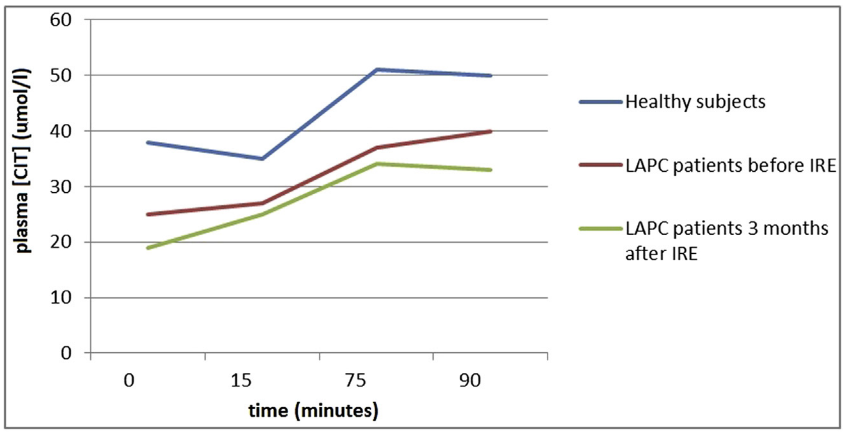

Fig. 3. Plasma [CIT] levels (μmol/L) of CGT in LAPC patients before and 3 months after IRE and healthy subjects.

Table 3
Results of feces collection and intestinal absorption capacity in patients with locally advanced pancreatic cancer before and 3 months after Irreversible Electroporation (IRE) and compared to healthy subjects.

	Before IRE ($n = 12$) median [IQR]	3 months after IRE ($n = 5$) median [IQR]	p-value (before/after IRE)	Reference healthy subjects ($n = 23$)[a] Median [IQR]	p-value before IRE vs healthy controls
Fecal weight (g/d)	237 [111–516]	382 [205–633]	0.093	135 [109–177]	**0.009**
Fecal energy loss (kcal/d)	349 [207–1019]	509 [370–1073]	**0.017**	216 [154–258]	**0.017**
Intestinal energy absorption capacity (%)	79 [51–88]	66 [43–82]	0.176	90 [86–93]	**0.003**
Fecal fat loss (g/d)	13 [5–56]	32 [14–60]	**0.036**	4.9 [3.9–6.3]	**0.009**
Fecal fat loss (kcal/d)	120 [44–526]	304 [135–566]	**0.036**	46 [33–59]	**0.009**
Intestinal fat absorption capacity (%)	81 [30–90]	51 [34–87]	0.398	93 [91–95]	**0.005**
Fecal energy loss explained by fecal fat excretion (%)	34	60	**0.036**	21 [19–27]	**0.001**

[a] Healthy subjects: 9 males/14 females; age mean ± SD: 43 ± 13 years (17).

Of note, patients with LAPC had low fasted [CIT] levels and very low fasted [GLU] levels, both before and after IRE procedure, when compared to reference values in healthy subjects. Critical illness is associated with low plasma glutamine in severely ill patients and probably similarly in cancer patients [18–20]. It has been demonstrated that a low plasma glutamine level is indicative for a catabolic state [11]. Therefore, we presume that the low fasted [GLU] and [CIT] in LAPC patients are consistent with catabolism. The slope of [CIT] curve was within normative values, showing that after challenge enterocyte function appeared to response adequately with generation of citrulline. Fasted [CIT] is a poor biomarker for enterocyte function, as has been reported before [9,10]. The normal plasma [CIT] increment (%) and slope of CGT curve after the fixed dose of dipeptide alanine–glutamine substantiated that enterocyte function remained adequate in LAPC patients, irrespective of IRE procedure.

On the contrary, severe malabsorption of energy and fat remained to be a highly relevant clinical problem in LAPC patients. Malabsorption was present in 50% of the patients before IRE and increased up to 80% after IRE. The poor intestinal absorption capacity and high fecal energy loss (mainly after IRE) were mainly due to increased fecal fat losses, most likely as a result of deterioration of the exocrine pancreatic insufficiency [7].

This study was performed as an exploratory study in a small group of patients with LAPC. Although numbers were small, the data were consistent with intact enterocyte function and loss of exocrine pancreatic function. Particularly after IRE, missing data were not at random as missing data were due to progression of disease and death. This must be regarded as a limitation.

5. Conclusion

Enterocyte function was generally within reference limits both before and after IRE. We found corroborative data that malabsorption has to be ascribed to exocrine pancreatic insufficiency in patients with LAPC.

Statements of authorship

Conception and design: NJ Wierdsma, MAE de van der Schueren, AA van Bodegraven.

Collection and assembly of data: JE Witvliet, HJ Scheffer, LG Vroomen, MR Meijerink.

Data analysis and interpretation: JE Witvliet, MAE de van der Schueren, NJ Wierdsma, AA van Bodegraven.

Manuscript writing: JE Witvliet, MAE de van der Schueren, NJ Wierdsma and AA van Bodegraven.

Final approval of the manuscript: all authors.

Conflicts of interest

None.

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