**Long term outcomes after perioperative treatment with *n*-3 fatty acid supplements in colorectal cancer**

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**Abstract**

**Background:** Perioperative supplementation with omega-3 fatty acids (*n*-3 FA) affect several aspects of cellular function. This study aimed to evaluate the effect of *n*-3 FA on survival in patients after colorectal cancer (CRC) surgery, the risk of disease recurrence and the effect on adjuvant chemotherapy.

**Methods:** Patients scheduled for an elective CRC resection between 2007 and 2010 were randomised to either an *n*-3 FA enriched oral nutrition supplement (ONS) twice daily, or a standard ONS (control) for seven days before and after CRC surgery. Outcome measures included postoperative complications, five-year survival and three-year incidence of disease recurrence. Groups were compared using the Kaplan Meier and the Aalen Johansen estimator along with the pseudo observation method for multivariable comparisons.

**Results:** A total of 148 patients were enrolled out of 610 patients treated during the study- time period. Of the 148 patients enrolled, 125 were analysed (65 receiving the *n*-3 FA enriched ONS and 60 receiving the standard ONS). There were no difference in postoperative complications immediately after surgery. The five-year survival for patients treated with *n*-3 FA was 69.2% (95% CI [56.5; 78.9]) compared to 81.7% (95% CI [69.4; 89.4]) in the control group (p = 0.193). After adjustment for age, stage of disease, and adjuvant chemotherapy, *n*-3 FA was associated with higher mortality (HR = 1.73, 95%CI [1.05; 2.83]; p = 0.029) compared to controls. The interaction between *n*-3 FA and adjuvant chemotherapy was, however, not statistically significant. The risk of disease recurrence after three years was not different between groups (RR = 1.66, 95%CI [0.65; 4.26]).

**Conclusion:** Perioperative supplementation with *n*-3 FA did not confer a survival benefit in patients undergoing CRC surgery. Nor did *n*-3 FA benefit the subgroup of patients treated with adjuvant chemotherapy or decrease the risk of disease recurrence.

**Key words**

Colorectal cancer, fatty acid supplementation, survival, adjuvant chemotherapy

**Introduction**

Colorectal cancer (CRC) surgery has been associated with a phase of hyper-inflammation followed by a relative immune incompetence.[1,2] The patients' nutritional status, and in particular, the availability of specific biologically active nutrients like the marine *n*-3 fatty acids (FA) (eicosapentaenoic acid (EPA), the docosahexaenoic acid (DHA)) and the docosapentaenoic acid (DPA), [3–7] have been investigated in literature as factors influencing the post-operative course. Especially *n*-3 FA have been associated with an improved immunonproliferative response. We therefore hypothesized, that perioperative treatment with *n*-3 FA could lead to a decrease in infectious and non-infectious complications after CRC surgery.

Several CRC cell line studies investigated also whether the *n*-3 FA could improve the response to chemotherapeutic agents. A previous study[8] demonstrated a synergistic anti-cancer effect between EPA and a regimen of 5-fluorouracil (5-FU) and oxaliplatin in vitro and in vivo, against the human colon cancer cells. In line with this, another research hypothesized that the EPA could have a role in adjuvant therapy for prevention of metastatic CRC.[9] The oral administration of EPA reduced growth of experimental CRC liver metastases and importantly, a preoperative treatment with EPA resulted in improved disease-free survival.[9] Finally, it has been proposed that *n*-3 FA might increase the susceptibility of tumor cells to chemotherapeutic regimens:[10] in an experimental study conducted on mice with colon cancer, the administration of 5-FU and *n*-3 FA increased both DNA damage and apoptotic index by activation of extrinsic and intrinsic apoptotic pathways. The increased pro-apoptotic effect by the synergism of 5-FU and *n*-3 FA could be attributed to the incorporation of *n*-3 FA into the cancer cell membranes, altering membrane fluidity thereby chemo-sensibilizing the tumour cells.

The aim of original study was to investigate if an oral supplement with *n*-3 FA in the perioperative period could be associated with an improved postoperative outcomes (decreased number of complications). In the current study, we evaluated if the treatment was associated with improved survival of patients after CRC surgery and a decreased risk of disease recurrence.

**Materials and Methods**

***Study design.*** *A* randomised, double-blind, placebo-controlled single-centre trial was conducted studying the effect of an n-3 FA-enriched ONS on the long term outcomes in patients scheduled for CRC surgery. The study was not sponsored and the authors have no commercial interests.

Patients scheduled for CRC surgery at The Department of Gastrointestinal Surgery, Aalborg University Hospital between July 2007 and January 2010 were screened for eligibility. Aalborg University Hospital treated between 260 and 350 patients each year in the aforementioned study period. Each patient was referred from the surgical outpatient clinic, and operated according to the current Danish guidelines. The inclusion and exclusion criteria have been described before[11] (ClinicalTrials Identifier: NCT00488904).

Briefly, patients in both the *n*-3 FA and control group received the ONS as a sip feed (200 ml twice daily, once in the morning and once in the afternoon) for 7 days before and 7 days after surgery. The feeds were isocaloric (1.5 kcal/ml) and isonitrogenous and were provided by Fresenius Kabi (Supportan®). Both feeds contained the same amounts of carbohydrates, proteins, total fat and *n*-6 FA, as well as vitamins and minerals. However, the fatty acid composition differed between the two feeds; both contained medium-chain triglycerides, sunflower oil and safflower oil, but the *n*-3 FA ONS also contained fish oil; hence patients in this group received 2.0 g EPA and 1.0 g DHA per day from the ONS, while no *n*-3 FA was provided to the controls.

**Outcome measures.** Outcomes included peri-operative results (pneumonia, wound infection, urinary tract infection, peritonitis (including anastomotic leakage) and sepsis) and survivals. The 5-year overall survival (defined as being alive five years after CRC surgery) and the risk of disease recurrence (defined as cumulative incidence of local or metastatic CRC recurrence) after three years according to treatment allocation were evaluated. According to the Danish national guidelines for CRC treatment, each patient was followed with a computed tomography of the thorax and abdomen one, and three years after treatment. If the patients did not receive a full colonoscopy prior to surgery, a new colonoscopy was planned three months after surgery, otherwise all patients received a colonoscopy five years after treatment. Data on patient survival and disease

recurrence were collected using the patient records, and the Danish National Patient Registry. After evaluation of the surgical resection specimen, the patients were re-staged and evaluated on a new MDT conference, after which high risk stage II and stage III patients were referred for adjuvant chemotherapy. Stage IV patients without surgically treatable liver metastasis received chemotherapy with palliative intent only. A subgroup analysis of all the patients receiving adjuvant chemotherapy with a curative intent was also performed. Patients receiving either neoadjuvant radio-chemotherapy or postoperative palliative chemotherapy (stage IV patients) were excluded from sub-group analysis. Post-trial analysis of survival was approved by The North Denmark Committee on Health Research Ethics committee (N-20140064) and the Danish Data Protection Agency.

***Statistical analyses.*** The original study was designed to detect a 20% difference in postoperative complication rates between groups (a reduction from 30% to 10% was hypothesised). The required sample size was 72 patients in each group (80% power, $\alpha$=0.05). Long term survival was not part of the original study, and therefore not part of the original sample size calculation. Baseline characteristics were compared using Student's t-test or Fisher's exact test where appropriate. The Kaplan Meier estimator was used to depict the association between treatment with the n-3 FA ONS and overall survival. The overall five-year survival was calculated from Kaplan Meier estimates and compared using the Log-rank test. To evaluate the effects of a supplement with n-3 FA along with interaction with adjuvant chemotherapy, a generalized linear model was constructed using the pseudo-observation method.[12] Estimates were presented as hazard ratios (HR) with 95% confidence intervals (95%CI). The risk of disease recurrence was estimated using competing risk regression. The Aalen Johansen estimator was used to compute the cumulative incidence plot and the pseudo-observation method was used to compute the relative risk (RR) of disease recurrence three years after surgery. All analyses were conducted using STATA® V.15.1 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorpLLC).

**Results**

*Population.* Figure 1 illustrates the study flowchart. A total of 610 patients were deemed eligible and asked to participate. However, 201 did not meet the inclusion criteria, 31 declined to participate and 230 could not be included because they received surgery within four to five days after diagnosis, and could therefore not receive the allocated seven day intervention prior to surgery. The remaining 148 patients were randomised, however 19 patients were subsequently excluded due patient refusal, postoperative death, lack of surgery, and logistics (did not received the appropriate intervention). These patients were analysed in the original study after the "intention to treat principle". For this study, we chose to exclude four patients in the control arm because, after enrolment, they were not treated with a CRC resection. Accordingly, 65 patients were enrolled in the treatment arm and 60 patients in the control arm. The baseline characteristics of the participants are shown in Table 1. Twenty-two patients received adjuvant chemotherapy either as monotherapy with 5-FU (n=5) or combined treatment (n=17) with folinic acid, 5-FU, and oxaliplatin (FOLFOX).

*Peri-operative outcomes*. The data on perioperative outcomes are reported elsewhere.[11] In brief, there was no statistically significant difference regarding the number of infectious, and non-infectious complications, 30 day mortality, or length of stay.

*Survival*. The five-year overall survival was 69.2% (95%CI [56.5; 78.9]) in patients treated with n-3 FA compared with 81.7% (95%CI [69.3; 89.4]) in the control group with no statistically significant difference between groups (p = 0.193). The Kaplan Meier survival curves are presented in Fig.2. As indicated by the survival curves, the assumption of proportional hazards was violated over the recorded time-period (which was also evident from the Log-Log plot). We therefore used the pseudo-observation method to construct a

generalized linear model to evaluate the effect of treatment with n-3 FA and adjuvant chemotherapy on five-year survival. The model was adjusted for age and stage of disease at the time of diagnosis. The unadjusted HR five-years after surgery was 1.67 (95%CI [0.87; 3.21]) suggesting no effect of n-3 FA treatment on survival of surgically treated CRC patients (Table 2). After adjustment for adjuvant chemotherapy, age and stage at the time of diagnosis supplementation with n-3 FA were associated with a higher overall mortality (HR = 1.73, 95%CI [1.05; 2.83]: p = 0.029). The interaction between treatment with n-3 FA and adjuvant chemotherapy was, however, not statistically significant, suggesting a spurious association. The stratified HR for patients in the n-3 FA group was 2.09 (95%CI [0.92; 4.75]) compared with 1.29 (95%CI [0.51; 3.25]) in the control group in patients treated with adjuvant chemotherapy (p = 0.138).

*Disease recurrence.* The effect of treatment with n-3 FA on the risk of disease recurrence using death as a competing risk was calculated (Fig 3). We only evaluated patients without residual disease immediately after surgery leaving 110 patients for analysis (55 in each group). The risk of disease recurrence did not differ between groups (RR = 1.66, 95%CI [0.65; 4.26]) and adjustment for stage and adjuvant chemotherapy did not alter the estimate.

**Discussion**

This study aimed to evaluate perioperative treatment with *n*-3 FA on the outcomes of CRC patients in a randomised clinical setting. Of note, the investigation has been designed as a randomised controlled trial, enrolling blinded participants: the sip feeds were nutritionally similar and had an identical appearance and taste, apart from content of marine *n*-3 FA. Compliance with the allocated intervention (*n*-3 FA vs. control) was acceptable and the study population was relatively homogeneous.[11]

However, the study was powered to show a difference in postoperative complications and not a difference in survival in patients receiving chemotherapy and *n*-3 FA, thus the number of patients might have been too low for this outcome. The low number of patients receiving adjuvant chemotherapy (11 patients in each group) also limited the study power and any association with reduced survival or other events should be interpreted with caution. Follow-up on postoperative dietary habits, other disease states and medications was not conducted which should also be regarded as a limitation. The intake of long-chain *n*-3 FA in western countries is generally low, but is traditionally higher in Scandinavian countries, and therefore the results may not be directly comparable for other populations. Finally, other *n*-3 FA dosages and different lengths of treatment duration were not considered. Prolonged treatment might be needed to ensure an effect on postoperative outcomes.

Some meta-analyses have identified that so-called immunonutrition, containing arginine, glutamine, nucleotides and *n*-3 FA, could reduce postoperative infections in selected patient groups.[13,14] However, it is unclear which of the components is responsible for the clinical effect. In the current study, daily supplementation with 3 g of EPA + DHA for 7 days before and 7 days after CRC surgery did lead to increased incorporation of these *n*-3 FA in the cell membranes and a significant increase in the production of leukotriene B5 from EPA and a significant decrease in the production of leukotriene B4, but had no effect on postoperative complications.[11,15,16]

It has been suggested that supplementation with *n*-3 FA could increase chemotherapeutic efficacy and thereby contribute to increased survival. In a combined analysis of The Nurses' Health Study and The Health

Professionals Follow-up Study, it was reported that a high intake of *n*-3 FA after a diagnosis of CRC was associated with a lower risk of mortality from CRC.[17] Thus, patients consuming at least 0.3 g *n*-3 FA/day had an adjusted HR for CRC-specific mortality of 0.59 (95% CI 0.35 to 1.01). Patients who increased their marine *n*-3 FA intake by at least 0.15 g/day after their diagnosis had a HR of 0.30 (95% CI 0.14 to 0.64, p for trend <0.001) for CRC deaths, compared with those who did not change their intake of marine *n*-3 FA. These results are indeed interesting, but the findings cannot be directly compared with the current study as residual confounding might be a factor in the aforementioned study, which should not be present in a randomised clinical setting.

A previous Phase II double-blind placebo RCT enrolled patients with colorectal cancer and liver metastases. Patients in the intervention group (n=43) received 2 g of EPA daily from randomization to the day of surgery (liver surgery).[9] Patients in the control group (n=45) received placebo capsules containing 500 mg mixed capric and caprylic acid medium-chain triglycerides twice-daily. The *n*-3 FA EPA was successfully incorporated into liver metastases removed at the operation and a trend towards reduced tumour vascularization in *n*-3 FA treated CRC liver metastases was recorded. The authors report a trend towards an improved overall survival from *n*-3 FA in the first 18 months after surgery (not statistically significant), and early CRC recurrence was similar in the treatment groups. [9]

A Meta-Analysis compared RCTs using pharmaconutrition with those using standard nutrition in elective adult surgical patients operated between 1980 and 2011[18]. No differences were reported in postoperative mortality, but there was a significant reduction in infectious complications and length of hospital stay. Regarding perioperative administration, there was a reduction in anastomotic dehiscence. Furthermore, a reduction in non-infectious complications was detected with postoperative administration. Pharmaconutrition given preoperatively could not demonstrate a notable advantage compared to standard nutrition in any of the assessed clinical outcomes. [18] The importance of timing is also shown in another review of published meta-analyses implying improved outcome after oncological gastrointestinal surgery, but before issuing recommendations further studies are warranted.[19] Additionally, two meta-analyses[18,20]

showed that preoperative pharmaconutrition did not confer any benefit over standard formulations. The earlier seen benefits of pharmaconutrition i.e., reduction in infectious complications, were only reported with peri- or postoperative administration. Currently, it seems premature to issue recommendations for the use of pharmaconutrition to these patients.

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**Figure legends**

**Figure 1** Flow chart of patients eligible for follow-up.

A total of 610 patients were deemed eligible, of which 201 failed to meet the inclusion criteria, 31 declined to participate and 230 received surgical resection within four to five days after diagnosis, making them unable to receive the allocated seven day intervention prior to surgery. We excluded four patients (excluded from analysis, n = 4) from the control group because they were deemed non-resectable during surgery.

**Figure 2** Overall survival according to treatment allocation

The blue line represents patients in the control group. The red line represents patients receiving the *n*-3 FA enriched oral nutritional supplement. The risk table represents the number of patients at risk according to treatment group.

**Figure 3** Risk of disease recurrence according to treatment groups

The risk of disease recurrence was analysed for patients who received curatively intended CRC surgery. The blue line depicts patients in the control group (N=55). The red line represents patients receiving the *n*-3 FA enriched oral nutritional supplement (N=55).