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Erythrocyte deformability and aggregability in patients undergoing colon cancer surgery and effects of two infusions with omega-3 fatty acids

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Abstract.
BACKGROUND: An adequate erythrocyte function is vital for tissue oxygenation and wound healing. The erythrocyte membrane phospholipid composition plays an important role in erythrocyte function and administration of omega-3 fatty acids may provide a means to improve it.
OBJECTIVE: To investigate peri-operative erythrocyte function and effects of omega-3 fatty acids
METHODS: Forty-four patients undergoing elective laparoscopic colon resection for non-metastasized cancer were randomized between intravenous omega-3 poly-unsaturated fatty acids (n-3 PUFAs) or placebo (saline). Peri-operative blood samples were analyzed with a Lorrca MaxSIS Ektacytometer and erythrocyte membrane phospholipids were determined with gas chromatography.
RESULTS: Patient and operation characteristics were equal between groups. There was a significant increase in erythrocyte membrane eicosapentaenoic acid (EPA) but not docosahexaenoic acid (DHA) in the n-PUFA group. There were no significant differences in erythrocyte deformability but the aggregation index (AI) was significantly lower and the aggregation half time (T½) was significantly higher in the n-3 PUFA group.
CONCLUSION: This study confirms rapid changes in erythrocyte membrane phospholipid composition after administration of intravenous n-3 PUFAs. Erythrocyte deformability parameters were not affected but erythrocyte aggregability was decreased in the n-3 PUFA group. Further investigation is necessary to gain more insights in the effects of n-3 PUFA and the postoperative inflammatory response on erythrocyte function.

Keywords: Erythrocytes, deformability, aggregability, colon cancer surgery, omega-3 fatty acids

1. Introduction

An important but less well known factor in the recovery after major abdominal surgery is a normal erythrocyte function, vital for an effective microcirculation and adequate tissue oxygenation. An impaired microvascular flow is associated with post-operative complications after major abdominal surgery [1].

Erythrocytes have the ability to undergo large passive deformations in order to pass the small capillaries of the microcirculation, up to one-third smaller than its normal diameter. There are 3 important factors that determine the ability to deform to this extend: the geometry of the cell, particularly cell surface area to volume ratio, the cytoplasmic viscosity determined by intracellular hemoglobin concentration, and membrane deformability [2].

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Erythrocyte aggregability is a reversible phenomenon under the influence of shear rate, which is the velocity gradient between the layers in a fluid under pressure. Erythrocyte aggregates can be divided into linear (rouleaux) and three dimensional structures (non-rouleaux). They can form at lower shear rates, dissolve at higher shear rates and have an impact on blood viscosity [3].

Critical illness and co-morbidities like diabetes and metabolic syndrome are associated with a decreased erythrocyte deformability and increased aggregability [4–6]. In addition, Langenfeld et al. demonstrated that trauma results in a decreased red cell deformability and that a further decrease in red cell deformability is an early indicator for infection [7].

Administration of omega-3 fatty acids is a way to change the composition of the erythrocyte membrane fatty acid composition and possibly enhance the erythrocyte function [8]. Intravenous administration of omega-3 fatty acids induces a rapid incorporation into erythrocyte membranes [9]. The positive effects of omega-3 fatty acids on erythrocyte function include improved morphology and blood viscosity, improved erythrocyte filtration time and improved erythrocyte deformability and plasma viscosity [10–13]. However, little is known about the effects of surgery and the accompanying inflammatory response on erythrocyte function.

In this study the effects of two intravenous administrations of omega-3 fatty acids on erythrocyte deformability and aggregability in patients undergoing colon cancer surgery were investigated. The hypothesis was tested that perioperative administration of intravenous omega-3 fatty acids improves erythrocyte deformability and aggregability, thereby improving the microcirculation and tissue oxygenation.

2. Methods

2.1. Trial design

The study was part of a randomised, controlled, double blind, placebo-controlled clinical trial with two parallel groups and a 1:1 allocation ratio where the effects of two perioperative intravenous n-3 PUFA infusions on *ex vivo* lipopolysaccharide (LPS) stimulated whole blood and serum interleukin-6 (IL-6) levels in colon cancer surgery were investigated. The n-3 PUFA solution (Omegaven®. Fresenius Kabi) was used off label as standalone infusions and not as part of parenteral nutrition. The perioperative care was structured in a standardized Enhanced Recovery after Surgery (ERAS) program [14]. The study was approved by the local regional Medical Ethics Committee of the VU medical center in Amsterdam and conducted according to the Declaration of Helsinki (as revised in 1983). Written informed consent was obtained from all patients. The study is registered at www.clinicaltrials.gov (NCT02231203).

2.2. Participants

Fourty-four patients were recruited with the following inclusion criteria: men and women, between the age of 55–85, having a body mass index (BMI) between 20 and 35 and undergoing elective laparoscopic resection for colon cancer. Exclusion criteria were participation in another trial, oral coagulants that could not be discontinued, a pre-operative haemoglobin level <5.0 mMol/L, metastatic disease, very poor venous access, current inflammatory or infectious disease, use of anti-inflammatory drugs, use of thyroid medication, use of fish oils or fish consumption >2 times a week or contraindications for the use of Omegaven® as indicated by the manufacturer (Fresenius Kabi Deutschland BmbH 61346 Bad Homburg). The trial was executed at the surgical department in the Northwest Clinics of Alkmaar, a large general hospital in the Netherlands.

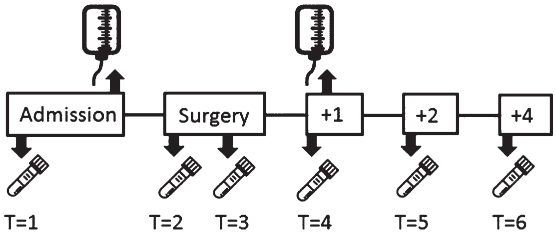

Fig. 1. Study design. Study medication was administered the night before and the morning after surgery. Blood was drawn at 6 different time points: T = 1 baseline, T = 2 day of surgery early morning, T = 3 during surgery, T = 4 first morning after surgery, T = 5 second morning after surgery, T = 6 fourth morning after surgery.

2.3. N-3 PUFA and placebo administration and blood sampling

Patients were randomly assigned (1:1), stratified by gender, to one of two treatment arms: omega-3 poly-unsaturated fatty acids (n-3 PUFAs, Omegaven® Fresenius Kabi) or placebo (saline: NaCl 0.9%). Omegaven® is a lipid emulsion containing 10% fish oil with a high percentage of n-3 fatty acids. Every 10 g of fish oil contains 1.25–2.82 g of eicosapentaenoic acid (EPA) and 1.44–3.09 g of docosahexaenoic acid (DHA). Patients, ward doctors, surgeons and investigators were blinded for treatment allocation. Study medication was administered in a dose of 2 ml/kg infusion (0.2 g fish oil/kg/d) during 4 hrs between 18:00 and 22:00 PM the evening before surgery and another infusion the morning after surgery between 08:00 and 12:00 AM. Blood samples were taken at 5 different time points: baseline (pre-admission, T = 1), morning of surgery (T = 2, after first infusion), during surgery (T = 3), first day after surgery (T = 4), second day after surgery (T = 5, after second infusion), fourth day after surgery (T = 6) (see Fig. 1). There are no red blood cell (RBC) membrane fatty acids analyses for T = 3 because no relevant changes were expected compared to T = 2.

2.4. Lorrca measurements

The principle of measuring erythrocyte deformability is based on ektacytometry. For erythrocyte deformability measurements Vacutainer® K2EDTA (Becton Dickinson, Plymouth, UK) anticoagulated blood was 1:200 diluted with a viscous isotonic (29.01 mPa.s) Poly Vinyl Pyrrollidone (PVP) phosphate-buffered saline solution (Elon ISO, RR Mechatronics Manufacturing BV, Zwaag, the Netherlands). The diluted sample (1 ml) was transferred into the Lorrca MaxSIS Ektacytometer (RR Mechatronics Manufacturing BV, Zwaag, the Netherlands) and subjected fully automatically to varying shear stresses by increasing the rotation speed. The Ektacytogram (deformability curve) was obtained by plotting the calculated values for the Elongation Index (EI) versus the corresponding shear stress (Pa) [15].

Erythrocyte deformability was expressed in EImax and SS ½. EI max is the theoretical maximum Elongation Index at infinite shear stress. SS1/2 is the shear stress at half maximal deformability [15, 16]. Reference ranges for erythrocyte deformability were determined as EImax 0.60–0.65 and SS ½ 1.68–3.02 Pa.

The principle of measuring erythrocyte aggregability is based on syllectometry. For erythrocyte aggregability K_2EDTA anticoagulated blood was fully oxygenized on a roller-bank for at least 15 minutes prior to measurement. Erythrocyte aggregability was expressed in the Aggregation Index (AI), extend of aggregation (amplitude) and kinetics of aggregation or aggregation half time (T ½). AI combines the static aspect of amplitude with the dynamic aspect of aggregation half time and describes a relevant part of the syllectogram (aggregation curve) from the top till 10 seconds thereafter. It is the ratio of the area above the curve and the area above + below the curve x 100% in a syllectogram of RBC aggregation. T ½ is the time till half of the amplitude in seconds. The method for erythrocyte aggregabilty with the Lorrca was earlier described by Hardeman et al. [17] Reference ranges for erythrocyte aggregability were determined as AI 52–80, amplitude 20–30 AU and T ½ 0.6–3.6 sec.

2.5. Red blood cell (RBC) membrane fatty acid analysis

A 10 ml venous sample collected in an K2EDTA tube was centrifuged to separate plasma, leukocytes and erythrocytes. For erythrocytes, the cells were washed with cooled NaCl 0,9% in three cycles and stored with a haematocrit of 50% in methanol/hydrochloric acid with buthylated hydroxytoluene. The buffy coat was removed as completely as possible. Fatty acid methyl esters were identified with gas chromatography after transesterification and trimethylsilylation according to the earlier described method of Muskiet et al. [18].

2.6. Statistical analysis

The intention to treat (ITT) population consists of all patients that have a value on the primary outcome parameter, received study medication on the day of admission and underwent surgery. Continuous variables are described as mean with standard deviation in case of a normal distribution and as median with interquartile range in case of a skewed distribution.

Nominal or ordinal variables were compared between groups with the chi-square or fisher exact test where appropriate. The groups were compared with the independent samples *t*-test in case of a normal distribution or the Mann-Whitney U test in case of a non-normal distribution. Linear mixed model analyses were performed to compare the course of perioperative continuous outcome variables between the intervention and control thereby controlling for baseline values. A *p*-value of 0.05 or less was considered statistically significant. The results were analysed with IBM SPSS® Statistics 23.0 or higher.

3. Results

3.1. Baseline characteristics

Twenty-three patients received 2 saline control infusions and 18 patients 2 n-3 PUFA infusions in the intention to treat population. In Table 1, the baseline and surgical characteristics are shown. There were 5 patients with an open surgical procedure in the saline control group ($p = 0.056$). Three patients were excluded from analysis, two because epidural anesthesia was not possible and one because of delivery problems of the study medication.

3.2. Membrane fatty acid analysis

A detailed description of changes in RBC membrane EPA and DHA is shown in Table 2. Comparing both groups there was a significant increase in RBC membrane EPA ($p = 0.006$) but not DHA ($p = 0.844$) in the n-3 PUFA group compared to the control group. There were no significant changes in n-6/n-3 fatty acid ratio between the groups per time-point, but compared to baseline the n-6/n-3 fatty acid ratio was lower at T = 5 and T = 6 in the n-3 PUFA group.

Table 1
Baseline and surgery characteristics

	Saline $n = 23$	N-3 PUFA $n = 18$	*P*-value
Male sex	15 (65.2)	13 (72.2)	0.632
Age (years)	69.0 (11)	65 (12)	0.653
BMI (kg/m^2)	26.5 (7.2)	26.9 (2.2)	0.906
Co-morbidity			
Cardiovascular	12 (52.2)	9 (50)	0.890
Diabetes	6 (26.1)	4 (22.2)	1.000
Tumour stage [30]			0.344
0	2 (8.7)	0 (0)	
1	10 (43.5)	9 (50)	
2	8 (34.8)	4 (22.2)	
3	3 (13)	5 (27.8)	
Laparoscopic resection	18 (78.3)	18 (100)	0.056
Type of resection			0.223
Right colectomy	12 (52.2)	4 (22.2)	
Left colectomy	3 (13)	4 (22.2)	
Sigmoid	6 (26.1)	7 (38.9)	
Low anterior	1 (4.3)	3 (16.7)	
Subtotal colectomy	1 (4.3)	0 (0)	
Operation time (minutes)	134 (50)	140 (60)	0.636
Blood loss (millilitres)	50 (90)	50 (100)	0.512
Study medication (millilitres in 4 hours)	158 (46)	167 (33)	0.635

Values are presented as percentage except for age, BMI, operation time and blood loss, which are presented as median (interquartile range). *P*-values were calculated with chi-square tests for percentages and with Mann-Whitney U tests for continuous variables. Abbreviations: BMI (body mass index), n-3 PUFA (omega-3 poly-unsaturated fatty acid).

3.3. Erythrocyte deformability

Erythrocyte deformability parameters SS1/2 and EImax are shown in Table 3. There were no significant differences in erythrocyte deformability parameters between the saline control group and the n-3 PUFA group. In both groups, there was a significant decline in SS ½ comparing baseline with $T = 5$ and $T = 6$.

3.4. Erythrocyte aggregability

Erythrocyte aggregability parameters AI, amplitude and t1/2 are shown in Table 4. The AI was significantly lower at $T = 2$ and $T = 4$ in the n-3 PUFA group. The T ½ was significantly higher at $T = 3$ and $T = 4$ in the n-3 PUFA group.

3.5. Subgroup analyses

Because there were more patients with infectious complications in the n-3 PUFA group (8 vs. 3, $p = 0.036$) we did subgroup analyses comparing patients with and without infections to evaluate possible differences. The results of the subgroup analyses are shown in Table 5. There were no differences in

Table 2
Erythrocyte membrane fatty acid analysis

		Saline $n = 23$	N-3 PUFA $n = 18$	Effect size	95% CI	*P*-value	*P*-value overall
EPA (mol%)	T1	0.755 (0.56)	0.700 (0.38)				0.006
	T2	0.796 (0.52)	0.687 (0.22)	–0.034	–0.110–0.042	0.377	
	T4	0.776 (0.46)	0.783 (0.24)	0.090	0.013–0.167	0.022	
	T5	0.791 (0.40)	0.910 (0.19)	0.202	0.126–0.279	0.000	
	T6	0.811 (0.33)	0.859 (0.29)	0.210	0.130–0.291	0.000	
DHA (mol%)	T1	4.280 (1.62)	3.306 (0.80)				0.844
	T2	4.227 (1.72)	3.217 (0.79)	–0.148	–0.540–0.243	0.448	
	T4	4.141 (1.64)	3.179 (0.87)	–0.095	–0.488–0.298	0.629	
	T5	3.872 (1.60)	3.374 (1.15)	0.046	–0.348–0.439	0.816	
	T6	4.270 (1.49)	3.553 (1.20)**	0.152	–0.251–0.556	0.451	
N-6/N-3 ratio	T1	3.634 (1.51)	4.505 (1.04)				0.872
	T2	3.555 (1.47)	4.289 (0.95)	0.132	–0.276–0.539	0.517	
	T4	3.631 (1.55)	4.132 (1.05)	0.008	–0.400–0.417	0.968	
	T5	3.660 (1.43)	4.019 (1.08)*	–0.149	–0.558–0.260	0.466	
	T6	3.527 (0.93)	3.884 (0.99)*	–0.242	–0.658–0.174	0.247	

Values are presented as median (interquartile range). *P*-values were calculated with linear mixed model for overall- and comparison per time point with a correction for baseline values. Effect size with 95% confidence interval is shown. Baseline values within groups compared with other time points with Wilcoxon signed rank test. Abbreviations: CI (confidence interval), DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), n-6 (omega-6), n-3 (omega-3), PUFA (poly-unsaturated fatty acids). *Significantly lower than baseline ($p < 0.05$). **Significantly higher than baseline ($p < 0.01$).

EPA, DHA or n-6/n-3 ratio between the groups (not shown). Erythrocyte deformability demonstrated a lower SS1/2 at T6 in patients with infections. There was a trend towards a lower EI max at T = 2 and T = 6 in patients with infections. Erythrocyte aggregability demonstrated a higher T1/2 at T = 3 in patients with infections compared to patients without infections. There were no differences in baseline parameters between patients with and without infectious complications.

4. Discussion

In this study two intravenous n-3 PUFA infusions resulted in a rapid increase of RBC membrane EPA and n-3 but not DHA content in patients undergoing colon cancer surgery. There were no significant changes in erythrocyte deformability parameters but AI was significantly lower and T1/2 significantly higher in the n-3 PUFA group.

4.1. Membrane fatty acids

The significant rise in RBC membrane EPA but not DHA is in line with the study of Senkal et al., where patients undergoing colorectal surgery received fish-oil enriched parenteral nutrition and had a significant increase in RBC membrane EPA but no significant increase in DHA [19]. The results are also in line with the study of Eltweri et al, where patients with advanced oesophagogastric cancer received repeated short term n-3 PUFA infusions and significantly increased RBC membrane EPA but not DHA was found [20]. The results are in contrast with a study by Delodder et al, where in

Table 3
Erythrocyte deformability

		Saline $n = 23$	N-3 PUFA $n = 18$	Effect size	95% CI	*P*-value	*P*-value overall
SS 1/2	T1	1.59 (0.34)	1.58 (0.39)				0.658
	T2	1.58 (0.42)	1.50 (0.34)	–0.045	–0.239–0.148	0.642	
	T3	1.52 (0.37)	1.53 (0.46)	–0.027	–0.227–0.173	0.787	
	T4	1.57 (0.43)	1.51 (0.31)*	–0.069	–0.266–0.128	0.489	
	T5	1.45 (0.27)*	1.36 (0.44)*	0.009	–0.185–0.203	0.926	
	T6	1.35 (0.31)*	1.31 (0.47)*	–0.046	–0.247–0.154	0.648	
EI max	T1	0.61 (0.05)	0.60 (0.03)				0.759
	T2	0.62 (0.03)**	0.61 (0.03)	–0.011	–0.029–0.006	0.207	
	T3	0.61 (0.03)	0.62 (0.02)**	0.011	0.008–0.030	0.249	
	T4	0.60 (0.04)	0.61 (0.04)	0.006	–0.012–0.280	0.496	
	T5	0.61 (0.03)	0.61 (0.03)	–0.001	–0.019–0.017	0.906	
	T6	0.60 (0.04)	0.59 (0.04)	–0.013	–0.032–0.006	0.190	

Values are presented as median (interquartile range). *P*-values were calculated with linear mixed model for overall- and comparison per time point with a correction for baseline values. Effect size with 95% confidence interval is shown. Baseline values within groups compared with other time points with Wilcoxon signed rank test. Reference ranges for erythrocyte deformability were determined as EImax 0.60–0.65 and SS ½ 1.68–3.02 Pa. Abbreviations: CI (confidence interval), EI max (theoretical maximum Elongation Index at infinite shear stress). n-3 (omega-3), PUFA (poly-unsaturated fatty acids), SS1/2 (shear stress at half maximal deformability). *Significantly lower than baseline ($p < 0.05$). **Significantly higher than baseline ($p < 0.05$).

healthy volunteers already 1 day after an intravenous n-3 PUFA infusion a significant increase in RBC membrane EPA but also DHA was found. However the higher dose used in that study (0.6 g/kg in three hours instead of 0.2 g/kg in four hours) may explain this difference [21].

4.2. *Erythrocyte deformability*

The elongation index in this study demonstrated to be quite constant, with minimal non-significant decline after surgery and no effect of the erythrocyte membrane fatty acid changes. This is in line with the study by Greco et al., where no significant changes in elongation index were found at the first and fifth postoperative day after major abdominal cancer resection [22]. All EI values in both groups were at the lower end of normal range, except for T = 6, four days after surgery in the n-3 PUFA group, which was just below the lower limit of normal range.

The decline in SS1/2 in both groups at T = 5 and T = 6 (half maximal elongation at lower shear rates), suggests that the postoperative state may have subtle effects on the ease of erythrocyte elongation without relevant effects on maximal deformability. Surprisingly, all SS1/2 values in both groups including baseline were below the lower reference limit (1.68 Pa). All patients underwent surgery because of a colon malignancy, which may explain these values below the reference values for normal healthy persons.

4.3. *Erythrocyte aggregability*

The AI at T = 2, T = 4 was significantly lower and T1/2 at T = 3, T = 4 was significantly higher in the n-3 PUFA group, suggesting a lower tendency of aggregation in patients that received intravenous n-3

Table 4
Erythrocyte aggregability

		Saline $n = 23$	N-3 PUFA $n = 18$	Effect size	95% CI	*P*-value	*P*-value overall
AI	T1	77 (9.8)	76 (16.4)				0.107
	T2	72 (9.7)*	69 (16.9)*	–4.18	–8.17 – –0.19	0.040	
	T3	69 (4.3)*	63 (18.8)*	–3.00	–7.27–1.27	0.167	
	T4	75 (10)*	69 (14.6)*	–5.12	–9.26 – –0.98	0.016	
	T5	78 (9.5)	77 (13.3)	–0.16	–4.16–3.83	0.935	
	T6	83 (5.0)**	83 (7.4)	–0.66	–4.92–3.60	0.761	
T 1/2	T1	1.12 (0.49)	1.09 (1.19)				0.080
	T2	1.50 (0.67)**	1.77 (1.64)**	0.30	–0.07–0.67	0.113	
	T3	1.68 (0.94)**	2.27 (2.41)**	0.59	0.19–0.99	0.004	
	T4	1.34 (0.66)**	1.76 (1.34)**	0.47	0.08–0.85	0.018	
	T5	1.03 (0.52)	1.04 (0.98)	–0.05	–0.42–0.32	0.780	
	T6	0.79 (0.25)*	0.74 (0.41)	–0.02	–0.42–0.37	0.906	
Amp	T1	25 (4.2)	24 (4.9)				0.909
	T2	24 (4.6)	25 (4.0)	0.10	–1.12–1.32	0.867	
	T3	24 (3.4)	24 (5.8)	–0.92	–2.23–0.38	0.165	
	T4	24 (4.4)	24 (4.4)	–0.18	–1.44–1.09	0.784	
	T5	22 (4.4)*	22 (5.6)*	0.23	–0.99–1.45	0.713	
	T6	22 (5.0)*	21 (5.0)	0.59	–0.71–1.90	0.372	

Values are presented as median (interquartile range). *P*-values were calculated with linear mixed model for overall- and comparison per time point with a correction for baseline values. Effect size with 95% confidence interval is shown. Baseline values within groups compared with other time points with Wilcoxon signed rank test. Reference ranges for erythrocyte aggregability were determined as AI 52–80, amplitude 20–30 AU and T ½ 0.6–3.6 sec. Abbreviations: AI (aggregation index), Amp (amplitude, extend of aggregation), CI (confidence interval), n-3 (omega-3), PUFA (poly-unsaturated fatty acids), T1/2 (time till half of the amplitude in seconds). *Significantly lower than baseline ($p < 0.05$). **Significantly higher than baseline ($p < 0.05$).

PUFA. This may be explained by the altered erythrocyte membrane fatty acid composition. However other influences on erythrocyte aggregation like plasma fibrinogen were not measured to rule out any differences. The AI, T1/2 and amplitude stayed within normal range for all time points except for AI at T = 6 in both groups.

The reduced aggregability in the n-3 PUFA group may have had an effect on haemostasis as erythrocytes play an important role in haemostasis and thrombosis, including by their rheological properties of aggregability and deformability [23]. However in this study no increased per-operative blood loss or bleeding complications occurred in the n-3 PUFA group. Other studies also found that n-3 PUFA do affect haemostasis but don't increase bleeding risk [24, 25].

In both groups, the extend of aggregation (amplitude) was lower at T = 5 (2 days after surgery), suggesting that the early post-operative phase may have an effect on erythrocyte aggregation independent of the changes in erythrocyte membrane EPA content. Early reports found a drop in blood viscosity (that is strongly related to erythrocyte aggregation) early after surgery as well, which may be explained by a drop in hematocrit due to per-operative blood loss [26, 27]. Significant blood loss is not common in laparoscopic abdominal surgery but postoperative intravenous fluid administration may have played a role in a smaller decrease in hematocrit.

Table 5
Patients with/without infectious complications

		Without infectious complications $n=30$	With infectious complications $n=11$	Effect size	95% CI	p-value	p-value overall
SS 1/2	T1	1.60 (0.36)	1.47 (0.25)				0.056
	T2	1.58 (0.38)	1.48 (0.67)	–0.155	–0.363–0.053	0.141	
	T3	1.60 (0.42)	1.44 (0.26)	–0.210	–0.426–0.006	0.056	
	T4	1.56 (0.51)	1.50 (0.72)	–0.116	–0.325–0.093	0.274	
	T5	1.52 (0.36)*	1.37 (0.25)	–0.131	–0.339–0.077	0.214	
	T6	1.40 (0.37)*	1.20 (0.23)*	0.231	–0.442 – –0.020	0.032	
EI max	T1	0.61 (0.04)	0.60 (0.03)				0.739
	T2	0.62 (0.02)**	0.60 (0.03)	–0.019	–0.039–0.003	0.053	
	T3	0.61 (0.03)	0.62 (0.01)	0.009	–0.012–0.030	0.396	
	T4	0.60 (0.04)	0.61 (0.03)	0.017	–0.003–0.037	0.089	
	T5	0.61 (0.03)	0.61 (0.02)	0.006	–0.014–0.025	0.548	
	T6	0.60 (0.03)	0.58 (0.03)	–0.019	–0.039–0.001	0.061	
AI	T1	76.9 (12.1)	77.5 (9.0)				0.980
	T2	70.8 (14.5)*	72.3 (7.4)*	–1.07	–5.68–3.53	0.646	
	T3	68.1 (16.2)*	62.8 (18.4)*	–4.48	–9.38–0.43	0.073	
	T4	70.4 (14.6)*	74.9 (7.8)*	–1.07	–5.73–3.59	0.650	
	T5	76.5 (8.7)	80.5 (4.4)	1.24	–3.37–5.84	0.595	
	T6	80.6 (6.1)**	84.9 (2.1)	2.59	–2.12–7.31	0.278	
T 1/2	T1	1.12 (0.80)	1.08 (0.57)				
	T2	1.58 (1.18)**	1.50 (0.65)**	–0.10	–0.53–0.32	0.635	0.853
	T3	1.78 (1.39)**	2.32 (2.26)**	0.64	0.19–1.10	0.006	
	T4	1.61 (1.18)**	1.35 (0.54)**	0.07	–0.36–0.50	0.744	
	T5	1.15 (0.55)	0.89 (0.22)	–0.11	–0.54–0.31	0.605	
	T6	0.87 (0.33)*	0.65 (0.10)*	–0.09	–0.53–0.35	0.681	
Amp	T1	24.3 (4.8)	24.1 (3.4)				
	T2	23.9 (6.1)	24.8 (2.2)	0.74	–0.64–2.11	0.291	0.653
	T3	23.7 (5.1)	24.3 (4.5)	0.85	–1.45–1.51	0.968	
	T4	23.7 (4.3)	24.1 (3.1)	0.29	–1.10–1.68	0.685	
	T5	22.0 (4.9)*	21.5 (5.0)*	0.06	–1.31–1.44	0.929	
	T6	21.7 (6.2)*	21.1 (4.9)*	0.02	–1.39–1.43	0.977	

Values are presented as median (interquartile range). P-values were calculated with linear mixed model for overall- and comparison per time point with a correction for baseline values. Effect size with 95% confidence interval is shown. Baseline values within groups compared with other time points with Wilcoxon signed rank test. Abbreviations: AI (aggregation index), Amp (amplitude, extend of aggregation), CI (confidence interval), n-3 (omega-3), EI max (theoretical maximum Elongation Index at infinite shear stress). SS1/2 (shear stress at half maximal deformability), T1/2 (time till half of the amplitude in seconds). *Significantly lower than baseline, $p < 0.05$. **Significantly higher than baseline, $p > 0.01$.

4.4. Infectious complications

Overall erythrocyte deformability parameters tended to be lower in patients with infections, but only ss1/2 at T = 6 was statistically significant. Several experimental and clinical studies have suggested a significant role for a decreased erythrocyte deformability in sepsis induced microvascular dysregulation and patient outcome [28]. With early diagnosis and treatment a septic state and an accompanying (prolonged) decrease in erythrocyte deformability was probably prevented in this study.

Overall erythrocyte aggregability parameters were not different between the groups, except for a significantly higher T1/2 at T = 3 (during surgery) and a trend for a lower AI at T = 3 in patients with infections. This is in contrast with a study of Ko et al., where in a rat sepsis model within hours after LPS injection AI increased and T1/2 decreased [29]. While most of the patients with infections were in the n-3 PUFA group (8/11), and the trends for aggregation parameters are similar to the results in Table 4, the results in this study may be explained as an effect of the n-3 PUFA rather than an effect of the infection.

5. Conclusions

This study confirms a rapid incorporation of RBC membrane EPA but not DHA after administration of intravenous n-3 PUFAs in patients undergoing elective colon cancer resection. Erythrocyte deformability parameters were not affected by n-3 PUFA administration but erythrocyte aggregability was decreased in the n-3 PUFA group. Only subtle effects of the postsurgical state were seen. Further investigation in other peri-operative settings and with other types of n-3 PUFA supplementation is necessary to gain more insights in the effects of n-3 PUFA and the postoperative inflammatory response on erythrocyte deformability and aggregability.

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