PHASE II RANDOMISED CONTROLLED TRIAL OF THE EFFECTS OF PARENTERAL FISH OIL EMULSION ON PRO AND ANTI INFLAMMATORY MARKERS AND CLINICAL OUTCOME IN CRITICALLY ILL SEPTIC PATIENTS IN INTENSIVE CARE UNIT

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By

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Abstract

Introduction

Sepsis is a leading cause of mortality in critically ill patients on the intensive care unit (ICU). Death from sepsis in the ICU is frequently preceded by the development of multiple organ failure as a result of uncontrolled inflammation. Treatment with omega-3 fish oil has been demonstrated to attenuate the effects of uncontrolled inflammation and may be clinically beneficial in reducing morbidity from organ dysfunction.

Trial design

A phase 2 randomised controlled trial investigating the effects of parenteral omega-3 on critically ill patients in ICU in a single institution.

Methods

Participants: Consecutive patients with sepsis were considered for the trial. Sepsis was defined as the presence of a known or suspected infection and two or more SIRS criteria (Systemic inflammatory response syndrome).

Interventions: 60 patients were randomised to receive either parenteral fish oil (FO) and standard medical care or standard medical care only.

Hypothesis: Administration of omega 3 fish oil emulsion will not alter the level of pro-inflammatory and anti-inflammatory markers in critically ill patients with sepsis on Intensive Care Unit as compared to controls.

Outcome: The primary outcome measure was the effect of omega-3 on various inflammatory markers including cytokine, complement, resolvins and protectins (measured using ELISA, LC-MS). The secondary outcome measure was clinical benefit measured using SOFA score and 28-day mortality. Cytokines and complement

were analysed used ELISA. Resolvins & protectins were analysed using LC-MS. Data was analysed using Strata statistical tool.

Results

Sixty patients were included in the study, 30 in the control group and 30 in the treatment group. The baseline demographics were matched for the two cohorts. A significant increase (p=0.001) was detected in the concentration of pro-inflammatory mediators PGE2, PGF2α, TXB2 in the control group while the anti-inflammatory mediators 4HDHA, 17HDHA were significantly higher in the FO group (p=0.01).

Omega-3 significantly reduced IL-17 in FO group (p=0.035). Also, the concentration of other pro-inflammatory cytokines (E-selectin, VCAM, ICAM, TNFR1, TNF-α, IL-17, IL-12, IL-6, IL-1b) were reduced in the FO group.

Omega-3 improved outcomes in C3 depleted patients by 50%.

Patients treated with parenteral fish oil were associated with a significant reduction in new organ dysfunction (delta-SOFA 2.2±2.2 vs. 1.0±1.5, p=0.005 and maximum-SOFA 10.1±4.2 vs. 8.1±3.2, p=0.041). Patients treated with fish oil demonstrated a reduction in 28-day mortality (26% in control vs 13% in FO, p=0.19).

Conclusion

This study has demonstrated that omega-3 altered the concentrations of various pro and anti-inflammatory mediators significantly resulting in clinical benefit. It was safe in critically ill septic patients in ICU.

Abbreviations used

AA	Arachidonic acid
ALI	Acute lung injury
APACHE	Acute physiology and chronic health evaluation
APC	Activated protein C
ARDS	Acute respiratory distress syndrome
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CRP	C-reactive protein
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic
FA	Fatty acid
FO	Fish oil
GCS	Glasgow coma score
GLA	Gamma linolenic acid
HDHA	Hydroxydocosahexaenoic acid
HDU	High dependency unit
HEPE	Hydroxyeicosapentaenoic acid
HETE	Hydroxyeicosatetraenoic acid
HODE	Hydroxyoctadecadienoic acid
IL	Interleukin
ICU	Intensive care unit
LCT	Long chain triglyceride
LOX	Lipoxygenase
LT	Leukotriene
LX	Lipoxin
MCT	Medium chain triglyceride
MI	Myocardial infarction
MODS	Multiple organ dysfunction score
PG	Prostaglandin
PMN	Polymorphonuclear leukocytes
PUFA	Polyunsaturated fatty acid
RCT	Randomised controlled trial
Rv	Resolvin
SAPS	Simplified acute physiology score
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
SOAP	Sepsis occurrence in acutely ill patients
TNF	Tumour necrosis factor
TPN	Total parenteral nutrition
TX	Thromboxane

Presentations and publications

Presentations

All ESPEN presentation abstracts were published in Supplement of Clinical Nutrition, "Nutrition and Metabolism – the ESPEN Journal"

Oral presentation-
Parenteral omega-3 reduced levels of pro-inflammatory interleukin-17, results of a randomised controlled trial in critically ill patients with sepsis, DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ESPEN 2017

Effect of parenteral omega-3 on mortality and complement-3 levels in septic patients. DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ESPEN 2017 Poster presentation

A randomised controlled trial investigating the effects of parenteral fish oil on pro-inflammatory resolving and protectin profile in critically ill patients with sepsis. University Hospitals of Leicester, ESPEN 2017 Poster presentation

Omega-3 significantly improved organ dysfunction in critically ill septic patients- results of a randomised controlled trial. DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ESPEN 2017 Poster presentation

A Randomised Controlled Trial Investigating the Effects of Parenteral Fish Oil on Cytokine profile in Critically Ill Patients With Sepsis. DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ASGBI 2016 Poster presentation - abstract published in BJS

A Randomised Controlled Trial Investigating the Effects of Parenteral Fish Oil on Anti-inflammatory Resolvin & Protectin profile in Critically Ill Patients With Sepsis. DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ASGBI 2016 Poster presentation - abstract published in BJS

A Randomised Controlled Trial Investigating the Effects of Parenteral Fish Oil on Pro-inflammatory Resolvin & Protectin profile in Critically Ill Patients With Sepsis. DK Bilku, T Hall, A Dennison. University Hospitals of Leicester, ASGBI 2016 Poster presentation - abstract published in BJS

Relationship between omega-3 fatty acids and complement activation in severe sepsis. DK Bilku, J Zimmer, CM Stover, TC Hall, WY Chung, AR Dennison. University Hospitals of Leicester SARS 2013 Poster presentation - abstract published in BJS

Effect of parenteral Omega-3 Fish oil on C3 levels and mortality in septic patients on Intensive Care Unit. University Hospitals of Leicester, ASGBI 2013 Poster presentation - abstract published in BJS

Publications

Can enhanced recovery programmes be further improved by the additional omega three fatty acids? Bilku DK, Hall TC, Al-Leswas D, Dennison AR. Ir J Med Sci. 2012 Dec;181(4):453-7. doi: 10.1007/s11845-012-0813-x. Epub 2012 Mar 22

A Randomized Controlled Trial Investigating the Effects of Parenteral Fish Oil on Cytokine profile in Critically Ill Patients With Sepsis, Dilraj K. Bilku, Thomas C. Hall, Dhya Al-Leswas, Cindy Horst, Jill Cooke**,** Matthew S. Metcalfe, Ashley R. Dennison – Accepted with corrections to HBSN May 2019.

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1. Introduction

1.1 Burden of sepsis in Intensive Care Unit (ICU)

Sepsis in critically ill septic patients is associated with high morbidity and mortality (40%). In addition, this has cost implications in management of the patient. The incidence of severe sepsis and the associated mortality in critical care in the first 24 hours is increasing in the United Kingdom as evidenced by analysis of clinical database. It demonstrated an increase in patients with sepsis in ICU from 23.5% in 1996 to 28.7% in 2004. The associated mortality decreased from 48.3% in 1996 to 44.7% in 2004 however, the total number of deaths increased from 9,000 to 14,000 (1, 2).

The SOAP study, a prospective, multi-centre, observational study analysed the incidence of sepsis in 3,147 European ICU patients and the various factors affecting it. It identified that mortality rate in septic patients was greater than the non-septic ones (27 vs 14%, p=0.001). A number of factors were associated with mortality including number of organ failures (calculated using SOFA score), age of patient, cirrhosis and mean fluid balance. Patients with zero organ dysfunction had 6% mortality however, four or more organ dysfunction increased the mortality to 65% (3).

In recent years, several studies have collected data from both National and International Intensive Care Units to determine the incidence of sepsis in critically ill patients, associated factors and costs. The epidemiology study of severe sepsis in the United States demonstrated that there were 751,000 cases of sepsis per year of which 38,300 (51.1%) required ICU care. The incidence varied with age, higher in infants

(5.3/1000), decreased in older children (0.2/1000) but increased in later life (5.3/1000) with a sharp rise in the elderly (26.2/1000) age group (4).

Alberti et al performed an international prospective cohort study involving 28 ICU in eight countries (Europe, Canada and Israel) between 1997 and 1998. A total of 14,364 patients were admitted. Of these, 21.1% developed sepsis with a mortality of 53.6%. This was in comparison to 16.9% mortality in the non-septic group (5).

1.2 Definition of sepsis

The definition of sepsis as stated by The American College of Chest Physicians and the Society of Critical Care Medicine is frequently used as the standard for consistent terminology (6). This is defined as the proven or suspected source of infection together with at least two of the four systemic inflammatory response syndrome features, namely, temperature >38°C or <36°C, heart rate >90 beats/min, white cell count >12 or <4 x 109 or respiratory rate >20 or PaCO2< 4.2 kPa. At present, there is no satisfactory objective biochemical marker with sufficient specificity or sensitivity to be routinely employed in clinical practice and hence the need for such definitions (7, 8).

The literature was searched using the terms, sepsis, omega-3, SOFA score and inflammatory mediators. The studies involving humans, adults were included. The studies were reviewed and summarised as follows.

1.3 Search for a new intervention to manage sepsis

Despite the introduction of surviving the sepsis campaign and a wealth of available antimicrobials, mortality from sepsis remains high (9). This is partly due to multi-factorial pathogenesis of sepsis including infection, trauma, ischaemia and severe injury. The inappropriate host response and hyper-inflammatory state is costly, both in terms of patient outcome and financially. The mean cost per case of severe sepsis in an ICU is £18,173 compared to cost per case of non-sepsis of £3,828 (10).

As a result of the high associated mortality, numerous studies have attempted to identify novel treatment strategies in septic patients often with inconclusive results. The various studies have tried to target specific mediators in the inflammatory pathway but with disappointing results. One such attempt is the PROWESS trial following which recombinant human protein C was approved as the first biological agent to be used in the treatment of severe sepsis and septic shock (11). It reduced mortality by 6.1%. However, it has been withdrawn recently due to increased incidence of severe bleeding (12). The search continues with the focus on manipulating the various mediators of inflammation.

1.4 Challenges of conducting a randomised controlled trial in ICU

Although there is a better understanding of the pathophysiology of sepsis, mortality still remains high in this group of patients. It is one of the vital clinical problem that needs an answer. Numerous inflammatory mediators have been identified to play a key role in the process leading to sepsis. There has been no success in developing a

therapeutic agent to target these mediators. The patient population in ICU is very heterogenous with differing age, gender, type of sepsis, source of sepsis, severity of sepsis and co-morbidities. The organisms causing sepsis in these complex patients also vary in type, virulence and antibiotic sensitivity. Their co-morbidities can be the cause of death rather than sepsis. Therefore, some trials use intent to treat analysis which analyses mortality and not the cause of death (13). Although, a randomised controlled trial is gold standard in analysing a new therapeutic agent, it is challenging to conduct one in ICU (14). In addition, there are numerous ethical barriers as patients are very unwell. It is very important to rigorously test the novel agent as poor study design and falsely reassuring results can do more harm to these already unwell patients. Much can be learnt from the experience of PROWESS study. The Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study was an international, multi-centre, randomised, placebo-controlled Phase 3 trial. It was conducted to demonstrate if the administration of activated drotrecogin alpha reduced the 28-day mortality in patients with sepsis. As a result of the PROWESS study investigating activated protein C, the drug has been given Food and Drug Administration approval. More recently it has been removed from the market due to concerns over increased bleeding (15).

1.5 Difficulties in evaluating new therapies in severe sepsis

Severe sepsis i.e. sepsis associated with organ failure is complex and therapeutically challenging. These patients present with different grades of systemic response to the clinical insult. Large doses of gram negative endotoxin are used to produce sepsis

artificially in experiments. However, sepsis in humans can be caused either by gram negative or gram positive organisms (16, 17) Although, there are similarities in mechanisms the host response may vary which has therapeutic implications. Moreover, the physiological reserve and level of immunity in every patient is influenced by their co-morbidities. These patient variables influence mortality. These cannot be completely investigated in animal experiments. It is a possible explanation for obtaining negative results in a trial when animal experiment was promising. This needs to be considered if power calculation of a study was based on animal experiments (18).

Numerous scoring systems are used to measure the severity of disease. These systems have been criticised for lacking a physiological basis, being misleading, complex and including criteria unrelated to the septic process (19, 20) More recently, scoring systems such as the Sepsis-related Organ Failure Assessment (SOFA) (21) and the PIRO (6) that incorporate the degree of organ failure have been used. In an attempt to ensure an independent level of inclusion criteria consistency, the PROWESS study group used a clinical coordinating centre which was available 24 hours a day (Vanderbilt Coordinating centre), to assess recruitment eligibility and safety (22).

In addition, the PROWESS trial used strict exclusion criteria for patients unlikely to benefit from the APC. Moribund patients and those with prolonged organ dysfunction were excluded (23). It is unlikely that patients with such severe disease could obtain benefit from an experimental agent.

1.6 Outcome measures in critically ill septic patients in ICU

Clinical trials evaluating septic patients have challenged the assumption that mortality is the gold standard end-point in the evaluation. Although mortality is easy to define, highly relevant and measurable endpoint it has drawbacks. Mortality is an appropriate endpoint when the mechanism of death is completely understood. Also, large homogenous sample size is needed to analyse with mortality as endpoint. However, ICU patients with sepsis are heterogeneous. Moreover, reduction in morbidity can produce a significant improvement in the quality of life of this already unwell patient. Targeting mediators in hyper inflammation can improve the hemodynamic instability. Antibiotic prophylaxis given in Colon cancer surgery does not affect mortality from cancer but reduces the post-operative infection from 30% to 10%(24).

1.7 Scoring systems in critically ill patients

1.7.1 The introduction of scoring systems to critically ill patients

The first scoring system used in medicine was the APGAR score for new born vitality. Patients in ICU are high risk with higher morbidity and mortality with associated high treatment costs. It is therefore useful to use scoring systems to predict outcomes. Some of these like APACHE (Acute Physiology and Chronic Health Evaluation) and SAPS (Simplified Acute Physiology Score) are only used in the first 24 hours of ICU admission. These scoring systems cannot be used to assess risk after 24 hours. Therefore, cannot be used in measuring outcomes when a continuous intervention is

applied. Different organs are affected to varying degree and at different time-points by the disease process. A scoring system which predicts the risk daily can miss the total organ dysfunction affecting the patient and therefore underestimate the risk. Scoring systems have become an important means to predict risk and outcome in medicine and particularly in critical illness. Since the first scoring system came into mainstream use, namely the APGAR score for new-born vitality (25), there has been a growth in both general and disease-specific scoring systems. Scoring systems are particularly useful to predict outcomes in ICU patients, where costs of treatment are so great and intervention can be directed towards specific groups. Some are used only in the first 24 hours of ICU admission such as the Acute Physiology and Chronic Health Evaluation (APACHE) and Simplified Acute Physiology Score (SAPS) scores (26, 27). More recently a model based on the UK critical care units has been developed by the Intensive Care National Audit & Research Center (ICNARC) based on data from a large, multi centre, high quality clinical database. This new scoring system has demonstrated better discrimination in mortality prediction than other previously published models (28).

These systems do not consider the organ dysfunction that develops after the first 24 hours. As the different organ systems can be affected at varying time-points in the course of the disease, (29) a daily prediction model can miss the total organ dysfunction sustained by the patient and, therefore, underestimate the risk. Mortality in ICU is due to multiple organ failure and therefore the outcome of any intervention cannot be measured by analysis at a single time-point (30-33). Newer scoring systems such as the Sequential Organ Failure Assessment (SOFA) score (34) and Multiple Organ Dysfunction Score (MODS) (30) are used over time to measure the evolution

of individual (or aggregated) organ dysfunction. The serial measurements done in these scores predict the dynamics of the disease process and the relationship of an intervention on patient outcome better.

1.7.2 The Multiple Organ Dysfunction Score (MODS)

The MODS was based on extensive literature reviews and past experience and was evaluated for its ability to predict mortality in an incremental manner. The scores were then validated on a separate group of patients. The score represents the most abnormal data for the entire ICU stay. The scoring is simple to apply but the MODS has been criticised for problems in evaluating the circulatory function score; measured as the cumbersome pressure-adjusted heart rate (PAR), which is treatment (vasopressor) independent (35).

1.7.3 The Sequential Organ Failure Assessment (SOFA) score

The degree of organ dysfunction is calculated using routinely collected data. This is then added to obtain the SOFA score. (table 2.2) This includes daily scores for respiratory, renal, cardiovascular, central nervous system, coagulation and hepatic failure. The higher value of score is associated with severe failure. The three types of SOFA score used are as follows:

1. Mean SOFA, the mean of the worst scores per day during the ICU stay.

2. Delta-SOFA, total ‘maximum SOFA score’ minus ‘admission total SOFA’
3. Max-SOFA, the sum of the worst scores during the ICU stay

The SOFA score calculated on admission to ICU indicates the degree of dysfunction already present. This can help stratify patients according to severity for inclusion into clinical trials. Prospective (36) and retrospective (34) scores in the first 24 hours of admission to ICU have demonstrated good correlation with mortality. Other studies have demonstrated that the delta-SOFA and maximum-SOFA scores also correlate to outcome. The delta SOFA measures the progress of the patient during their ICU stay and can potentially be influenced by an intervention. Moreno and colleagues demonstrated that the delta SOFA was a good prognostic indicator after controlling for the admission SOFA score, suggesting that strategies directed at the prevention of further organ dysfunction will have a significant impact on prognosis, independent of the physiological condition of the patient on admission to the ITU (37). The area under the receiver operating characteristic (ROC) curve (AUC) was 0.742 (SE 0.017) for delta SOFA in predicting mortality. The same study also demonstrated that maximum-SOFA can be used to quantify the impact of therapeutic interventions on overall or organ-specific morbidity. The total maximum SOFA score had an AUC value of 0.847 (SE 0.012) in their study. Other work has shown good correlation with mortality from the mean-SOFA score with AUC values as high as 0.88 (SE 0.03) (38).

1.7.4 The limitations of the SOFA score

Although SOFA score is widely used in ICU, it has its own limitations. A high delta SOFA score does not indicate higher mortality because the delta SOFA score will be low if the SOFA score at admission to ICU was very high and did not consider improvement in organ dysfunction. To overcome these limitations and improve the prediction of mortality some studies have used a combination of serial scores and score at admission. For example, combining SAPS with maximum SOFA score. But this still needs to be validated in larger studies (39, 40).

Some scoring systems use only one marker to assess the severity of a particular organ system. An example being, giving renal replacement therapy in ICU will improve the Creatinine and may not influence urine output. Therefore, if only Creatinine is used then it will underestimate the true extent of organ dysfunction. SOFA score considers both Creatinine and urine output (41).

The scoring systems are an essential part of describing ICU patients and predicting their mortality. There are numerous potential errors related to interpretation of various scoring systems, it is important to use these with knowledge of science of severity scoring. Some of the scoring systems make serial measurements during the stay in ICU while some only consider data in the first 24 hours of admission. The sequential measurements reflect the progress of the patient over the period of stay in ICU and response of patient to the various therapeutic interventions.

1.8 The concerns of sample size

To investigate an intervention with mortality as an end-point, hundreds of patients will be required. Although all septic patients in ICU have a definite physiological abnormality, they are heterogenous. Even in a homogenous group of patients with Myocardial Infarction (MI) with a clear intervention 10,000 patients were needed to demonstrate a 5% reduction in mortality (42). Since mortality in ICU is higher than following MI, size of population to conduct a trial in ICU investigating critically ill septic patients will be lower (43). However, it is still challenging to recruit these smaller numbers. Multi-centre trials are conducted to recruit more patients to improve sample size. It removes “investigator fatigue” which may happen due to novelty of trial wearing off. But it can introduce more heterogeneity due to variable Intensive care practice and access to various resources. For example, a patient in a UK Intensive Care Unit has more organ dysfunction and subsequently higher mortality as compared to one in US as the number of beds in ICU are less in the UK. As a consequence, patients admitted to Intensive Care Unit in UK are more unwell resulting in higher mortality (44).

Also, the initial success of the PROWESS study is principally attributed to its design. It had a large sample size enough to detect even the smallest improvement in survival. As a consequence of this, sub-group analysis could be performed to identify patients who would optimally benefit from therapy. It identified that only patients with severe sepsis and a high risk of death should be treated. Further trials have shown no benefit and also an increase in bleeding complications in those patients with a low mortality risk (23).

1.9 Inflammation and omega-3 fatty acids

Inflammation is the basis of several acute and chronic diseases such as asthma, rheumatoid arthritis, cardiovascular diseases, Alzheimer's disease, cancer, inflammatory bowel disease and sepsis (45, 46). It is body's response to insults such as infection, injury and surgical procedures. It is characterised by the five classical cardinal signs, redness (rubor), increased heat (calor), swelling (tumor), pain (dolor) and loss of function (functio laesa). It results from the increased movement of plasma and leukocytes (especially granulocytes) from the systemic circulation into the injured tissues. This is followed by a cascade of events involving complement, clotting cascade and the immune system. The primary aim of this response is to protect the host from the insulting agent but unfortunately (and not uncommonly) hyper or inappropriate inflammation can occur.

Hyper-inflammation can occur as part of body's response to sepsis or surgical stress and attention has turned towards those products which could potentially address both the nutritional status and this inappropriate inflammation. These immunomodulatory dietary products rather than just being a source of energy provide nutritional agents which have specific well-defined effects and are particularly effective in producing modifications which help protect the immune system and modulate the production and effect of inflammatory mediators.

1.10 Mechanisms of action of omega-3

The beneficial effects of omega-3 fatty acids (the metabolic products of dietary omega-3 lipids) were highlighted when over an eight year period, the epidemiological studies of Dyerberg *et al* in Greenland Eskimos clearly demonstrated their anti-thrombotic effect and the consequent protective role in heart disease (47). Omega-3 fish oils include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Humans cannot synthesize these to any significant extent and obtain them from cold water fish that themselves derive them from consumed plankton and algae (where they are synthesized).

The active ingredients, DHA and EPA have been extensively studied in the clinical as well as the epidemiological setting. The cell membranes of all cells are composed of phospholipids and the polyunsaturated fatty acid components (omega-3 and omega-6 fatty acids defined by the first carbon atom with a double bond counted from the omega [methyl] end) are metabolised to produce a bewildering array of products. These include virtually all the molecules included in the inflammatory cascade which result from a competitive metabolism of the omega-3 and omega-6 components by the cyclo-oxygenase (COX) and 5-lipoxygenase (LOX) pathways. The competitive nature of these metabolic pathways mean that the relative proportions of the omega-3 and omega-6 fatty acids determine which products are produced. Principle amongst these are the eicosanoids (the term is derived from the Greek eicos meaning twenty and referring to the number of carbon atoms) and although they can be derived from either the omega-3 or omega-6 fatty acids, those derived from the omega-6 series are strongly pro-inflammatory whereas those from the omega-3 series are much less so

or even anti-inflammatory. The resulting eicosanoids comprise four groups; the prostaglandins, prostacyclins, leukotrienes and thromboxanes (named from the tissue in which they are first described). They are very powerful “local hormones” acting only at the site of production. Consequently, any adjustment of the diet which affects the ratio of omega-3 and omega-6 fatty acids in the cell wall has the potential to significantly influence the type of eicosanoids which are produced and it is this effect which can modulate the cells of the immune system (48). The aim of omega-3 supplementation is thus to reduce the amount of substrate available for the synthesis of harmful inflammatory mediators by competing with arachidonic acid for metabolism via COX and LOX. They also inhibit the release of arachidonic acid from phospholipids by phospholipase A2 meaning that less is available for metabolism. The overall result is the formation of several inflammatory mediators with a different structure to those derived from arachidonic acid which are biologically less potent and include the 3-series prostaglandins and thromboxanes and 5-series leukotrienes (49). It was shown by Lee *et al* in1985 and later in1993 by Sperling *et al* that there was a 40-70 % reduction in LTB4 and 5-hydroxyeicosatetraenoic acid production by neutrophils and monocytes following dietary enrichment with EPA and DHA (50). There were similar findings by Stenson *et al* and Hawthorne *et al* in patients with Ulcerative colitis whose diets were enriched with fish oil over a prolonged period (51, 52).

Products derived from the metabolism of omega-3 significantly decrease the generation of inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 (53-55). Animal models have shown that omega-3 also suppresses inflammatory gene expression especially for TNF-α, IL-6 and IL-1ß as this gene expression is regulated by eicosanoids derived from arachidonic acid. In addition, it is also now known that

some of this effect is due to a direct effect of omega-3 on intracellular signaling pathways which leads to activation of one or more transcription factors particularly nuclear factor kappa-ß (56).

The body also responds to stress by producing a temporary state of immunosuppression and it was traditionally believed that this immunosuppression follows the initial hyper-inflammation. It is however now recognised (and has been demonstrated by several groups) that they can co-exist. Immunosuppression is characterised by failure of antigen presentation and impairment of the T-helper lymphocyte type-1 response and both these responses are responsible for the production of cytokines (57-59). This was examined in a study by Lin *et al* in 2005 that randomised rats undergoing gastrectomy to receive TPN with either 50% soybean emulsion and 50% fish oil emulsion or soybean emulsion alone (the control group). Their results suggested that omega-3 administration promoted lymphocyte T-helper 1 cytokine production, enhanced peritoneal macrophage activity and reduced leucocyte adhesion molecule expression (60). These findings supported the concept of an improved immune function following supplementation with omega-3 which did not appear to be at the expense of any immunosuppression. The administration of the omega-3 appears to protect or enhance cell mediated immunity with no deleterious effect.

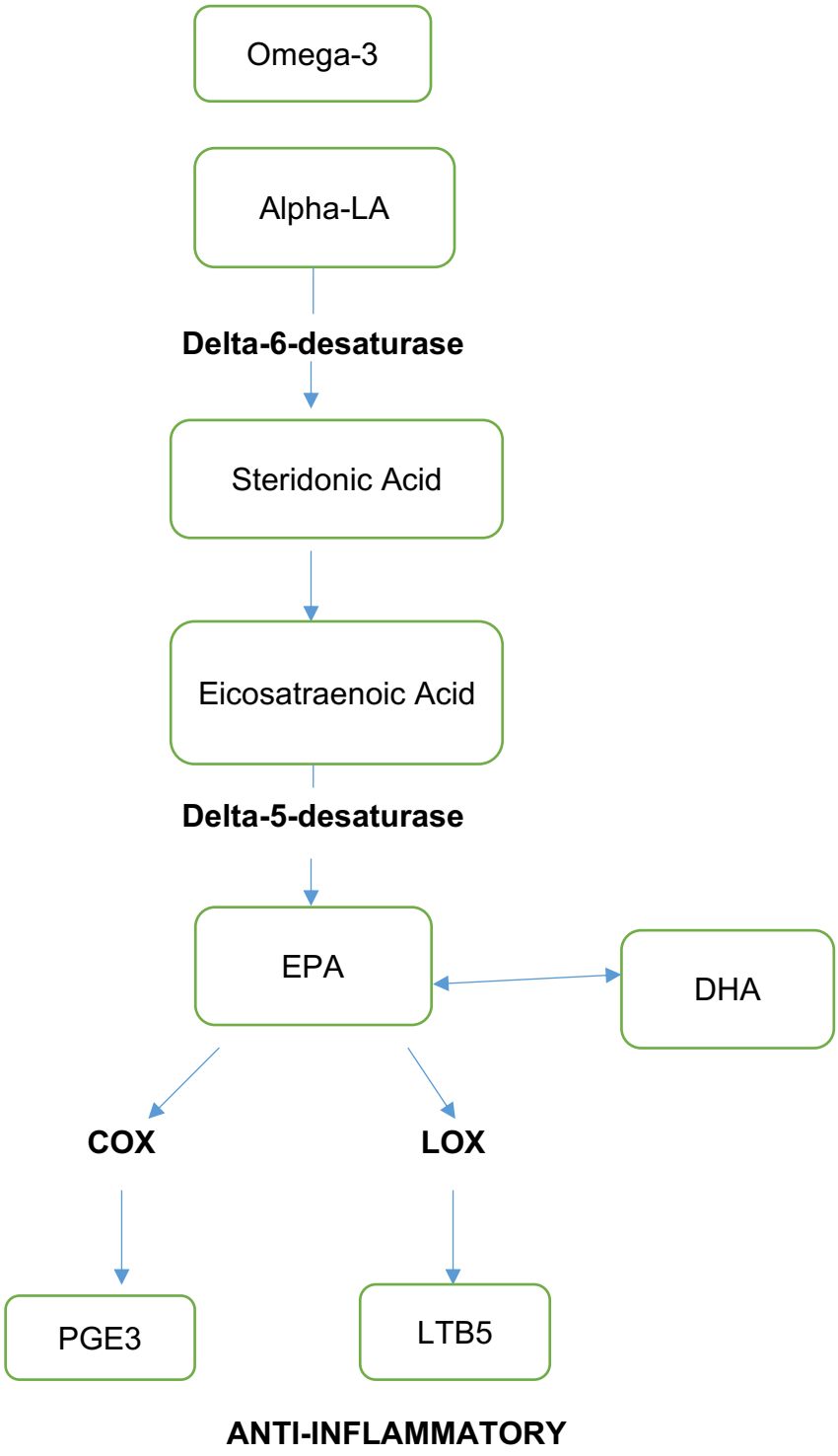

Figure 1.1: Pathway demonstrating actions of omega-3 and omega-6

1.11.1 Omega 3 in critically ill patients

A literature search was performed using the terms sepsis, inflammation randomised, blind, clinical trial, nutritional support, parenteral nutrition, omega-3 fatty acids, fish oils, lipid emulsions, critical illness, and critically ill. Studies were reviewed and summarised below.

Parenteral omega-3 has shown improvement in inflammation and immune function when used in surgical patients. This has resulted in shorter stay in hospital and ICU (61-63). Patients have tolerated fish oil well (64) with improved liver function and antioxidant status (65). It also improved the morbidity of the patient as measured by different scores such as APACHE and SAPS (66). The role of omega-3 has also been explored in other conditions secondary to hyperinflammation. Beneficial effects have been demonstrated in patients with pancreatitis (67) after five days of parenteral nutrition with FO. The authors proposed that omega-3 reduced the hyper inflammatory systemic response triggered by pancreatitis resulting in decreased pro-inflammatory cytokine production and diminished organ injury. This was reflected in reduction in inflammatory parameters such as C-reactive protein (CRP), better oxygenation index and reduced period of renal replacement therapy.

Heller and colleagues (68) reported a dose-response effect of parenteral fish oil on antibiotic demand, length of hospital stay and mortality (using doses of >0.05 g and >0.1 g fish oil/kg/day respectively) in critically ill patients. The need for antibiotic treatment was higher in the patients receiving fish oil doses of <0.15 g/kg/day suggesting a threshold dose for n -3 efficacy. The study by Khor and colleagues (66)

supported this by finding that organ dysfunction could be improved by fish oil supplementation in their blinded RCT of 28 patients. They found no difference in length of stay or serum TNF-α levels.

In recent years, three systematic reviews and meta-analysis have been performed. Manzares et al carried out a meta-analysis on 10 RCTs evaluating clinical outcomes in ICU patients and concluded that infectious complications were significantly reduced in FO containing lipid emulsion group (risk ratio (RR) = 0.64; 95% confidence interval (CI), 0.44 to 0.92; P=0.02; heterogeneity I^2 = 0%). Hospital length of stay (LOS) in FO group was reduced with statistical significance (weighted mean difference = − 7.42; 95% CI, − 11.89 to − 2.94; P=0.001). However, there was no reduction in mortality (69). Palmer et al analysed 8 RCTs in critically ill ICU patients receiving FO as parenteral nutrition. No significant effect on infectious complications (RR = 0.78; 95% CI, 0.43 to 1.41; P=0.41), mortality and LOS was demonstrated(70). Chen et al analysed 12 RCTs in ICU patients receiving n-3 FO through enteral or parenteral route and found no significant effect on mortality (RR = 0.82, 95% CI, 0.62 to 1.09; P=0.18). Although, sub-group analysis demonstrated that mortality was significantly reduced in the group receiving enteral n-3 (RR=0.69, 95% CI (0.53, 0.91), p = 0.007) (71).

1.11.2 Role of Omega-3 in septic patients

Numerous studies have investigated the role of immune-modulating agents in sepsis but have used a mixture of fish oil, arginine, glutamine and antioxidants. It is therefore difficult to assess the impact of fish oil on the beneficial effects. Despite this fact,

studies investigating role of immunomodulation have demonstrated improvement in ventilation requirement and mortality (72-74).

Pontes-Arruda and colleagues conducted a single centre, prospective, double blinded, placebo-controlled randomised trial of 115 septic patients and showed that enteral nutrition containing EPA, GLA and antioxidants decreased mortality as compared to controls (73). Intention to treat analysis showed that patients receiving the EPA/GLA diet had reduced incidence of cardiovascular failure (36.2% versus 21%, respectively; $p = 0.0381$) and respiratory failure (39.6% versus 24.6%, respectively; $p = 0.0362$). Similar improvement in respiratory function, oxygenation, ventilation requirement and organ dysfunction has been seen in another study by Gadek and colleagues investigating the role of enteral nutrition in a randomised study (75). Two meta-analyses also confirmed above findings. Pontes-Arruda et al demonstrated in their meta-analysis of three studies (76) in patients with acute lung injury and acute respiratory distress syndrome that enteral nutrition improved the clinical outcome and mortality significantly. This was due to reduction in new organ failures, ventilation requirements and improved oxygenation. Marik and colleagues (77) performed a meta-analysis of 1,918 high risk patients (21 studies) undergoing elective surgery receiving FO and arginine. They concluded a reduced length of stay, acquired infections and risk of wound infection.

However, some studies investigating role of immune-modulating diets have demonstrated conflicting evidence. Grau-Carmona (78) found that although administration of FO reduced length of stay in ICU, there was no improvement in organ dysfunction. Bertolini and colleagues conducted a study in 47 patients from thirty-three

ICUs in Italy. The treatment group received a combination of FO, arginine and Vitamin E while the control received standard parenteral nutrition. Mortality rates were analysed in the sub-group of patients with severe sepsis. Interim analysis demonstrated excess mortality in the group treated with immuno-nutrition (44.4% vs 14.3%; p= 0.039) leading to premature cessation of the trial (79). Similar results were obtained by Friesecke and colleagues (80) who analysed a mixture of MCT/LCT/FO lipid emulsion in critically ill ICU patients. They showed no beneficial effect on levels of IL-6, monocyte expression of HLA-DR (a marker of immune competence) or on overall clinical outcome compared with MCT/LCT. The authors hypothesized that the beneficial effect may have been because patients entered the trial after the inflammatory process was fully activated, in contrast with studies investigating therapy in surgical patients who receive omega-3 prior to surgical trauma (81). In two further studies, Mayer and colleagues (82, 83) reported diminished inflammation, including reduced TNF-α, IL-1β, IL-6, IL-8 and IL-10 production by cultured monocytes, in septic patients receiving soybean oil together with fish oil compared to those receiving soybean oil alone. The administration of the omega-3 rich emulsion induced an increase in n-3 free FA's in plasma and reversed the n -3/ n -6 ratio, favoring EPA and DHA over AA. These changes reached a maximum effect in 3 days (83). There was no difference in serum cytokine levels between the groups. The omega-3 group demonstrated reduced ventilation requirements with decrease in CRP and leucocyte count but this was not statistically significant (82). An increase in LTB5 (an anti-inflammatory leukotriene) was observed in the group receiving omega -3 fish oil. Trends were also seen with increased plasma omega -3 free FA's, the TXA3/TXA2 ratio, and platelet-activating factor (PAF) synthesis in the group receiving the omega -3 rich lipid emulsion.

Barbosa and colleagues reported the results of a randomised clinical trial investigating the effects of fish oil containing lipid emulsion on patients with sepsis in a single unit (84). Twenty-five patients with systemic inflammatory response syndrome or sepsis, and who were predicted to need parenteral nutrition were randomised to receive either a 50:50 mixture of medium-chain FAs and soybean oil or a 50:40:10 mixture of medium-chain FA's, soybean oil and fish oil. They demonstrated that parenteral fish oil increased plasma EPA, lowered IL-6 and improved gas exchange. These changes were associated with a trend towards shorter length of hospital stay.

1.12 Effect of omega -3 on complement profile

The development of septic shock is multi-factorial. Initially the host immune system responds to sepsis to protect the host but this immune response can become excessive and inappropriate. This can then become harmful to the host rather than being protective. Complement system which is part of the innate immunity plays an important role in escalating this immune response. The severity of sepsis and the clinical course is influenced by numerous variables including etiology of sepsis, co-morbidities of the patient, the severity of immune response and the degree of disturbance of body's homeostasis. The complement system plays an important role in building up this immune response during sepsis. It consists of numerous protein molecules which are precursors and in inactive form in the blood circulation. Presence of sepsis stimulates these inactive proteins resulting in a cascade of reactions and activation of membrane attack complex. There are three pathways for complement activation, the classical, lectin and alternative (85). All the pathways generate various

forms of C3 convertase which cleaves and activates C3 into C3a and C3b. This is followed by a cascade of reactions leading to formation of membrane-attack-complex (MAC). The key function of complement is to kill target cells with MAC, phagocytosis by macrophages and leucocytes and release of anaphylotoxins i. e. C3a, C4a, C5a.

However, excessive stimulation of the complement system can result in depletion of C3 resulting in worse outcomes (86, 87) Jianan Ren et al (86) in their prospective study in patients with abdominal sepsis demonstrated that the C3 depleted group had a poor prognosis with higher rate of postoperative and coagulopathy related complications. It was observed that the patients who died had severe C3 depletion. (C3 levels in non-survivors 0.36 +/- 0.18 vs survivors 0.78 +/- 0.27, $p<0.001$) and SOFA scores (11.0 +/- 0.9 vs. 9.3 +/- 1.2, $P<0.001$). They demonstrated that C3 levels of 0.578mg/mL had a sensitivity of 78.4% and specificity of 99.8% for predicting 28-day mortality in these patients. Role of numerous complement inhibitors have been studied to prevent depletion or reduce complement consumption. Silasi-Mansat et al demonstrated that the inflammatory process caused by E coli sepsis in baboons can be reduced by inhibiting C3 convertase thus improving outcome (88).

1.13 Effect of omega-3 on resolvins and protectins

1.13.1 Role of mediators in resolution of inflammation

The host protects itself from harmful invading pathogens by building a localised acute inflammatory reaction. This is mounted by polymorphonuclear neutrophil (PMN) as it

is the first line of defence in injury or infection. When this protective response becomes uncontrolled and excessive it can lead to numerous acute or chronic systemic inflammatory disorders including cardiovascular disease, inflammatory bowel disease, alzheimer's disease, age related macular degeneration, diabetes, periodontal disease and rheumatoid arthritis etc. (48, 89). More than 100 years ago, it was observed by Metchnikoff that neutrophils were ingested by macrophages causing resolution of inflammation. Later, researchers identified cytokines, complement etc. which stimulated recruitment of leucocytes to the damaged tissues (90). Researchers believed that removal of inciting agent and dilution of chemoattractants stopped further leucocyte recruitment thus causing resolution of inflammation. It has now been established that resolution of inflammation is not a passive process as believed earlier. Serhan and his group have done a lot of work and have demonstrated numerous specialised pro-resolving mediators from polyunsaturated fatty acids. These mediators include mainly resolvins, protectins, lipoxins and maresins. Resolvins are derived from omega-3 polyunsaturated fatty acids and exist as two series, D and E. D-series resolvins are products of docosahexaenoic acid (DHA) metabolism involving 15-lipoxygenase (LOX) and 5-LOX. E-series resolvins are synthesized from eicosapentaenoic acid (EPA) involving 5-LOX. Protectins are also omega-3 polyunsaturated fatty acid derivatives, generated from docosahexaenoic acid through a 15-lipoxygenase mediated pathway (91).

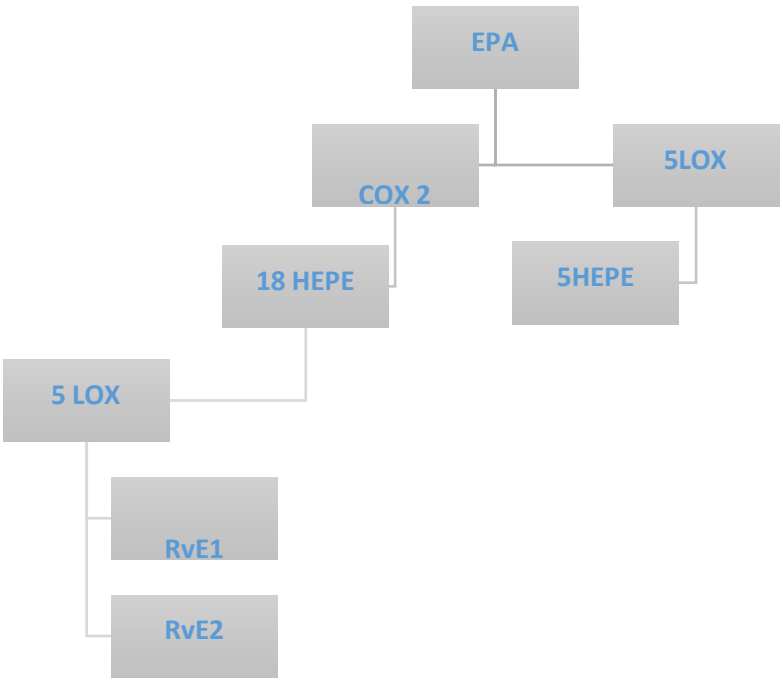

Figure 1.2 Metabolism of EPA

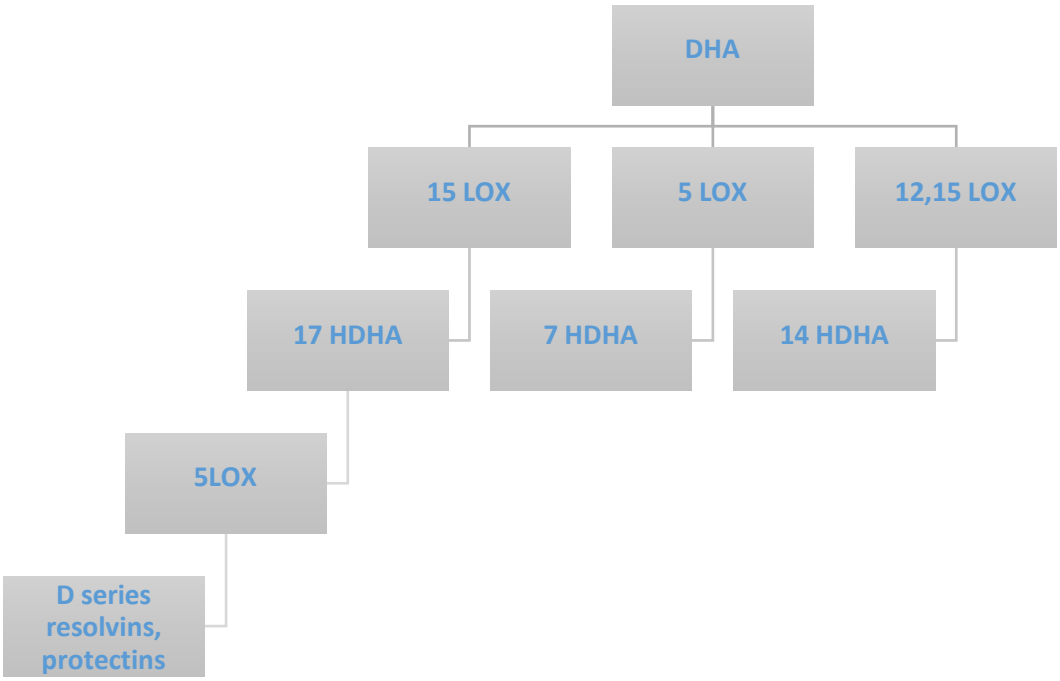

Figure 1.3 Metabolism of DHA

Following infection or tissue injury, the first event is the release of pro-inflammatory mediator, leukotrienes and prostaglandins. LTB4 attracts the PMNs to the damaged tissue while PGE2 and PGD2 stimulate the inflammatory process further resulting in development of acute inflammation. Despite being protective in the beginning this acute inflammatory reaction can develop into chronic inflammation and can lead to the pathogenesis of various diseases. This recent finding that resolution is an active process has generated new models for developing treatment options for diseases secondary to inflammation by targeting the active mediators. This process would prevent the development of chronic inflammation. Each of these pro-resolving mediators have specific roles such as blocking movement of neutrophils, activation of monocytes, macrophages, phagocytosis etc. This cascade of events will ultimately result in resolution of inflammation and block the destructive process (92).

1.13.2 Role of Arachidonic acid in the formation of pro-inflammatory mediators

Phospholipase enzymes act on phospholipids in the beginning of inflammation and cause release of polyunsaturated fatty acids such as arachidonic acid, EPA and DHA. Arachidonic acid initiates the acute phase of inflammation by producing LTB4, LTC4, PGE2 and PGD2. These act on G-coupled receptors and stimulate inflammatory enzymes, lipoxygenases and cyclooxygenases, numerous cytokines, chemokines and growth factors which are both pro and anti-inflammatory in action. Inhibition of various enzymes and receptors has been extensively studied and applied to develop treatment strategies to treat various inflammatory diseases (93, 94).

1.13.3 Role of Arachidonic acid in the formation of anti-inflammatory mediators

Interestingly, despite having a key role in initiation and development of acute inflammation AA is also associated with formation of anti-inflammatory mediators. Most important being lipoxins, LXA4 and LXB4 which are formed through the actions of lipoxygenase (LO5, LO12). These have potent anti-inflammatory and pro-resolving properties. This is the basis of action of aspirin triggered LXA4 formed from cyclooxygenase -2 (92).

1.13.4 Role of resolvins in various diseases

Topical application of RvE1 in periodontitis in rabbit model reduced inflammation by blocking the formation of superoxide by TNFα. RvE1 was used to treat mice with inflammatory bowel disease such as Crohn's disease and ulcerative colitis. These mice demonstrated improved histological appearances, maintained body weight and reduced mortality. RvE1 reduced serum levels of 2,4,6 trinitrobenzenesulfonic acid (TNBS), reduced expression of pro-inflammatory genes such as TNFα, IL-12, nitric oxide synthase and COX2 and polymorphonuclear leukocytes in the colonic mucosa. When RvE1 was given to a murine model of asthma, it reduced the infiltration of eosinophils and T lymphocytes. It also decreased the production of pro-inflammatory cytokines IL-23, IL-6 and IL-17 thus improved hyper responsiveness of allergic airways (89).

DHA is a precursor for protectin which are also anti-inflammatory and protective in action. This was first observed in neural tissues and therefore named with the prefix neuroprotection. The protectins block infiltration by PMNs and decrease secretion of cytokines. NPD1 reduces damage due to stroke and improves corneal wound healing in mouse models. Role of Protectin D1 (PD1) was studied in zymosan induced peritonitis models. After 4 hours PD1 had blocked majority of the PMN migration and infiltration into the site. The effect of RvE1 and PD1 together in a murine peritonitis model was explored. Studies demonstrated that presence of RvE1 and PD1 had an additive effect (95).

Sepsis remains a challenge despite wide spectrum of anti-microbials and introduction of sepsis bundle. If acute inflammation is not controlled, it quickly progresses to chronic inflammation resulting in immune suppression, multi-organ failure and mortality. Role of omega-3 was studied in mouse model of sepsis initiated by caecal ligation and puncture (CLP). After 12 hours, huge bacterial burden associated with significant leucocyte infiltration was noted both in the peritoneum and systemically. The group of mice who received omega-3 remained active as compared to the control group where the mice were hypothermic and less active. Treatment with RvD2 reduced the bacterial load and influx of inflammatory cells. The survival was 50% higher in the RvD2 group at 7 days. Secondly, the mice who did not survive in the control group died after 36 hours while in the omega-3 group died after 48 hours thus providing an additional window during which interventions can be applied such as antibiotics to improve outcome. Further analysis of peritoneal exudates 12 hours after CLP demonstrated that RvD2 diminished the levels of cytokines and pro-inflammatory lipid mediators such as LTB4 and PGE2. Similar findings of increased phagocytosis

were obtained when human PMNs were exposed to RvD2 and Escherichia coli in vitro. These in vivo and in vitro findings suggest that RvD2 is protective against breakdown of mucosal barrier leading to sepsis. RvE1 has also been found to improve survival in mouse with pneumonia by increasing clearance of bacteria.

Thus, it has been demonstrated that PMNs with inflammatory exudates can change phenotype and can produce pro-resolving and protective mediators from essential fatty acids. Secondly, this process can be augmented by administration of resolvins, protectins and maresins which are derived from omega-3 fatty acids. These mediators actively resolve inflammation by acting on specific receptors and producing specific by-products (95).

18 HEPE has been demonstrated to block neutrophil infiltration in a murine model of zymosan induced peritonitis. It was also found to reduce levels of LPS triggered TNF formation in murine macrophages. 18 HEPE is a pathway marker for the formation of numerous anti-inflammatory E-resolvins. 18 HEPE and 17 HDHA which are pathway markers for E and D resolvins respectively were also found in human and mouse blood samples. 4 HDHA which is derived from DHA also has potent biological action and acts as a pathway marker for DHA derived mediators. Since there are numerous mediators formed from DHA and EPA which are pro-resolving and protective in their effect, it might be useful to assess the common pathway products 18 HEPE and 17 HDHA instead (96).

The scoring systems are an essential part of describing ICU patients and predicting their mortality. There are numerous potential errors related to interpretation of various

scoring systems, it is important to use these with knowledge of science of severity scoring. Some of the scoring systems make serial measurements during the stay in ICU while some only consider data in the first 24 hours of admission. The sequential measurements reflect the progress of the patient over the period of stay in ICU and response of patient to the various therapeutic interventions.

There are contradictory findings on the effect of fish oil on various inflammatory mediators in septic patients in ICU (67, 84, 97, 98). Because of poor methodology, limited sample size and heterogeneity there is inadequate evidence to support the routine use of fish oil containing lipid emulsions in critically ill patients.

The studies published so far have not recognized any adverse effects and demonstrated that n-3 fatty acids are safe in critically ill patients (99). The major drawback of the studies to date is the different combination of preparations being used, via different routes (parenteral and enteral) and underpowered studies. Secondly, FO is usually given in combination with other immune-modulators. Consequently, it is difficult to quantify the exact contribution of omega-3 to the positive effect.

Therefore, a randomised controlled trial was conducted to investigate the effects of n-3 fatty acids on the inflammatory mediator profile in critically ill septic patients in ICU.

1.14 Statement of aims and null hypothesis

The primary outcome of this study was to analyse the effect of n-3 fatty acids on pro-inflammatory and anti-inflammatory markers in critically ill patients with sepsis on an Intensive Care Unit. These markers included cytokines, complement, resolvins and protectins. The secondary outcome was to evaluate the relationship of measured markers to the degree of new organ dysfunction and 28-day mortality. The organ dysfunction was measured using the SOFA score. This study is novel as no previous study has investigated the role of parenteral omega-3 in sepsis. All of the data collected will be used, if parenteral fish oil is associated with advantageous clinical outcomes, to form the largest pilot study and basis for a multi-centre randomised control trial.

The **null hypothesis** for this study was that the administration of omega 3 fish oil emulsion will not alter the level of pro-inflammatory and anti-inflammatory markers in critically ill patients with sepsis on Intensive Care Unit as compared to controls.

2. Methods

2.1 Study design

This is a phase II, single centre, randomised controlled trial analysing the effects of omega-3 fish oil in septic patients admitted to the Intensive Care Unit (9 ICU and 4 HDU beds) in a tertiary-referral hospital. Patients were recruited from the University Hospitals of NHS Trust, Leicester, United Kingdom. The study was approved by the South East Research Ethics Committee and was conducted in accordance with the Helsinki Declaration (approval attached as appendix 1).

Power analysis for the trial was carried out, based on the literature, to see a 50% reduction in the number of new organ dysfunctions measured using the SOFA score (51, 52). In order to have 80% power to find a difference of at least 50% reduction in the new organ dysfunction, with alpha value of 0.05, we required a total of 140 patients, 70 per group. To assess the safety of Omegaven in these very unwell septic patients and due to major difficulties in recruitment which was not predicted before the start of trial as explained below an interim analysis performed at 27 patients. It demonstrated significant improvement in delta SOFA score and mortality. A repeat power analysis performed on the basis of these results indicated that we required a total of 60 patients, 30 in each group.

2.1.1 Difficulties in the trial

This study was ethically complex which caused a number of issues even before the study was started. The fish oil emulsion, Omegaven, (Fresenius kabi) is licensed to be used as a supplement to parenteral nutrition. But the aim of this study was to analyse the role of Omegaven as a medicinal product. Secondly, some of the patients that were planned to be recruited from ICU were critically unwell and lacked capacity as they were unconscious and ventillated. There were concerns as to who would consent for these patients if next of kin was not available as the protocol of the study stated recruiting these patients within a narrow window of admission to ICU. There are only three Ethics committees in the UK who evaluate the ethics of trial protocol including London. This trial was evaluated and approved by the ethics committee in London who recommended that medical professionals not related to the trial team be trained to act as legal representatives when next of kin were not available. Therefore, a number of ICU consultants were trained to act as legal representatives.

During the initial stage of this trial, due to redistribution of services all the acute medical admissions were moved to another site in the trust. This significantly reduced the number of admissions to ICU. In addition, there were two other trials running concurrently in ICU with this trial. As one patient could only be enrolled into one study, the patients on ICU were shared between three studies on an alternate basis. To maximize recruitment, we approached suitable patients continuously over a 24 hour period with 2 researchers working alternately. This was done so that admissions to ICU during the night can be recruited as soon as possible allowing early treatment of patients with fish oil. The nursing staff on ICU were also helpful in highlighting suitable

patients, again this helped in screening all new admissions to ICU for eligibility into the trial and early recruitment. However, despite a team of researchers working continuously over a 24hour period it was not easy to approach patients and their families who were admitted during the night.

Secondly, some patients were not suitable for recruitment due to strict inclusion and exclusion criteria. Also, some patients had been unwell for prolonged periods before coming to ICU and they didn't want to be part of "a trial". They felt they were emotionally and physically drained for anything more than their essential treatment.

This caused problems in recruiting patients to the trial which was not anticipated. In view of shortage of suitable patient recruitment and to ensure safety of Omegaven in patients, a repeat power calculation was performed on the basis of an interim analysis of 27 patients. It was calculated that for a power of 95%, a total of 60 patients would be required. (figure 2.1, power calculations)

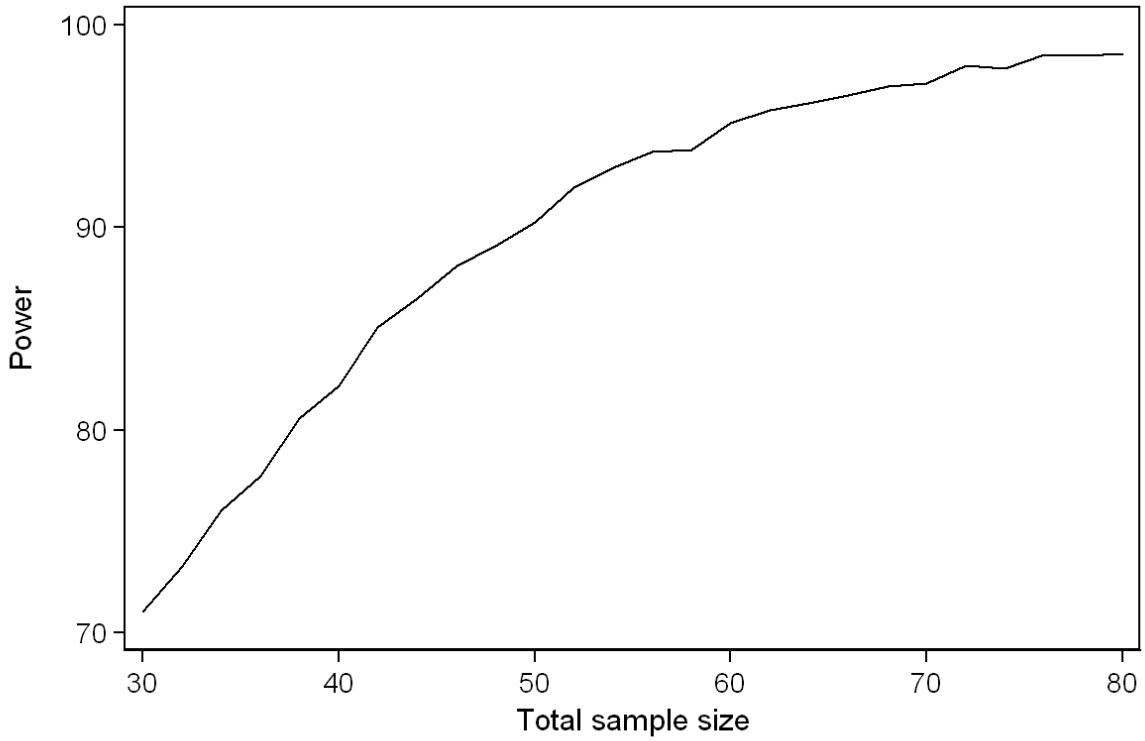

Figure 2.1: Power calculations

2.1.2 Primary outcome measures

The primary outcomes were effects on various inflammatory markers in plasma. These include the following:

- Cytokines
- Complement cascade
- Resolvins
- Protectins

2.1.3 Secondary outcome measures

- Routine biochemical markers
- Physiological markers
- Clinical outcome as measured using SOFA score
- 28-day mortality

2.1.4 Patient inclusion criteria

All critically ill septic patients admitted to the Intensive Care Unit at Leicester General Hospital from May 2010 through to July 2012.

Sepsis was defined as the presence of a known or suspected infection and two or more SIRS criteria (Systemic inflammatory response syndrome). SIRS criteria included temperature >38 C or <36 C, heart rate >90bpm, respiratory rate >20bpm or PaCO2 <32mmHg, white cell count >12g/l or <4g/l.

2.1.5 Patient exclusion criteria

The following exclusion criteria were used after advice from the manufacturer of Omegaven taking into consideration safety of its use in septic patients.

Patients were excluded from this trial if they had:

- Planned ICU admission following surgery
- Hypersensitivity to fish, egg or soy protein or other active substances of parenteral nutrition
- Uncontrolled hyperlipidaemia
- Severe primary blood coagulation disorder
- Acute pancreatitis secondary to hyperlipidaemia
- Ketoacidosis
- Acute thromboembolic disease
- Chronic liver disease or cirrhosis
- Acute phase of myocardial infarction or stroke
- Undefined coma state
- Pregnancy

87 consecutive patients were assessed for eligibility. 27 patients were excluded from the trial, 8 declined to participate, 2 were expected to die within 24 hours, 3 had active bleeding, 4 were unconscious and could not contact next of kin, 1 had suffered a recent coronary event, 2 had reduced GCS from an unknown cause, 5 were enrolled in another study, 1 was breast feeding and 1 was pregnant.

2.2 Trial treatments

2.2.1 Administration of Omegaven

- Study treatment was administered daily over a maximum of two weeks.
- Omegaven was infused intravenously either through the lumen of the central venous catheter or peripherally inserted central catheter or via a peripheral venous access cannula. Central venous access was part of the standard clinical care for patients in the Intensive Care Unit and was inserted by the patient's own medical team. Parenteral route was selected so that there was no doubt of effect of absorption of omega-3 if the gut barrier was affected due to sepsis in these unwell patients.
- Omegaven (10g/100ml) was given in a dose of 2ml per Kg body weight (eg: 70kg man received 140ml). It was infused at the rate of 0.5 ml Omegaven/kg body weight/hour. Therefore, a 70kg man received 140ml of Omegaven at the rate of 35ml/hour over a period of four hours. This rate of 0.5ml was used after consultation with manufacturer with regards to safe infusion rate.
- At the point of commencing Omegaven emulsion, patients were monitored for any signs of allergy or adverse reaction.
- Further enteral or parenteral feeding continued as per clinical requirement of patient.

2.2.2 Omegaven

Omega-3 (Omegaven 10gram/100ml) was supplied by Fresenius Kabi. It can be administered with other fat emulsions but ensuring that Omegaven should constitute 10-20% of this intake.

Omegaven composition data:

100 ml of Omegaven emulsion contains 10 grams of highly refined fish oil which consists of the following:

- eicosapentaenoic acid (EPA) 1.25 - 2.82 g
- docosahexaenoic acid (DHA) 1.44 - 3.09 g
- myristic acid 0.1 - 0.6 g
- palmitic acid 0.25 - 1.0 g
- palmitoleic acid 0.3 - 0.9 g
- stearic acid 0.05 - 0.2 g
- oleic acid 0.6 - 1.3 g
- linoleic acid 0.1 - 0.7 g
- linolenic acid ≤ 0.2 g
- octadecatetraenoic acid 0.05 - 0.65 g
- eicosaenoic acid 0.05 - 0.3 g
- arachidonic acid 0.1 - 0.4 g
- docosaenoic acid ≤ 0.15 g
- docosapentaenoic acid 0.15 - 0.45 g
- dl-α-Tocopherol (as antioxidant) 0.015 - 0.0296 g
- Glycerol 2.5 g
- Purified egg phosphatide 1.2 g

The total energy provided by 100 ml of Omegaven is 112 kcal/100 ml

2.2.3 Monitoring during Omegaven infusion

Patients were carefully monitored for any signs or symptoms of anaphylactic reaction. If present, the infusion was immediately interrupted. Patients were also monitored for signs of fat overload syndrome. Overdose leading to fat overload syndrome can occur when the triglyceride level during lipid infusion rises above 3 mmol/l, acutely, as a result of too rapid infusion rate, or chronically at recommended rates of infusion in association with a change in the patient´s clinical condition e.g. renal function impairment or infection.

Potential risks of Omegaven infusion were: (Reference document attached in appendix 2)

- Anaphylactic reactions (e.g. erythema)
- Prolonged bleeding time and an inhibited platelet aggregation
- Dyspnoea (1/1000-1/10000)
- Hypoglycaemia and other metabolic disturbances associated with TPN
- Fat overload syndrome
- Hyper and hypotension (1/1000-1/0000)
- Lack of appetite (1/100-1/1000)
- Nausea and vomiting (1/100-1/1000)
- Headache, pain in the chest, back and loins, bone-pain
- Priapism (<1/10000)

2.2.4 Fat Overload syndrome

This is caused by impaired capacity to eliminate triglycerides (levels >3mmol/l) which may be caused by an overdose. There are various causes such as genetic predisposition, renal impairment and sepsis.

It is characterised by hyperlipidaemia, fever, fat infiltration, hepatomegaly with or without jaundice, splenomegaly, anaemia, leukopenia, thrombocytopenia, coagulopathy, haemolysis, reticulocytosis, abnormal LFTs and coma. The symptoms are usually reversible if the fat emulsion is discontinued.

2.2.5 Drug storage and drug accountability

The hospital clinical trials pharmacist ensured that all study drugs were stored in a secure area, under recommended storage conditions and in accordance with applicable regulatory requirements. To ensure adequate records, all Omegaven was accounted for in the drug accountability inventory forms.

2.2.6 Concomitant medications and therapies

All patients on the trial had different source of sepsis with varying severity. The various sources of sepsis included chest, abdomen, urinary tract and skin. Patients received appropriate medical care including antibiotics, inotropes, renal replacement,

intravenous fluids and parenteral and enteral nutrition as per their individual requirement and ICU protocol.

2.3 Trial procedures

2.3.1 Screening

All patients admitted to ICU who were critically ill with sepsis from May 2010 through to July 2012 were screened. If the patient fulfilled the inclusion criteria they were approached by the investigator for recruitment. Some patients either did not fit the inclusion criteria or declined to participate in the trial.

2.3.2 Randomisation of patients

Once eligible patients were identified and consent was obtained, the patients were randomised into two groups by an independent professional, not related to the study using sealed envelopes. The block randomisation method was used to ensure a balance in sample size across both the groups over time. Blocks were small consisting of 10 patients each which kept the number of subjects similar in each group at all times. Group one was the treatment group (N=30) while group two was the control group (N=30). No blinding was done as there was no appropriate product available.

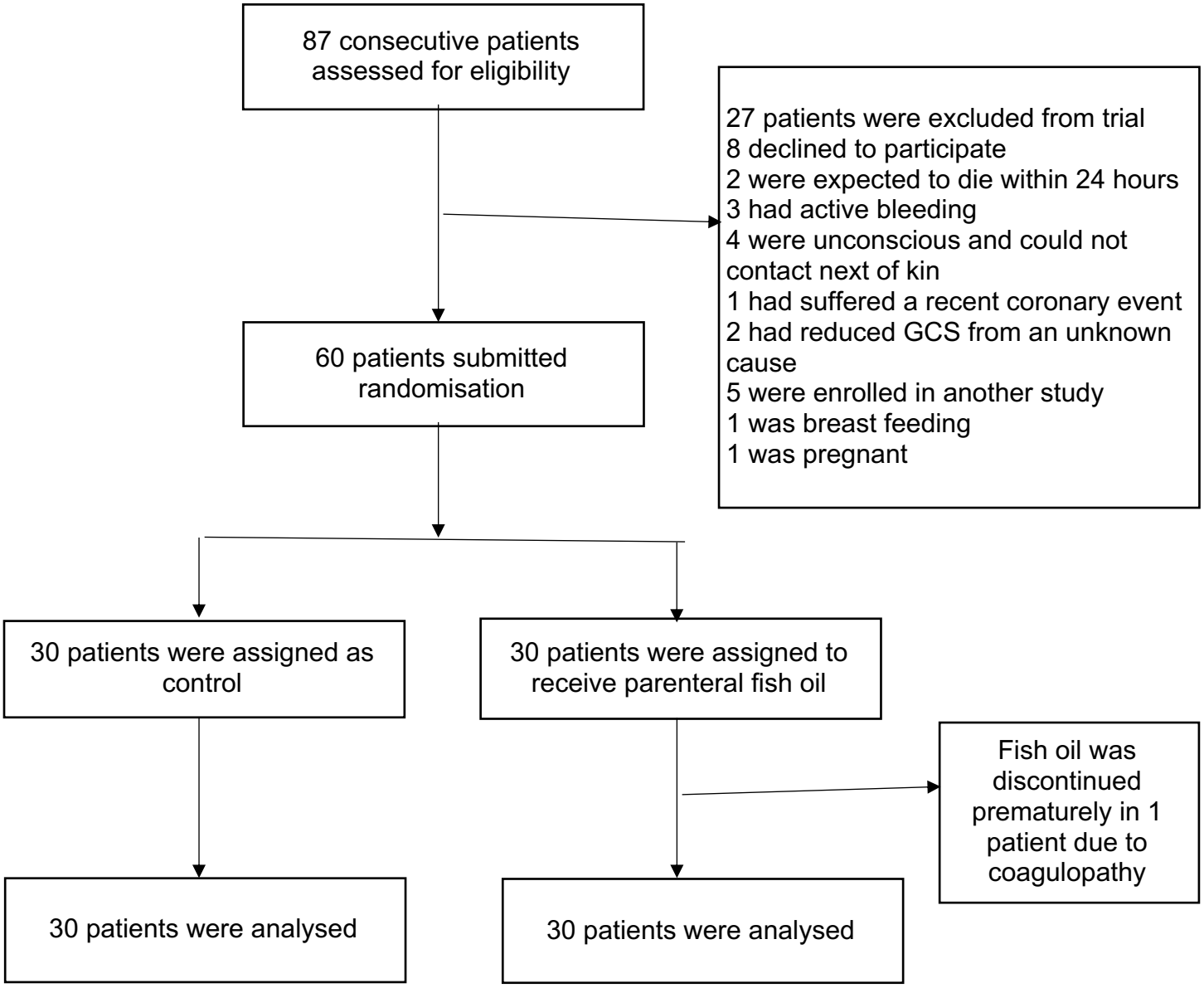

Figure 2.2: Flow diagram of patients screened, included and excluded with reasons for exclusion

The treatment group received intravenous omega-3 infusion (Omegaven, Fresenius Kabi) in addition to standard medical care while the second group received the standard medical care as per clinical requirement and no omega-3 infusion.

2.3.3 Consent of patients

For patients who were conscious, consent was obtained after detailed information was given to them regarding the trial by the investigator (consent form attached as appendix 3). 27 patients were recruited via assent and 33 patients via consent. Patient information leaflet was also provided (copy attached as appendix 4).

For patients who were unconscious or unable to consent at the time of admission, assent was sought for the patient from the patient's legal representative i.e. next of kin if available or a professional consultee (according to the department of health recommendations). Consent was then sought from the patient once they regained consciousness or improved clinically to a stage where they can consent. After consent was obtained, the patient continued to receive Omegaven emulsion according to randomisation. However, if patient on regaining consciousness refused to participate, his/her wishes were respected and Omegaven infusion was stopped. Routine medical management on the Intensive Care Unit was continued.

2.3.4 Trial period

Patients received treatment with Omegaven for a maximum of fourteen days or less if patient was discharged from ICU earlier, whichever came first.

2.3.5 Follow-up

The patient was followed to calculate the 28-day mortality and any instances of readmission to ICU.

Adverse events that were serious and suspected to be due to Omegaven or considered significant by the investigator were followed after the therapy was discontinued until the sequelae resolved or stabilised at a level acceptable to the investigator. Each serious adverse event was reported to the hospital Research and Development department.

2.3.6 Subject Withdrawal

Subjects could withdraw from the trial at any time at their own request or could be withdrawn at any time at the discretion of the investigator for safety. Also, unconscious patients for whom the consent was provided by their legal representative had the right to refuse participation after regaining consciousness.

2.4 Blood samples

2.4.1 Routine laboratory blood samples

The following haematological and biochemical tests were performed:

Full blood count

Coagulation screen

Urea, creatinine, sodium

2.4.2 Cytokine evaluation

Blood samples for changes in pro-inflammatory and anti-inflammatory cytokines were collected at a maximum of eight time points depending on the length of stay of patient in ICU. Each cytokine was analysed separately which is summarized in tables and graphs in the results section. The association of each cytokine with gender, age, source of sepsis and 28-day mortality was explored. The list of cytokines measured were as follows:

VCAM

ICAM

E-selectin

IL-17

TNFα

IL-1b

IL-12

IFN-g

IL-6

IL-10

TNFR1

IL-1ra

The first blood sample was collected before the infusion was started on day 0, and then infusion commenced. This was repeated on days 1, 2, 3, 5, 7, 10 and 13 for a maximum of fourteen days or less if patient was discharged early from ICU whichever came first. Two serum gel and two heparinised blood collection tubes were used to collect whole blood. These were centrifuged at 1000G for 15minutes at 4°C to produce 4x250uL aliquots of serum and plasma respectively. This was stored at -80°C until analysis.

2.4.2.1 Cytokine quantification by multiplex array

The whole blood collected from patients was centrifuged to separate plasma which was stored in eppendorf tubes at -80°C. At the time of analysis, the plasma was thawed and subjected to cytokine concentration quantification using a combination of electrochemiluminescence and patterned arrays as per manufacturer's instructions (Meso Scale Discovery, MSD).

The following pro-inflammatory and anti-inflammatory cytokines were evaluated in the multiplex array, IL-1β, IL-6, IL-8, TNFα, IL-10, IL-12, IL-17, TNF-R1, IL-1RA, E-selectin, ICAM-1 and VCAM-1. Plasma samples were thawed at room temperature and centrifuged for 10 minutes at 13,000 rpm in a bench top micro-centrifuge. The protocol used to process the samples is summarised in the table below. The light emitted from microplates was measured using MSD photodetectors. Efficient signal processing algorithms converted the measured signal into data. The concentrations were then entered into an excel spreadsheet to provide data on changes with treatment and time for each patient.

Table 2.1: Details of cytokine quantification

	1	2	3	4	5	6	7
Block	PI-4 II	3 plex custom (IL-10, IL-12, IL-17)	IL-23	TNFRI	IL-1RA	E-selectin	ICAM-1, VCAM-1
Wash	25µl dil2, 30min	25µl dil2, 30min	25µl dil2, 30min	25µl dil2, 30min	25µl dil2, 30min	150µl 5%A, 1h	150µl 5%A, 1h
Sample	25µl, Neat 2h, Cal range 2500pg/ml 4 fold in dil2	25µl, Neat 2h, Cal range 2500pg/ml 4 fold in dil2	25µl, Neat 2h, Cal range 2500pg/ml 4 fold in dil2	25µl, 10fold in dil2, 2h, Cal range 2500pg/ml 4 fold in dil2	25µl, 100fold in 1%A, 2h, Cal range 2500pg/ml 4 fold in dil2	40µl dil10, 10µl Neat, 2h Cal range 1000ng/ml 7 fold in dil 10	40µl dil15, 10µl sample 200 fold in 1%A, 2h Cal range 500ng/ml 5 fold in dil 15
Wash	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST
Det Ab	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h	25µl at 1Xin dil3, 1h
Wash	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST	3x150µl PBST
Read Buffer	150µl 2x	150µl 2x	150µl 2x	150µl 2x	150µl 2x	150µl 2x	150µl 2x

2.4.3 Complement quantification by ELISA

A homogenous group of 20 patients with similar origin of sepsis i.e. abdominal sepsis was selected. 10 patients were from the treatment group i.e. those who received omega-3 fish oil, while 10 patients were from the control group. The C3 component of complement was measured. Serum samples separated from whole blood collected at two time points were stored at -80°C. Time point 1 (t1) was day 0 and time point 2 (t2) was the last day of stay on Intensive Care Unit.

Samples were thawed at the time of analysis. The AssayMax© human complement C3 ELISA KIT was used. The serum samples were diluted to 1:800 in Mix diluent. To generate a standard curve, serial 1:2 dilutions were made starting with a C3 standard concentration of 10 µg/ml. All samples were measured in duplicates. 25µl of each serum sample or C3 standard were used and 25µl C3 conjugate was added directly. The plate was sealed and incubated at room temperature for 2 hours. After washing the plate 5 times with a wash buffer, 50µl SP-conjugate was added for 30 minutes. The plate was washed 5 more times and incubated with 50µl Chromogen substrate until it turned blue. To stop the reaction, 50µl of a stop solution was added until a change from blue to yellow colour was observed. The plate was read directly at 450nm with an ELISA reader and the data was analysed by creating a standard curve and estimating the C3 concentrations of the serum samples with EXCEL.

2.4.4 Resolvins & Protectins

Blood samples for changes in resolvins and protectins were collected at a maximum of eight time points depending on the length of stay of patient in ICU. First blood sample was collected before starting the infusion as day 0, and then infusion commenced. This was repeated on days 1, 2, 3, 5, 7, 10, 13 for a maximum of fourteen days or less if patient was discharged early from ICU whichever came first.

Whole blood collected in one heparinised blood collection tube was centrifuged at 1000G for 15 minutes at 4°C to separate plasma. 2ml of plasma was mixed with 4ml ice cold methanol and stored at -80°C until further analysis. During the extraction process, the recovery from plasma was 30-40% lower as compared to serum. The reasons for this were not well understood.

The author travelled to the University of California to join a group of researchers at Berkeley, University of California. There she learnt the techniques involved in preparation and analysis of these samples. The author assisted the research team at the University of California with preparation and analysis of samples for analysis of resolvins and protectins.

The analysis was performed in two stages. The first stage involved Liquid-chromatography (LC) followed by analysis using Mass-spectrometry (MS).

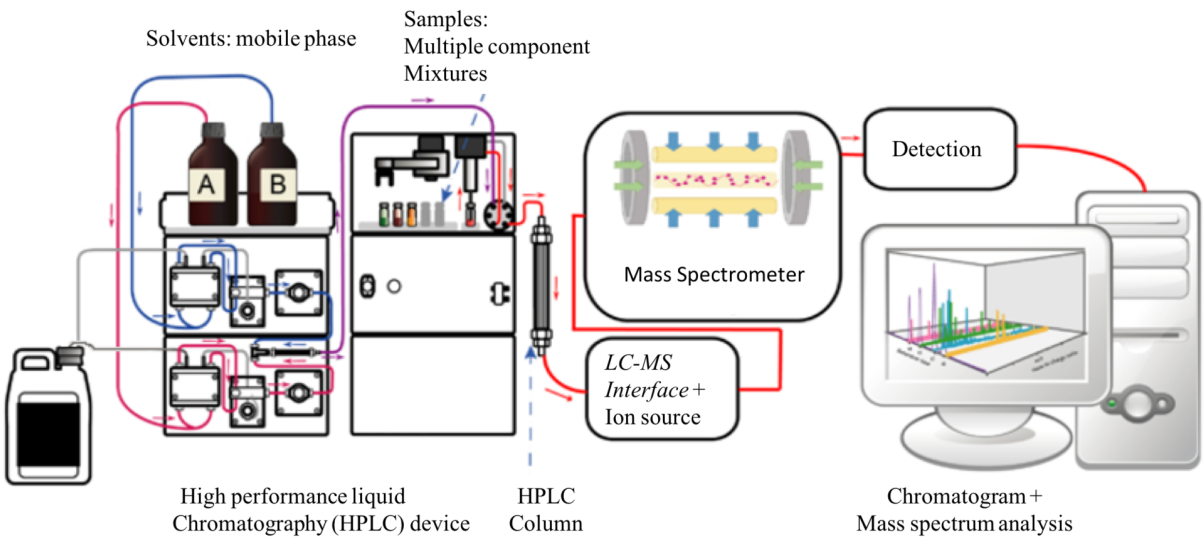

Figure 2.3: Schematic representation of Liquid chromatography and mass spectrometry

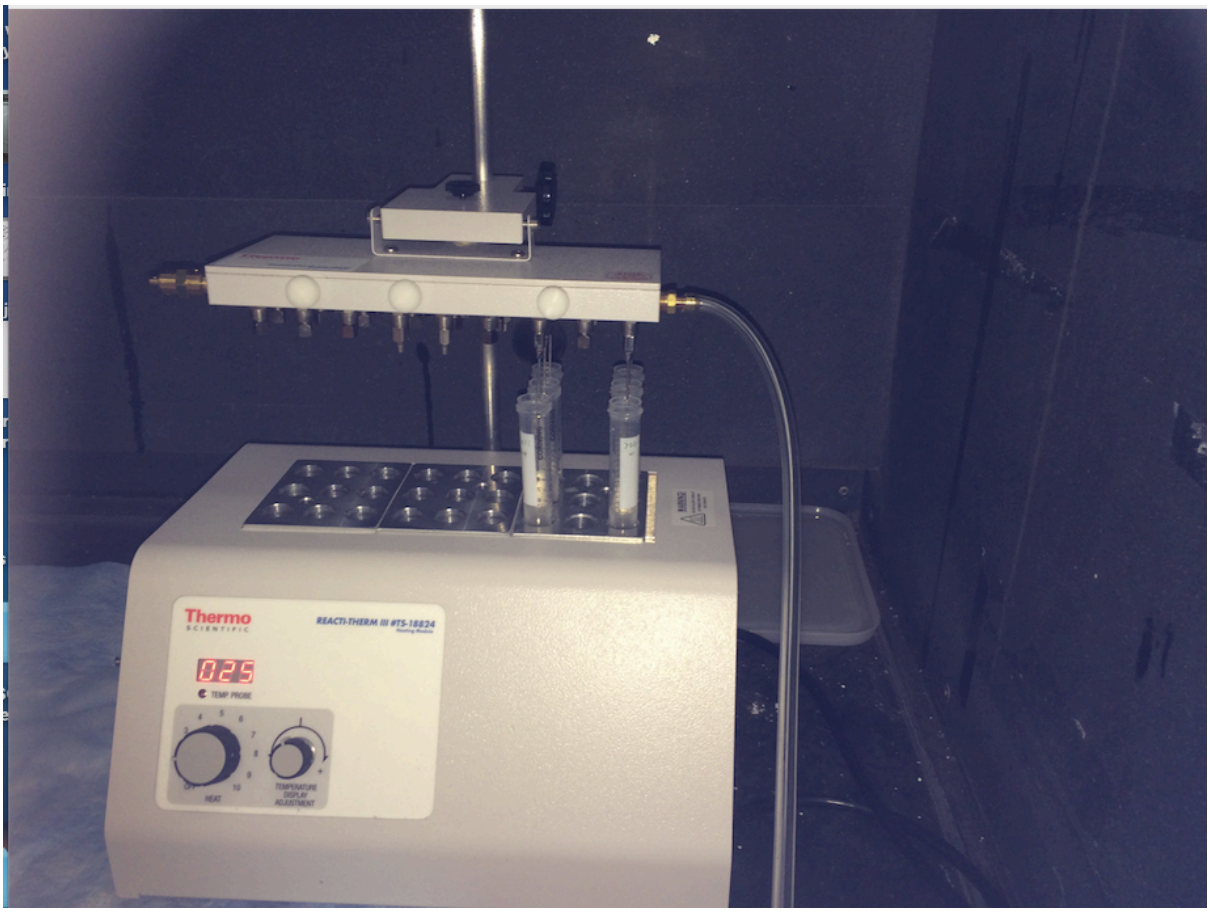

Figure 2.4: Demonstration of drying of samples on the block

2.4.4.1 Liquid Chromatography

LC coupled with MS was used for analysing resolvins and protectins in the patient's plasma samples. This coupling is advantageous due to high sensitivity and specificity and its ability to handle complex mixtures as compared to other chromatographic detectors.

The entire procedure was performed on ice. The samples were centrifuged at 4500 rpm at 4°C for 20 minutes. The supernatant was carefully pipetted into a new 50ml tube without disturbing the pellet. Ice cold LC/MS grade water was added to make 10fold dilution. The solution was acidified to a pH of 4 by adding 5µl of 1N HCl which was confirmed by using pH indicator strips. 20µL of the Internal Standard (IS) was added to each sample using an Agilent glass syringe. Also 20µL of the IS was placed in a separate amber HPLC (High performance liquid chromatography) vial with glass insert which was used as the Standard for 100% recovery. The IS contained 400pg each of PGE2-d4, LTB4-d4 and 15s HETE-d8 (Cayman Chemicals). While the samples were in the centrifuge, the columns were primed twice using 10ml LC/MS grade methanol. This process allowed removal of impurities from the columns and prepared them for the actual sample. The flow rate was maintained at 1-2 drops per second. Care was taken to ensure that the columns do not run completely dry. The columns were then washed twice with 10ml LC/MS grade H2O. The sample was loaded onto the columns (Agilent, SampliQ C18 6ml, 500mg column Part No. 5982-1165) and allowed to drip slowly out of the columns at a flow rate of 1 drop per second making sure not to let the columns run completely dry. The columns were again rinsed twice with 10ml LC/MS grade H2O maintaining a flow rate of 1-2 drops per second.

After the final rinse the columns were allowed to run dry. All markers (mobile phase) moved through the columns (stationery phase) at varying speeds. This speed depended on the polarity of markers.

The sample was then transferred to a sterile 15ml conical Polypropylene test tube for drying on a block which was turned on to 25°C. The metal needles were cleaned by vortexing them with HPLC grade methanol. Needles were screwed into the evaporation manifold. The nitrogen valve was opened and manifold lowered into the tubes. Nitrogen was started at the rate of 1psi and slowly increased until light bubbling was heard. Methanol was added as the level in the tubes was approaching about 1ml. This was continued until the tube ceases to feel cold, checking every 10 minutes. Methanol was added in decreasing quantities, starting at 1ml of methanol, then 500µl, then 100µl, etc until completely dry. This drying process took between 1 to 1.5 hours. The sample was re-suspended in 100µl of methanol.

The above sample contained all the extracted markers including resolvins and protectins. The levels of markers were then read using a Mass spectrometer.

2.4.4.2 Mass spectrometry (MS)

Mass spectrometry consisted of two stages, first ionisation of the analyte molecule, followed by analysis of ions produced based on their mass to charge ratio. Although lipids were eluded from the columns at different times there was still some overlap. Therefore, to differentiate them fragmentation was performed. Fragmentation was

achieved using electron spray. Each molecule has a unique structure which when bombarded with electrons got fragmented in unique characteristic ways. Combination of initial molecular weight and fragmentation ion molecular weight made it unique and helped in identification and quantification of particular ions. All parts of MS including electron spray ionisation, temperature and gas flow were calibrated before samples were loaded. This was done to get the strongest possible signal for each ion making the detection more specific.

The samples were pumped through a metal capillary kept at 3 kilo volt which nebulised the sample into a fine spray of charged droplets. Dry nitrogen and heat were applied to the droplets to vaporise them and transfer the residual charge to the analytes. These analytes were then transported through a number of small openings and focused voltages into the Mass spectrometer. MS detected molecular weight of the original molecule and fragmented ions. These pairs of ion weights were unique and were used to provide a method of identification for lipid molecules. The methods programmed in the computer were designed to allow the MS to analyse dozens of ion pairs in a single sample. This data along with retention time was used to positively identify target lipids. Calibration curves were set up at the beginning of analysis which were used to quantify data into concentrations. Data was entered into excel sheet and manually quantitated by using ABSciex-Analyst software.

2.5 Clinical outcome analysis

2.5.1 SOFA score

The total SOFA score was calculated by combining six separate organ dysfunction scores including respiratory, coagulation, liver, cardiovascular, central nervous system and renal. The most abnormal clinical and laboratory parameters were used to calculate the SOFA score daily (table 2.2). How best to calculate GCS in a sedated or paralysed patient has been a topic of debate for a number of years. Since many of the patients in this study were either sedated or ventilated calculation of GCS was omitted as its measurement could be confusing.

Organ System	0	1	2	3	4
Respiration PaO2/FiO2	>400	<400	<300	<200 with respiratory support	<100 with respiratory support
Coagulation Platelets (103/mm^3)	>150	<150	<100	<50	<20
Liver Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6-11.9	>12
Cardiovascular (hypotension)	No hypotension	MAP<70	Dopamine</=5 or dobutamine	Dopamine>5 or norepinephrine</=0.1	Dopamine>15 or norepinephrine >0.1
Central Nervous System (GCS)	15	13-14	10-12	6-9	<6
Renal Creatinine (mg/dl) or urine output (ml/d)	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

Table 2.2: Sequential Organ Functional Assessment (SOFA) score

2.6 Statistical analysis

Changes in cytokines, resolvins and protectins were analysed using a statistical model in STATA software. This included mixed effects linear regression to take into account the repeated measures taken over time for each patient. Clinical outcomes were correlated with complement changes using excel spreadsheet. A p value of < 0.05 was taken as significant.

3. Results

The results are summarised in 4 parts as follows:

1. Analysis of clinical data
2. Analysis of cytokines
3. Analysis of complement
4. Analysis of resolvins and protectins

3.1 Clinical results

A number of clinical parameters were measured for all the patients in both the groups during their stay on ICU. This included general demographic features such as age, gender, details of surgery, APACHE II, co-morbidities and details of the origin of sepsis. No significant differences were detected in the demographics between the two groups. Also, baseline biochemistry and haemodynamic parameters were recorded. These are all summarised below with the corresponding p-values.

Characteristic	Control Group (n=30)	Fish oil Group (n=30)	p value
Age (years)	64.5 ± 13.4	63.8 ± 11.7	0.830
Gender – female:male	15:15	12:18	0.436
Recent surgery – no. (%)	12 (40)	18 (60)	0.121
Elective (post-op sepsis)	5 (16.7)	9 (30)	0.222
Emergency	7 (23.3)	9 (30)	0.559
APACHE II	17.9 ± 6.2	19.1 ± 6.7	0.473
Corresponding mortality risk (%)	29.2	31.8	0.562
SOFA score	7.6 ± 3.2	7.2 ± 3.0	0.582
Comorbidities – no. (%)			
Hypertension	16 (53.3)	20 (66.7)	0.292
Ischaemic heart disease	2 (6.7)	7 (23.3)	0.145
Congestive heart failure	1 (3.3)	2 (6.7)	1.000
Chronic obstructive pulmonary disease	11 (36.7)	8 (26.7)	0.405
Chronic renal failure	5 (16.7)	8 (26.7)	0.347
Diabetes	6 (20)	5 (16.7)	0.739
Liver disease	0 (0)	0 (0)	n/a
Alcoholism	0 (0)	2 (6.7)	0.492
Cancer	9 (30)	8 (26.7)	0.774
Immunocompromised	1 (3.3)	5 (16.7)	0.195
Steroid use	1 (3.3)	7 (23.3)	0.052
Solid organ transplant	1 (3.3)	4 (13.3)	0.353
Intravenous drug abuse	0 (0)	1 (3.3)	1.000
Baseline biochemistry			
Albumin	25.8 ± 6.0	25.9 ± 6.0	0.949
CRP	234.1 ± 95.0	180.2 ± 104.6	0.105
Blood glucose	8.18 ± 2.6	8.72 ± 2.56	0.348
Haemodynamic variables			

Mean arterial pressure (mmHg)	63.5 ± 7.6	67.3 ± 12.6	0.238
Arterial pH	7.27 ± 0.1	7.28 ± 0.1	0.704
Serum lactate (mmol/litre)	2.5 ± 2.4	2.4 ± 2.0	0.717
Respiratory variables			
PaO_2/FiO_2	227.9 ± 115	197.0 ± 111	0.304
Ventilated – no. (%)	11 (36.7)	18 (60)	0.071
Focus of sepsis – no. (%)			
Chest	8 (26.7)	8 (26.7)	0.774
Abdomen	16 (53.3)	18 (60.0)	0.436
Urinary tract	6 (20.0)	2 (6.7)	0.253
Skin	0	2 (6.7)	0.49
Pathogen type cultured – no. (%)			
Gram + alone	3 (10)	7 (23.3)	0.166
Gram – alone	10 (33.3)	4 (13.3)	0.067
Mixed	7 (23.3)	8 (26.7)	0.766
Other	2 (6.7)	1 (3.3)	1.000
No pathogen	8 (26.7)	10 (33.3)	0.573

Table 3.1.1: Demographic and baseline characteristics of the patients

The only significant baseline variant was the number of patients with a haematological dysfunction, which was more prevalent in the control than the fish oil cohort (46.7% vs. 10%, p=0.002). Whilst, there was a trend towards more organ dysfunction free days and fewer developments of new cardiac arrhythmias, this was not significant. The study was unable to demonstrate any significant difference in mortality or length of ICU or acute hospital stay.

Characteristic	Control Group (n=30)	Fish oil Group (n=30)	p value
Baseline Organ Failure - no. (%)			
Cardiovascular	17 (56.7)	16 (53.3)	0.795
Respiratory	13 (43.3)	18 (60)	0.196
Renal	6 (20)	7 (23.3)	0.754
Hepatic	4 (13.3)	2 (6.7)	0.671
Haematological	2 (6.7)	1 (3.3)	1.000
Baseline Organ Dysfunction - no. (%)			
Cardiovascular	9 (30)	7 (23.3)	0.559
Respiratory	16 (53.3)	10 (33.3)	0.118
Renal	13 (43.3)	11 (36.7)	0.598
Hepatic	11 (36.7)	8 (26.7)	0.405
Haematological	14 (46.7)	3 (10)	0.002

Table 3.1.2: Organ dysfunction score

The total SOFA score was calculated by combining six separate organ dysfunction scores including respiratory, coagulation, liver, cardiovascular, central nervous system and renal. The most abnormal clinical and laboratory parameters were used to calculate the SOFA score daily. The delta SOFA (p=0.005) and max SOFA (p=0.04) score were significantly lower in the FO group. Also, CRP (C-reactive protein) one of the markers of monitoring degree of inflammation was significantly reduced (p=0.01) in the FO group.

Variable	Control Group (n=30)	Fish Oil Group (n=30)	p Value
SOFA score			
Delta-SOFA	2.2 ± 2.2	1.0 ± 1.5	0.005
Max-SOFA	10.1 ± 4.2	8.1 ± 3.2	0.041
Day 1 SOFA	0.9 ± 1.3	0.5 ± 1.2	0.030
Day 3 SOFA	1.1 ± 1.9	0.2 ± 0.6	0.038
Day 7 SOFA	0.7 ± 0.9	0 ± 0	0.014
Day 13 SOFA	0.4 ± 0.8	0 ± 0	0.173
Inflammatory markers			
Mean CRP	186.7 ± 78	141.5 ± 62.6	0.019
Days free of organ dysfunction			
Cardiovascular	8.7 ± 4.5	10.2 ± 3.8	0.174
Respiratory	7.2 ± 5.2	7.8 ± 4.7	0.888
Renal	8.4 ± 4.8	11.2 ± 2.7	0.052
Hepatic	10.8 ± 4.7	13.0 ± 2.0	0.117
Haematological	11.3 ± 3.8	12.7 ± 2.4	0.058
Days free of organ support			
Vasopressors	9.5 ± 4.4	11.4 ± 3.4	0.091
Ventilation	11.3 ± 4.3	10.0 ± 5.4	0.348
Renal replacement therapy	12.3 ± 3.4	12.9 ± 2.9	0.471
Mortality			
28-day mortality	8 (26.7)	4 (13.3)	0.197
Total inpatient mortality	9 (30)	4 (13.3)	0.117
Length of stay (days)			
In ITU	12.3 ± 12.4	8.8 ± 7.7	0.858
In hospital	33.5 ± 30.4	26.7 ± 18.2	0.796
New arrhythmia	6 (20%)	1 (3.3%)	0.103

Table 3.1.3: Outcome measure results

3.2 Analysis of cytokines

3.2.1 Changes in serum cytokine concentration with treatment

A total of 12 cytokines were measured in serum samples of 60 patients in both the control and fish oil group as listed below.

3.2.2 Summary of cytokine analysis with details to follow:

Cytokine IL-17 was significantly ($p=0.035$) higher in the control group. Linear regression demonstrated that the concentration of other pro-inflammatory cytokines (E-selectin, VCAM, ICAM, TNFR1, TNF-α, IL-17, IL-12, IL-6, IL-1b) was also higher in the C group as compared to the FO group. While the level of anti-inflammatory cytokine, IL-10 was higher in FO group when compared to control although not significant ($p=0.33$). IL-1RA varied significantly over time ($p=0.07$) across all patients but demonstrated no significant difference between the FO and C group. Similarly, IF-Ng concentration varied significantly over time ($p=0.002$) across both groups but presented no significant difference between the two groups.

Max-SOFA scores for cytokines IL1RA ($p=0.001$), IL-6 ($p=0.01$) and TNFR1 ($p<0.001$) were significantly associated with cytokine concentration.

There was significant association between 28-day mortality and concentration of VCAM on day 1 ($p=0.05$) and day 5 ($p=0.03$). Similarly, significant association was observed between mortality and concentration of IL-17 on day 3 ($p=0.02$). ICAM and mortality were associated on day 1 ($p=0.05$) and day 5 ($p=0.05$).

3.2.3 Pro-inflammatory cytokines

1) VCAM (Vascular Cell Adhesion Molecule)

The concentration of VCAM did not vary significantly over time. This was analysed by performing mixed effects linear regression. The fixed dependent variable was the concentration of VCAM, the fixed independent variable was time and the random variable was the patient ID. This analysis considered the repeated measures that were taken over time for each patient. The p values for days 0, 1, 2, 3, 5, 7, 10 and 13 were above 0.05. These results are summarised in the table below.

When fish oil was added to the model, the p value for fish oil was 0.64. This suggested that VCAM concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.83. This suggested that VCAM concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.62. This suggested that VCAM concentration did not vary significantly between patients of different age.

Model	Coef.	P-value	95% CI	
Day	-38.34	0.27	-106.44	29.76
Fish oil	-203.20	0.64	-1057.84	651.45
Day	-38.38	0.27	-106.50	29.74
Gender	-93.65	0.83	-952.74	765.43
Day	-37.29	0.28	-105.56	30.98

Age	8.83	0.62	-26.50	44.15

Table 3.2.1: Effect of fish oil, gender and age on VCAM

Mortality and VCAM concentration

Logistic regression was used to explore the possible association between 28-mortality and VCAM concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and VCAM concentration for days 0, 2, 3, 7, 10 and 13. However, there was a significant association between 28-day mortality and concentration on days 1 and 5. Mortality was not associated with age (p=0.53), gender (p=0.13), fish oil (p=0.20) or source of sepsis (p=0.81)

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.2: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

2) ICAM (Intercellular Adhesion Molecule)

The concentration of ICAM did not vary significantly over time. The p value for all days other than day 0 (p=0.05) and day 1 (p=0.04) were more than 0.05. When fish oil was added to the model, the p value for fish oil was 0.53. This suggested that ICAM concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.52. This suggested that ICAM concentration did not vary significantly between males and females. When age was added to the model, the p value was 0.90. This suggested that ICAM concentration did not vary significantly between patients of different age. This is summarised in table below.

Concentration	Coef.	P-value	95% CI	
Day	-15.46	0.69	-92.51	61.59
Fish oil	-379.44	0.53	-1575.51	816.62
Day	-15.43	0.70	-92.50	61.63
Gender	-394.94	0.52	-1595.10	805.21
Day	-15.84	0.69	-93.01	61.33
Age	-3.10	0.90	-52.36	46.16

Table 3.2.3: Effect of fish oil, gender and age on ICAM

Mortality and ICAM

Logistic regression was used to explore the possible association between 28-day mortality and ICAM concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and ICAM concentration for days 0, 2, 3, 7, 10 and 13. However, there was a significant association between mortality and concentration on days 1 (p=0.05) and 5 (p=0.05). Age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with mortality as shown below.

28-day mortality	Coef.	P-value	95%CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.4: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

3) E-selectin

The concentration of E-selectin varied significantly over time. This was analysed by performing mixed effects linear regression. The fixed dependent variable was the concentration of E-selectin, the fixed independent variable was time and random

variable was the patient. This type of analysis considered the repeated measures taken over time for each patient. The results are shown below. The p values for time (days) was <0.05. However, when fish oil is added to the model, the p value for fish oil was 0.14. This suggested that, although the E-selectin concentration varied over time, there was no significant difference between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.65. This suggested that, although the E-selectin concentration varied over time, there was no significant difference between males and females. When age was added to the model, the p value for gender was 0.39. This suggested that, although the E-selectin concentration varied over time, there was no significant difference between patients of different ages.

Mortality and E-selectin concentration

Logistic regression was performed to explore the possible association between 28-day mortality and E-selectin concentration. This analysis was repeated for each day. No significant association was found between 28-day mortality and E-selectin concentration for any of the study days. Mortality was not associated with age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81)

28-day mortality	Coefficient	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.5: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

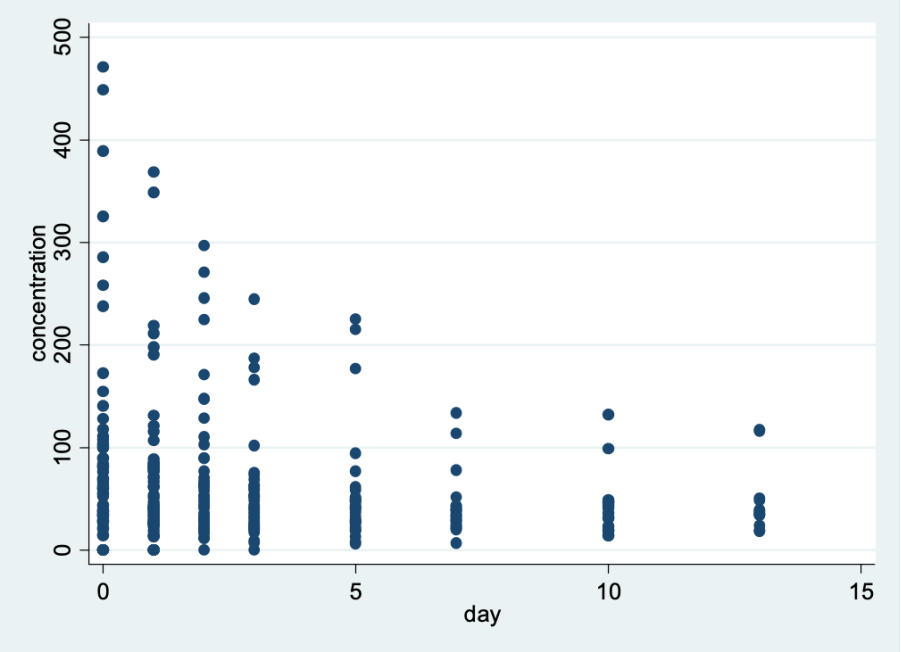

Figure 3.2.1: Scatter plot showing E-selectin concentration measured at different days for the entire cohort of patients

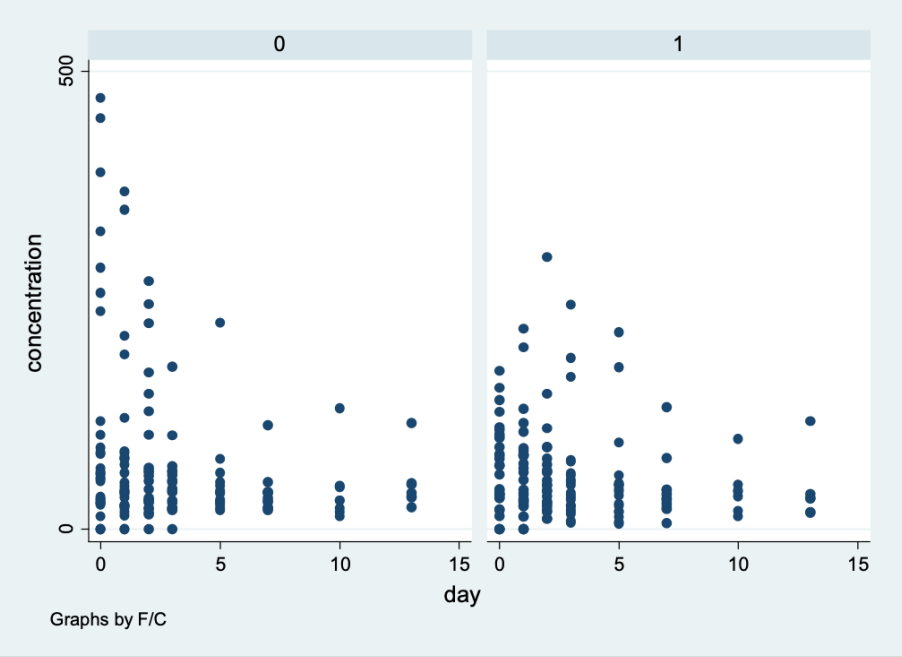

Figure 3.2.2**:** Scatter plot showing E-selectin concentration for the fish oil (1) and control (0) group.

4) Interleukin-17 (IL-17)

The concentrations of IL-17 in both the groups is summarised in graph below. The concentration of IL-17 varied significantly between days 0, 10 and 13 in both the groups. However, the p-values for day 1, 2, 3, 5 and 7 were >0.05.

Concentration	Coef.	P-value	95% CI	
Day 0	-20.15	0.0.02	-31.42	-7.11
Day 1	-2.15	0.65	-11.42	7.11
Day 2	-6.44	0.20	-16.18	3.29
Day 3	-6.28	0.24	-16.67	4.11
Day 5	6.67	0.24	-4.47	17.81
Day 7	-9.94	0.12	-22.31	2.43
Day 10	-21.61	0.003	-36.09	-7.13
Day13	-20.60	0.01	-36.36	-4.85

Table 3.2.6: IL-17 at 8 time-points in all the patients

Fish oil was significantly associated with IL-17 concentration. The concentration of IL-17 was significantly (0.035) higher in the control group. Gender (p=0.40) and age (p=0.74) were not found to be significant factors.

Concentration	Coef.	P-value	95% CI	
Day	-1.42	0.004	-2.39	-0.46
Fish oil	-17.57	0.035	-33.89	-1.25
Day	-1.42	0.004	-2.39	-0.46
Gender	-7.29	0.400	-24.24	9.67
Day	-1.42	0.004	-2.38	-0.45
Age	0.11	0.747	-0.58	0.81

Table 3.2.7: Effect of fish oil, gender and age on IL-17

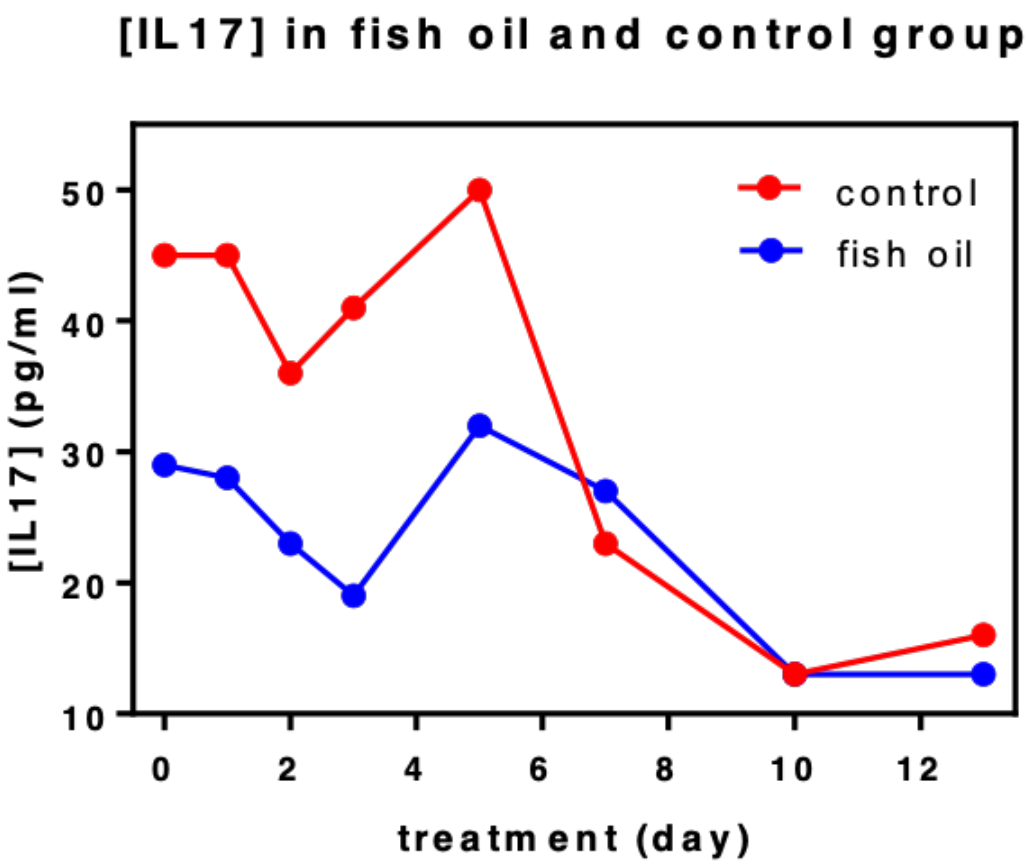

Figure 3.2.3: IL17 in fish oil and control group

Mortality and IL-17 concentration

The concentration of IL-17 was significantly associated with mortality on day 4 (p=0.02) but there was no significant difference (0.20) between the fish oil and control group.

Mortality was not associated with gender (p=0.13), age (p=0.53) or source of sepsis (p=0.81).

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.8: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

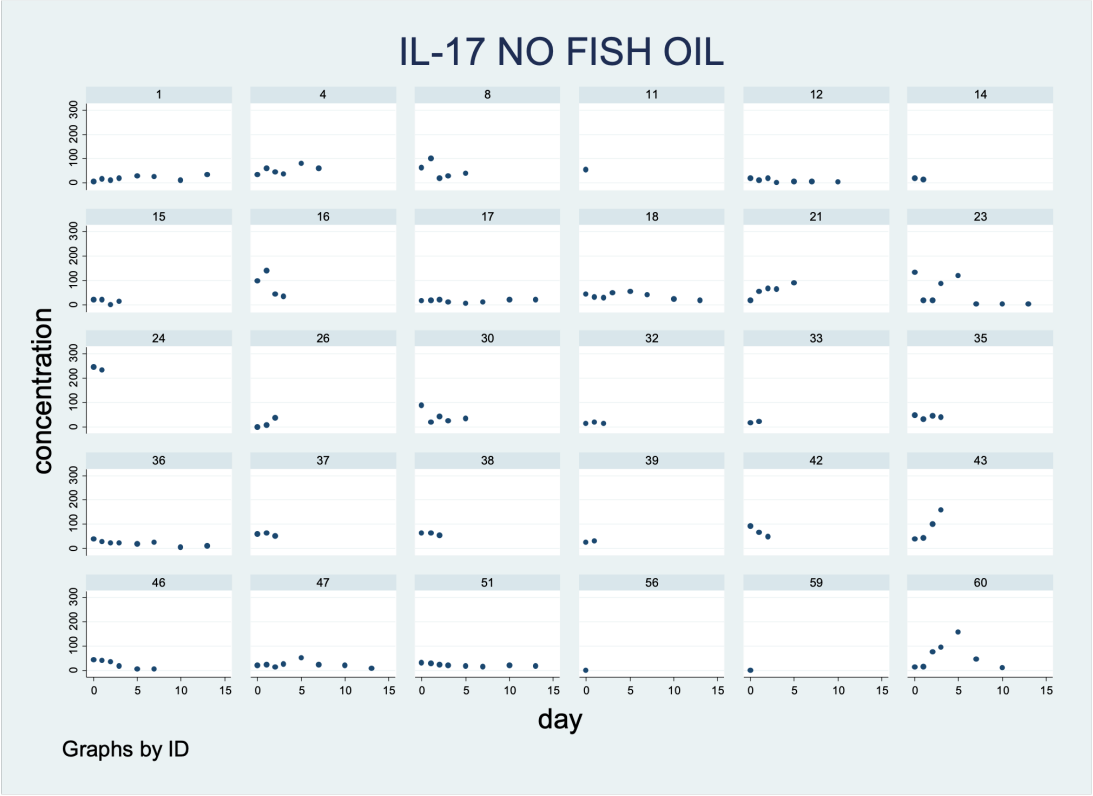

Figure 3.2.4 Individual patients in the control group

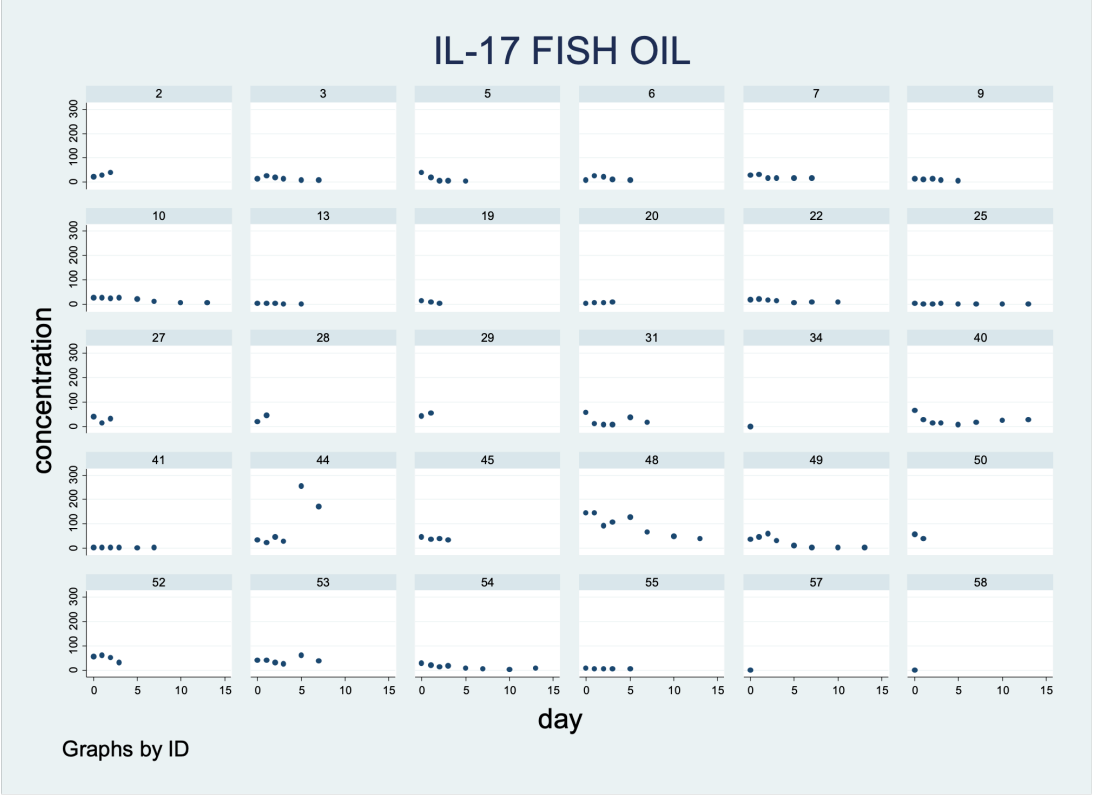

Figure 3.2.5: Individual patients in the fish oil group

5) TNFα (Tumour Necrosis Factor- alpha)

The concentration of TNFα varied significantly (p=0.02) over time in both the control and fish oil group. The concentration was higher in the control group as compared to the fish oil group but this difference was not statistically significant (0.82).

Fish oil (p=0.82), gender (p=0.78) and age (p=0.20) were not significantly associated with TNFα concentration.

Model	Coef.	P-value	95% CI	
Fish oil	-1.45	0.82	-13.78	10.89
Gender	-1.76	0.78	-14.17	10.65
Age	0.33	0.20	-0.17	0.82

Table 3.2.9: Effect of fish oil, gender and age on TNF-α

Mortality and TNFα concentration

Logistic regression was used to explore the possible association between mortality and TNFα concentration. This analysis was repeated for each individual day. No significant association was found between status and TNFα concentration for any of the days.

Age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not found to be associated with mortality in both the control and fish oil group.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47

Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.10: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

6) IL-1b (Interleukin-1b)

The concentration of IL-1b varied significantly over time in the entire group. Although the concentration was higher in the control group, the difference in the concentration between the fish oil and control group was not significant (p=0.83).

Fish oil (p=0.83), gender (p=0.24) and age (p=0.61) were not significant factors

The relationship between IL-1b concentration and mortality was explored. This analysis was repeated for each individual day. No significant association was found between status and IL-1b concentration for any of the days. These results are reported below. In addition, age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47

Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.11: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

7) IL-12 (Interleukin-12)

IL-12 concentration did not vary significantly over time in both the groups (p=0.15). However, the concentration was higher in the control group. When fish oil was added to the model, the p value for fish oil was 0.84. This suggested that IL-12 concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.22. This suggests that IL-12 concentration did not vary significantly between males and females. When age was added to the model, the p value for gender was 0.69. This suggested that IL-12 concentration did not vary significantly between patients of different age. The results are summarised in table below.

Concentration	Coef.	P-value	95% CI	
Day	-4.31	0.15	-10.25	1.62
Fish oil	-10.63	0.84	-112.42	91.15
Day	-4.30	0.16	-10.23	1.63
Gender	-63.34	0.22	-164.49	37.80

Day	-4.26	0.16	-10.19	1.68
Age	0.86	0.69	-3.30	5.03

Table 3.2.12: Effect of fish oil, gender and age on IL-12

Logistic regression was used to explore the possible association between 28-day mortality and IL-12 concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and IL-12 concentration for any of the days. These results are reported below.

In addition, age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.13: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

8) IFN-g (Interferon gamma)

The concentration of IFN-g varied significantly (p=0.002) over time in both the control and fish oil group. When fish oil was added to the model, the p value for fish oil was 0.24. This suggested that IFN-g concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.68. This suggested that IFN-g concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.64. This suggested that IFN-g concentration did not vary significantly between patients of different ages. Results are summarised in table below.

Concentration	Coef.	P-value	95% CI	
Day	-6.17407	0.002	-10.1251	-2.22307
Fish oil	31.21164	0.24	-20.8572	83.28045
Day	-6.20428	0.002	-10.159	-2.24961
Gender	11.07342	0.682	-41.959	64.10584
Day	-6.24268	0.002	-10.2023	-2.28301
Age	-0.52397	0.643	-2.7379	1.689956

Table 3.2.14: Effect of fish oil, gender and age on IFN-g

Logistic regression was used to explore the possible association between mortality and IFN-g concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and IFN-g concentration for any of the days.

IFN-g concentration, age (p=0.53), gender (p=0.13) and fish oil (p=0.20) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47

Table 3.2.15: Effect of age, gender and fish oil on 28-day mortality

9) IL-6 (Interleukin-6)

The concentration of IL-6 varied significantly ($p<0.001$) over time in both the control and fish oil group other than day 1 ($p=0.07$). When fish oil was added to the model, the p value for fish oil was 0.36. This suggested that IL-6 concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.16. This suggested that IL-6 concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.70. This suggested that IL-6 concentration did not

vary significantly between patients of different age. Results are summarised in tables below.

Concentration	Coef.	P-value	95% Conf. Interval	
Day	-58.32	0.000	-89.97	-26.66
Fish oil	-126.48	0.362	-398.66	145.71
Day	-57.73	0.000	-89.34	-26.12
Gender	-192.03	0.162	-461.26	77.20
Day	-59.00	0.000	-90.83	-27.17
Age	-2.27	0.700	-13.80	9.26

Table 3.2.16: Effect of fish oil, gender and age on IL-6

Logistic regression was used to explore the possible association between mortality and IL-6 concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and IL-6 concentration for any of the days.

IL-6 concentration, age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with 28-day mortality as summarised in table below.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.17: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

3.2.4 Anti-inflammatory cytokines

1) IL-10 (Interleukin 10)

The concentration of IL-10 did not vary significantly (p=0.35) over time in both the control and fish oil group. When fish oil was added to the model, the p value for fish oil was 0.33. This suggested that IL-6 concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.36. This suggested that IL-10 concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.71. This suggested that IL-10 concentration did not vary significantly between patients of different age. Results are summarised in table below.

Concentration	Coef.	P-value	95% CI	
Day	-1.79	0.35	-5.52	1.94
Fish oil	305.74	0.33	-304.71	916.18
Day	-1.79	0.35	-5.52	1.94
Gender	-286.40	0.36	-900.56	327.76
Day	-1.79	0.35	-5.52	1.95
Age	4.74	0.71	-20.13	29.62

Table 3.2.18: Effect of fish oil, gender and age on IL-10

Logistic regression was used to explore the possible association between mortality and IL-10 concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and IL-10 concentration for any of the days.

IL-10 concentration, age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.19: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

2) TNFR-1 (Tumour Necrosis Factor Receptor-1)

The concentration of TNFR-1 did not vary significantly over time in both the control and fish oil group other than day 1 (p=0.03). When fish oil was added to the model, the p value for fish oil was 0.17. This suggested that TNFR-1 concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.56. This suggested that TNFR-1 concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.73. This suggested that TNFR-1

concentration did not vary significantly between patients of different age. Results are summarised in table below.

Model	Coef.	P-value	95% CI	
Day	-11.61	0.97	-678.64	655.41
Fish oil	-7777.18	0.17	-18981.31	3426.94
Day	-13.58	0.97	-680.81	653.65
Gender	-3399.53	0.56	-14813.13	8014.07
Day	-20.91	0.95	-689.16	647.34
Age	-82.37	0.73	-548.13	383.38

Table 3.2.20: Effect of fish oil, gender and age on TNFR1

Mortality and TNFR1 concentration

Logistic regression was used to explore the possible association between mortality and TNFR1 concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and TNFR1 concentration for any of the days.

Fish oil (p=0.20), age (p=0.53), gender (p=0.13) and source of sepsis (p=0.81) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Fish oil	-0.86	0.20	-2.19	0.47
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.21: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

3) IL-1ra (Interleukin-1 receptor antagonist)

The concentration of IL-1ra varied significantly ($p<0.001$) over time in both the control and fish oil group other than day 13 ($p=0.31$). When fish oil was added to the model, the p value for fish oil was 0.42. This suggested that IL-1ra concentration did not vary significantly between the fish oil and control group. Similarly, when gender was added to the model, the p value for gender was 0.66. This suggests that IL-1ra concentration did not vary significantly between males and females. When age was added to the model, the p value for age was 0.47. This suggested that IL-1ra concentration did not vary significantly between patients of different age. Results are summarised in table below.

Concentration	Coef.	P-value	95% CI	
Day	-539.16	0.07	-1118.88	40.57
Fish oil	-1657.64	0.42	-5653.68	2338.39
Day	-534.64	0.07	-1114.88	45.59
Gender	-894.49	0.66	-4905.90	3116.91
Day	-512.95	0.09	-1096.38	70.48
Age	63.56	0.47	-110.44	237.57

Table 3.2.22: Effect of fish oil, gender and age on IL-1ra

Logistic regression was used to explore the possible association between mortality and IL-1ra concentration. This analysis was repeated for each individual day. No significant association was found between 28-day mortality and IL-1ra concentration for any of the days.

IL-1ra concentration, age (p=0.53), gender (p=0.13), fish oil (p=0.20) and source of sepsis (p=0.81) were not associated with 28-day mortality.

28-day mortality	Coef.	P-value	95% CI	
Age	0.02	0.53	-0.04	0.07
Gender	-1.10	0.13	-2.52	0.33
Fish oil	-0.86	0.20	-2.19	0.47

Source of sepsis	0.06	0.81	-0.41	0.53

Table 3.2.23: Effect of age, gender, fish oil and source of sepsis on 28-day mortality

3.2.5 Correlation of cytokine concentration and Organ dysfunction

Organ dysfunction was measured using maximum SOFA score (max-SOFA). This was defined as the sum of the worst scores during the ICU stay. Calculation of SOFA score is explained in results 4. Analysis was performed to explore correlation between the cytokines and max-SOFA score. Maximum SOFA score was significantly associated with the concentrations for the cytokines IL1ra (p=0.001), IL-6 (p=0.01) and TNFR1 (p<0.001).

Cytokine	Concentration	Coef.	P-value	95% CI	
IL1ra	Day	-644.12	0.03	-1217.89	-70.36
	Max SOFA	884.99	0.001	347.63	1422.35
IL6	Day	-60.66	<0.001	-92.17	-29.15
	Max SOFA	46.77	0.01	11.77	81.77
TNFR1	Day	-58.68	0.86	-726.52	609.16
	Max SOFA	2049.56	<0.001	654.46	3444.66

Table 3.2.24: Relationship of cytokines and max-SOFA score

Fish-oil group was added to the above model to explore the possible association between cytokine concentration and taking fish-oil after adjusting for time and max SOFA score. The p values for fish-oil suggest that taking fish oil was not significantly associated with the cytokine concentrations if the time and max SOFA scores were considered.

Cytokine	Concentration	Coef.	p value	95% CI	Cytokine
IL1RA	Day	-646.88	0.03	-1220.82	-72.94
	Fish oil	659.84	0.76	-3527.47	4847.15
	Max SOFA	916.20	0.002	343.58	1488.82
IL6	Day	-60.64	<0.001	-92.16	-29.11
	Fish oil	-13.69	0.92	-287.24	259.85
	Max SOFA	46.19	0.01	9.29	83.10
TNFR1	Day	-54.28	0.87	-721.93	613.37
	Fish oil	-3684.67	0.51	-14631.41	7262.07
	Max SOFA	1913.79	0.01	464.13	3363.46

Table 3.2.25: Comparison of max-SOFA score in fish oil and control groups

3.3 Analysis of complement: Changes in serum C3 component of complement in patients with abdominal sepsis

The complement system plays an important role in building up the immune response during sepsis. It consists of numerous protein molecules which are precursors and in inactive form in the blood circulation. Presence of sepsis stimulates these inactive proteins resulting in a cascade of reactions and activation of membrane attack complex. There are three activation pathways for complement, the classical, lectin and alternative (1). All the pathways generate various forms of C3 convertase which cleaves and activates C3 into C3a and C3b. This is followed by a cascade of reactions leading to formation of membrane-attack-complex (MAC). The key function of complement is to kill target cells with MAC, phagocytosis by macrophages and leucocytes and release of anaphylotoxins i.e. C3a, C4a, C5a. Depletion of C3 component has demonstrated worse outcomes.

This randomised controlled trial analysed the effect of omega-3 fish oil in 60 patients with sepsis in the Intensive Care Unit. The source of sepsis was from various sources including chest, abdomen, urinary tract and skin. From this group, a homogenous group of 20 patients with similar origin of sepsis i.e. abdominal sepsis was selected. 10 patients were from the treatment group i.e. those who received omega-3 fish oil, while 10 patients were from the control group who received standard medical treatment and no fish oil. The C3 component of complement was measured to examine the role of C3 depletion and effect of omega-3. Serum samples separated from whole blood collected at two time points were stored at -80C. Time point 1 (t1) was day 0 and time point 2 (t2) was the last day of stay on Intensive Care Unit.

Time point 1 (t1) indicated as red in graph below revealed higher levels of C3 compared to t2 for 12 out of 20 patients. The mean C3 level in these patients was 0.5mg/ml. Patient ID7 showed an increase up to 2.4mg/ml C3 during t1, but the levels reduced to a “normal” concentration at second time-point (t2). Patient ID 46 showed an increase in C3 concentration from 0.5 at t1 to 1.3 mg/ml at t2 (figure 3.3.1).

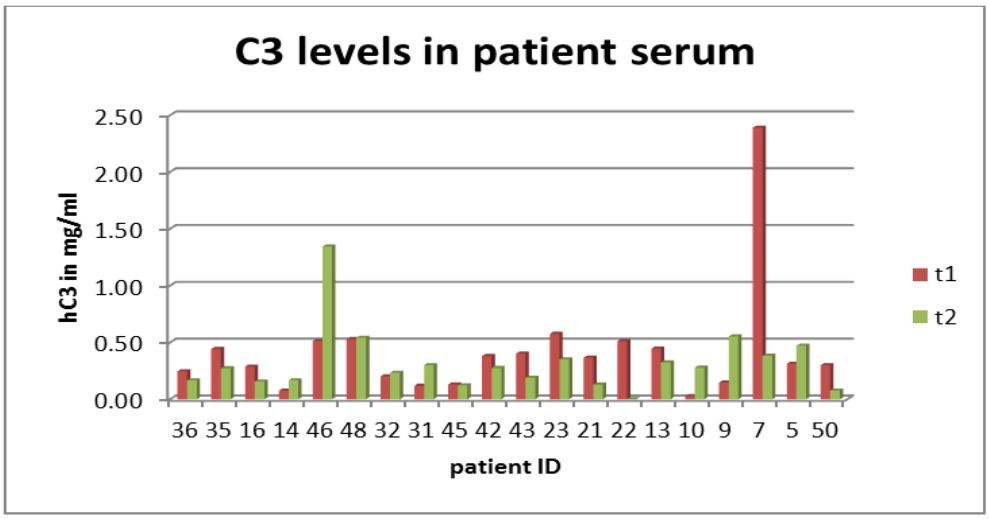

Figure 3.3.1: C3 level in 20 patients at time point 1 (red) and time point 2 (green)

All patients were found to be C3 depleted at t1 (<750mcg/ml at baseline). They were divided into two groups, group1 (N=8) were patients with lower levels of C3 at t2, group 2 (N=12) were patients with stable or higher C3 levels at t2. The two groups were compared with regards to type of organism, mortality and effect of omega-3 on mortality.

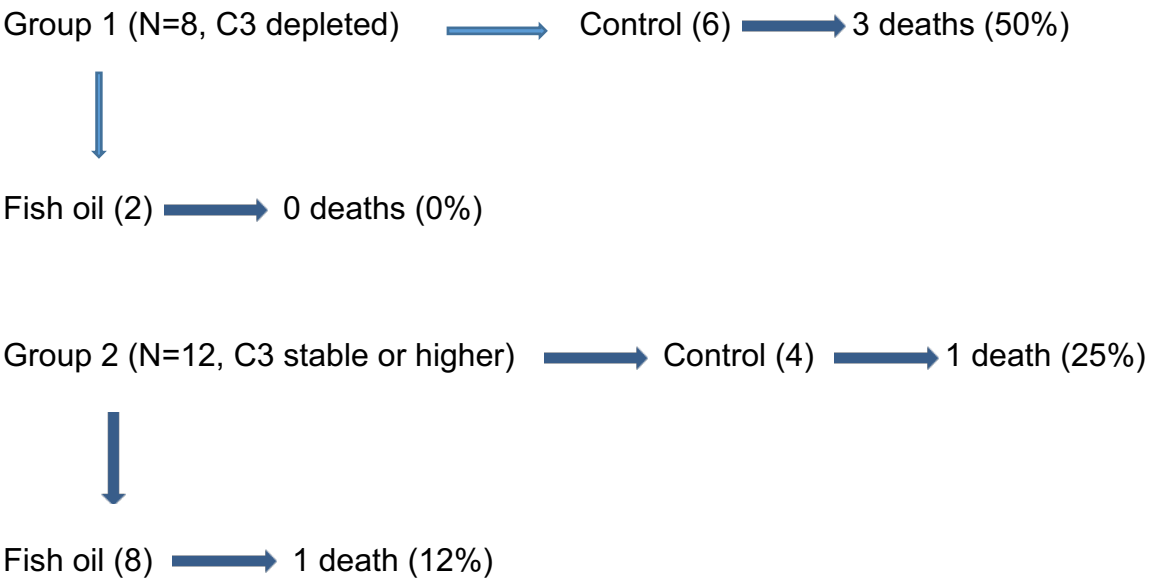

Figure 3.3.2: Flow chart demonstrating the two groups of patients

In group1, there were 3 deaths (50%) all of which were controls and had progressive depletion of C3. There were no deaths (0%) in patients receiving omega-3 in group1. In group 2, 6 of 7 patients receiving omega-3 survived (12% mortality) while 3 of 4 control patients survived (25% mortality) (figure 3.3.2).

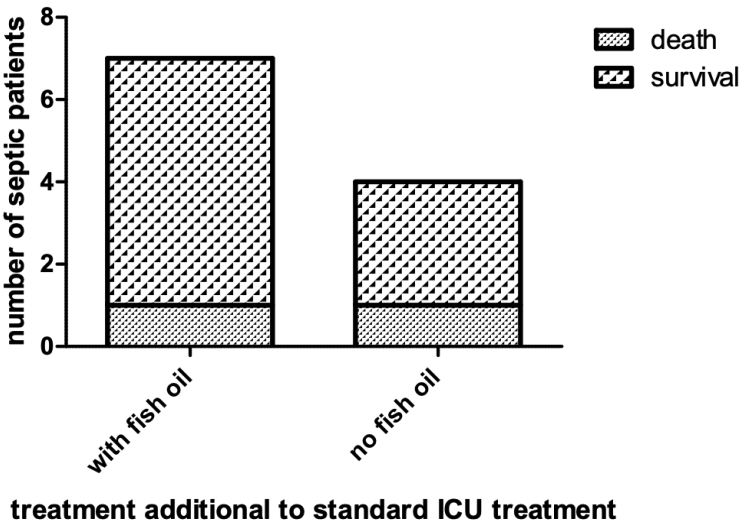

Figure 3.3.3: Survival in control and fish oil group

In group1, majority of the sepsis was due to gram negative organism whereas in group 2 it was due to gram positive organism. In group 1 with gram negative sepsis, the C3 concentrations decreased between the two time-points (Figure 1). Moreover, these patients had worse outcome measured using 28-day mortality. C3 concentrations increased between the two time-points in group 2 with gram positive sepsis. However, the other two groups (mixed pathogen and no pathogen found on blood culture) demonstrated no specific pattern.

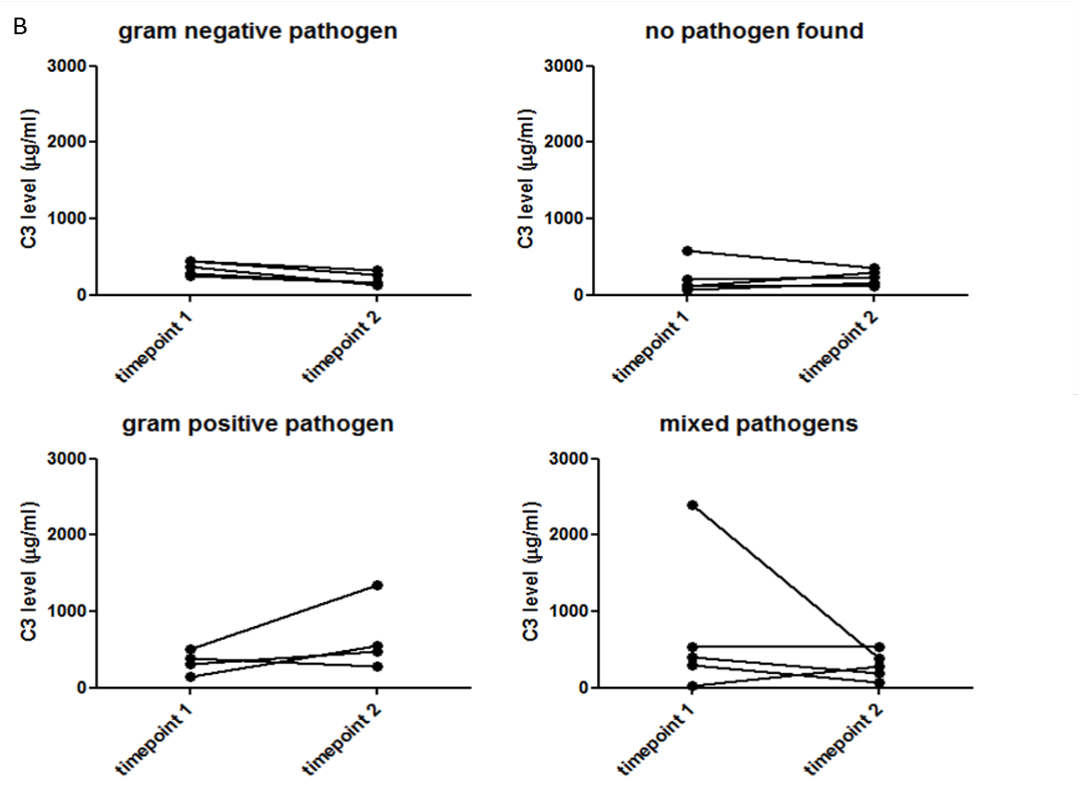

Figure 3.3.4: Correlating change in C3 level (timepoint 1 to timepoint 2) with different types of pathogens.

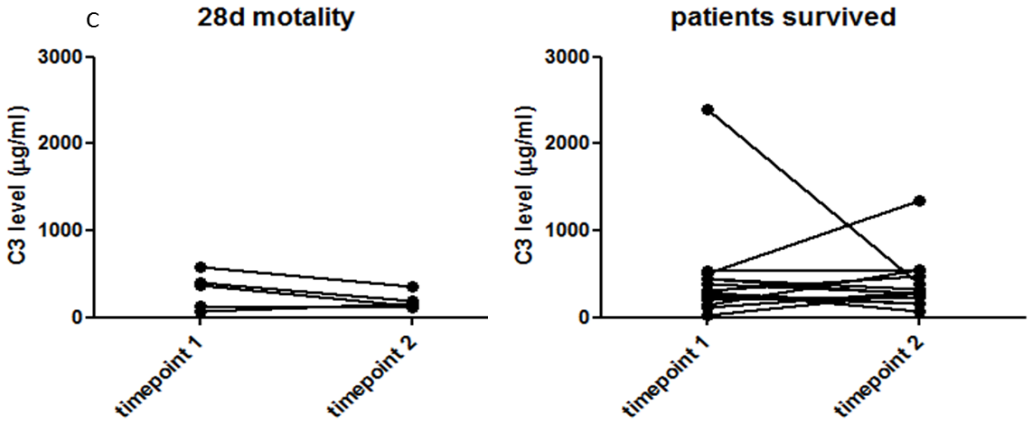

Figure 3.3.5: Correlating change in C3 level with outcome (28-day mortality)

We concluded that parenteral omega-3 reduced mortality by up to 50%. Progressive depletion of C3 was associated with poor outcome and may be used as a marker for clinical outcome.

No correlation was demonstrated between C3 level and gender (6 women and 14 men). Furthermore, no relationship was shown between the white cell count and C3 levels.

3.4 Analysis of Resolvins and Protectins

A sub-group of 20 patients with abdominal sepsis including 10 receiving fish oil and 10 controls (without fish oil but all other supportive treatment) were analysed using mixed effect linear regression. Summary of all the measured markers is as follows:

Marker	**Role- Pro/anti inflammatory**	**Result**	**p-value**
5-HETE	Pro-inflammatory	The concentration in the fish oil group was overall higher than the control group but this difference was not found to be significant	0.10
12-HETE	Pro-inflammatory	The concentration in the fish oil group was overall higher than the control group but this difference was not found to be significant	0.49
15-HETE	Pro-inflammatory	There was no significant difference between the fish oil and control groups	0.13
LTB4	Pro-inflammatory	There was a significant increase in the LTB4 concentration over time in the control group ($p<0.001$). No significant change in the LTB4 concentration over time was detected in the fish-oil group (p value- 0.1)	
LXA4	Anti-inflammatory	The concentration was higher in FO group but not statistically significant.	0.14
PGD2	Anti-inflammatory	No significant difference between the fish oil and control groups was detected	0.12
PGE2	**Pro-inflammatory**	**A significant increase was detected in the PGE2 concentration over time in the control group**	**0.001**
PGF2alpha	Pro-inflammatory	No significant difference between the fish oil and control groups.	0.10
TXB2	**Pro-inflammatory**	**A significant increase was detected in the TXB2 concentration over time in the control group**	**0.02**
Hipoxillin	Anti-inflammatory	No significant difference in between the two groups	0.74

4HDHA	**Anti-inflammatory**	**Significant difference between the fish oil and control group was detected, sign higher in FO**	**0.01**
14 HDHA	Anti-inflammatory	No significant difference was detected between the fish oil and control groups, but the conc is higher in FO group	0.75
17 HDHA	**Anti-inflammatory**	**The concentration was significantly higher in the fish oil group compared to the control group**	**0.01**
12 HEPE	Anti-inflammatory	Concentration was higher in the fish oil group although the difference was not significant	0.09
13 HODE	Anti-inflammatory	No significant difference between the two groups	0.38
15 HEPE	Anti-inflammatory	Conc is higher in the FO group but not statistically sign	0.38
18 HEPE	Anti-inflammatory	Concentration was higher in fish oil group although not significant	0.53

Table 3.4.1: Summary of resolvins & protectins

3.4.1 EPA metabolome

3.4.1.1 Markers

1) **12 HEPE (Hydroxyeicosapentaenoic acid)**

A significant reduction in the 12 HEPE concentration was detected on day 7 (p=0.05). However, no significant difference was detected between the fish oil (FO) and control group (p=0.09). Results are summarised below.

Subgroup analysis of the control and fish oil groups was performed. A significant decrease was detected in the 12HEPE concentration over time in the control group (p=0.002). No significant change over time was detected in the 12HEPE concentration in the fish oil group (p=0.27).

Group	Coefficient	P value	95% CI	
Control	-135.31	0.002	-222.51	-48.12
Fish oil	51.04	0.27	-39.92	142.00

Table 3.4.2: Comparison of 12 HEPE over time in the control and fish oil groups

2) 13HODE (Hydroxy octadecadienoic acid)

No significant change in the 13HODE concentration was detected over time. No significant change was detected between the fish oil and control group (p=0.38).

Subgroup analysis of the control and fish oil groups was performed. No significant change was detected in the 13 HODE concentration over time in the control (p=0.84) and fish oil group (p=0.78).

Group	Coefficient	P-value	95% CI	
Control	-3.64	0.84	-37.82	30.54
Fish oil	-2.74	0.78	-22.01	16.53

Table 3.4.3: Comparison of 13 HODE over time in the control and fish oil groups

3) 15HEPE (Hydroxyeicosapentaenoic acid)

No significant change in the 15HEPE concentration was detected over time. No significant change was detected between the fish oil and control group (p=0.38). Subgroup analysis of the control and fish oil groups was performed. A significant increase was detected in the 15HEPE concentration over time in the control group (p<0.001 and 95% CI 156.18 to 378.35). No significant change over time was detected in the 15HEPE concentration in the fish oil group (p=0.59).

Group	Coefficient	P-value	95% CI	
Control	267.26	0.000	156.18	378.35
Fish oil	-64.21	0.59	-298.10	169.67

Table 3.4.4: Comparison of 15 HEPE over time in the control and fish oil group

4) 18HEPE (Hydroxyeicosapentaenoic acid)

A significant increase in the 18HEPE concentration was detected between day 2 and day 0 (p=0.01 and 95%CI 1101.18 to 7180.15). No significant change was detected between the fish oil and control group (p=0.53).

Subgroup analysis of the control and fish oil groups was performed. A significant increase was detected in the 18HEPE concentration over time in the control group (p<0.001 and 95%CI 416.91 to 1183.85). No significant change over time was detected in the 18HEPE concentration in the fish oil group (p=0.64).

Group	Coefficient	P value	95% CI	
Control	800.38	0.000	416.91	1183.85
Fish oil	110.06	0.64	-352.24	572.35

Table 3.4.5: Comparison of 18 HEPE over time in the control and fish oil groups

3.4.1.2 Relationship of EPA metabolome, max SOFA score and fish oil

Linear regression showed a significant association between fish oil and max SOFA score in these patients. There was a significant difference in the max SOFA scores of the control and fish oil groups, being lower in the fish oil group ($p<0.001$).

Max SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.90	0.000	-3.80	-2.00

Table 3.4.6: Effect of fish oil on max-SOFA in EPA metabolome

3.4.1.3 Delta SOFA score and fish oil

Linear regression showed a significant association between fish oil and delta SOFA score in these patients. There was a significant difference in the delta SOFA scores of the control and fish oil groups being lower in the fish oil group ($p<0.001$).

Delta SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.60	0.000	-3.19	-2.01

Table 3.4.7: Effect of fish oil on delta-SOFA in EPA metabolome

3.4.2 DHA metabolome

3.4.2.1 Markers

1) 4HDHA (Hydroxydocosahexaenoic acid)

No significant change in the 4HDHA concentration was detected over time. However, a significant reduction in the fish oil group was detected as compared to the control group (p=0.01).

Concentration	Coefficient	P-value	95% CI	
Day 0	-	-	-	-
Day 1	8.56	0.59	-22.38	39.50
Day 2	13.19	0.40	-17.75	44.13
Day 3	-11.91	0.45	-42.85	19.03
Day 5	28.73	0.07	-2.20	59.67
Day 7	-11.12	0.49	-42.58	20.34
Day 10	16.41	0.35	-17.76	50.58
Day 13	-12.10	0.53	-49.43	25.24
Fish oil	37.21	**0.01**	10.87	63.55

Table 3.4.8: 4HDHA at 8 time points in all the patients

Subgroup analysis of the control and fish oil groups was performed. No significant change was detected in the 4HDHA concentration over time in the control (p=0.43) and fish oil group (p=0.92).

Group	Coefficient	P-value	95% CI	
Control	-0.87	0.43	-3.04	1.29
Fish oil	0.22	0.92	-3.89	4.34

Table 3.4.9: Comparison of 4 HDHA over time in the control and fish oil groups

2) 14HDHA (Hydroxydocosahexaenoic acid)

A significant reduction in the 14HDHA concentration was detected on days 3, 7 and 13 compared to day 0. However, no significant difference was detected between the fish oil and control groups (p=0.75).

Subgroup analysis of the control and fish oil groups was performed. A significant reduction was detected in the 14HDHA concentration over time in the control group (p=0.004 and 95%CI -141.38 to -25.94). No significant change was detected in the 14HDHA concentration in the fish oil group (p=0.70).

Group	Coefficient	P-value	95% CI	
Control	-83.66	0.004	-141.38	-25.94
Fish oil	-5.64	0.70	-34.20	22.92

Table 3.4.10: Comparison of 14HDHA over time in the control and fish oil group

3) 17HDHA (Hydroxydocosahexaenoic acid)

A significant increase in the 17HDHA concentration was detected on days 2, 3 and 5 compared to day 0. The concentration was significantly higher in the fish oil group as compared to the control group (p=0.01 and 95%CI 113.36 to 764.97).

Concentration	Coefficient	P-value	95% CI	
Day 0	-	-	-	-
Day 1	150.11	0.30	-134.83	435.05
Day 2	346.72	**0.02**	61.78	631.67
Day 3	334.83	**0.02**	44.37	625.29
Day 5	492.05	**0.001**	201.59	782.52
Day 7	265.46	0.08	-30.89	561.80
Day 10	209.20	0.21	-118.87	537.27

Day 13	-89.62	0.65	-476.34	297.11
Fish oil	439.17	**0.01**	113.36	764.97

Table 3.4.11: 17HDHA at 8 time points in all patients

Subgroup analysis of the control and fish oil groups was performed. No significant change was detected in the 17HDHA concentration over time in the control group (p=0.80) and fish oil group (p=0.95).

Group	Coefficient	P-value	95% CI	
Control	2.00	0.80	-13.70	17.70
Fish oil	1.43	0.95	-40.90	43.77

Table 3.4.12: Comparison of 17 HDHA over time in the control and fish oil groups

3.4.2.2 Max SOFA score and fish oil in DHA metabolome

Linear regression showed a significant association between fish oil and max SOFA score in these patients. There was a significant difference in the max SOFA scores of the two groups, lower in the FO group ($p<0.001$).

Max SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.90	0.000	-3.80	-2.00

Table 3.4.13: Effect of fish oil on max SOFA score

3.4.2.3 Delta SOFA score and fish oil

Linear regression showed a significant association between fish oil and delta SOFA score in these patients. There was a significant difference in the delta SOFA scores of the two groups, lower in the FO group ($p<0.001$).

Delta SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.60	0.000	-3.19	-2.01

Table 3.4.14: Effect of fish oil on delta SOFA score

3.4.3 AA metabolome

3.4.3.1 Markers

1) 5HETE (Hydroxyeicosatetraenoic acid)

There was significant reduction in the 5HETE concentration between day 0 and day 7 in all the patients (p=0.07 and 95%CI -133.42 to 4.92). The concentration in the fish oil group was overall higher than the control group (positive coefficient 48.36) but this difference was not found to be significant (p= 0.10).

Subgroup analysis of the control and fish oil groups was performed. There was a significant reduction of the 5HETE concentration over time in the control group (p=0.04 and 95%CI -11.52 to -0.30). No significant change in the 5HETE concentration was detected over time in the fish-oil group (p=0.77).

Group	Coefficient	P-value	95% CI	
Control	-5.91	0.04	-11.52	-0.30
Fish oil	-1.07	0.77	-8.39	6.25

Table 3.4.15: Comparison of 5HETE over time in the control and fish oil groups

2) 12HETE (Hydroxyeicosatetraenoic acid)

There was a significant reduction in the 12HETE concentration over time in both the groups. The concentration in the fish oil group was overall higher than the control group (positive coefficient 289.52) but this difference was not found to be significant (p=0.49).

Subgroup analysis of the control and fish oil groups was performed. There was a significant reduction of the 12HETE concentration over time in the control group (p=0.004 and 95%CI -191.56 to -35.52) but not in the fish-oil group (p=0.06).

Concentration	Coefficient	P-value	95% CI	
Control	-113.54	0.004	-191.56	-35.52
Fish oil	-93.33	0.064	-192.18	5.53

Table 3.4.16: Comparison of 12HETE over time in FO and C groups

3) 15HETE (Hydroxyeicosatetraenoic acid)

There was no significant change in the 15HETE concentration over time. There was no significant difference between the fish oil and control groups (p=0.13).

Subgroup analysis of the control and fish oil groups was performed. There was no significant change in the 15HETE concentration over time in the control (p=0.56) and fish oil group (p=0.54). It may not be a good marker to predict effectiveness of fish oil.

Group	Coefficient	P-value	95% CI	
Control	-7.12	0.56	-30.80	16.56
Fish oil	-10.01	0.54	-42.31	22.29

Table 3.4.17: Comparison of 15 HETE over time in the control and fish oil groups

4) LTB4 (Leukotriene B4)

There was a significant increase in the LTB4 concentration between day 0 and day 10 (p=0.001 and 95%CI 118.17 to 462.93). However, there was no significant difference between the fish oil and control groups (p=0.35).

Subgroup analysis of the control and fish oil groups was performed. There was a significant increase in the LTB4 concentration over time in the control group (p<0.001 and 95%CI 5.00 to 16.29). No significant change in the LTB4 concentration over time was detected in the fish-oil group (p=0.10).

Group	Coefficient	P-value	95% CI	
Control	10.65	0.000	5.00	16.29
Fish oil	15.96	0.10	-3.21	35.14

Table 3.4.18: Comparison of LTB4 over time in the control and fish oil groups

5) LXA4 (Lipoxin A4)

There was no significant change in the LXA4 concentration over time and no significant difference was detected between the fish oil and control groups (p=0.14).

Subgroup analysis of the control and fish oil groups was performed. There was a significant increase in the LXA4 concentration over time in the control group ($p<0.001$ and 95%CI 2.57 to 7.65). No significant change in the LXA4 concentration over time was detected in the fish-oil group (p=0.96).

Group	Coefficient	P-value	95% CI	
Control	5.11	0.000	2.57	7.65
Fish oil	0.19	0.961	-7.48	7.87

Table 3.4.19: Comparison of LXA4 over time in the control and fish oil groups

6) PGD2 (Prostaglandin D2)

A significant increase in the PGD2 concentration was detected on days 3 and 7 compared to day 0. No significant difference between the fish oil and control groups was detected (p=0.12). It should be noted that this may be due to the scarcity of the data.

Subgroup analysis of the control and fish oil groups was performed. No significant change was detected in the PGD2 concentration over time in the control group (p=0.20) and fish oil group (p=0.06).

Group	Coefficient	P value	95% CI	
Control	0.61	0.20	-0.32	1.55
Fish oil	0.26	0.06	-0.01	0.53

Table 3.4.20: Comparison of PGD2 over time in FO and C groups

7) PGE2 (Prostaglandin E2)

No significant change in the PGE2 concentration was detected over time. No significant difference was noted between the fish oil and control groups (p=0.19). It should be noted that this was probably due to the scarcity of the data.

Subgroup analysis of the control and fish oil groups was performed. A significant increase was detected in the PGE2 concentration over time in the control group (p=0.001 and 95%CI 1.29 to 5.16). However, no significant change over time was detected in the PGE2 concentration in the fish oil group (p=0.32).

Group	Coefficient	P value	95% CI	
Control	**3.22**	**0.001**	**1.29**	**5.16**
Fish oil	4.71	0.32	-4.59	14.01

Table 3.4.21: Comparison of PGE2 over time in FO and C groups

8) PGF2α (Prostaglandin F2α)

A significant increase in the PGF2α concentration was detected on days 1 and 2 compared to the baseline (p=0.004 and <0.001 respectively). No significant difference between the fish oil and control groups was detected (p=0.10).

Subgroup analysis of the control and fish oil groups was performed. A significant increase was detected in the PGF2α concentration over time in the control group (p<0.001, 95%CI 0.98 to 2.29) and fish oil group (p<0.001, 95%CI 79.17 to 90.65).

Group	Coefficient	P value	95% CI	
Control	1.63	0.000	0.98	2.29
Fish oil	84.91	0.000	79.17	90.65

Table 3.4.22: Comparison of PGF2α over time in the control and fish oil group

9) TXB2 (Thromboxane B2)

No significant change in the TXB2 concentration was detected over time. However, a significant difference between the fish oil and control group was detected (p=0.03 and 95%CI 10.20 to 161.73). This was calculated by comparing concentrations on each day in both groups.

Concentration	Coefficient	P-value	95% CI	
Day 0	0	0	0	0
Day 1	-23.54	0.66	-126.67	79.58
Day 2	-40.14	0.45	-143.26	62.98
Day 3	-30.30	0.57	-133.42	72.82
Day 5	-9.65	0.86	-114.38	95.08
Day 7	22.34	0.68	-84.08	128.76
Day 10	68.91	0.24	-46.65	184.48

Day 13	-19.63	0.76	-145.68	106.42
Fish oil	85.96	**0.03**	10.20	161.73

Table 3.4.23: TXB2 at 8 time points in all patients

Subgroup analysis of the control and fish oil groups was performed. A significant increase was detected in the TXB2 concentration over time in the control group (p=0.02 and 95% 0.81 to 9.08). No significant change over time was detected in the TXB2 concentration in the fish oil group (p=0.55 and 95%CI -13.00 to 18.70).

Group	Coefficient	P-value	95% CI	
Control	4.94	0.02	0.81	9.08
Fish oil	2.85	0.73	-13.00	18.70

Table 3.4.24: Comparison of TXB2 over time in the control and fish oil groups

10) HIPOXILLIN

A significant increase in the hipoxillin concentration was detected on days 2 and 5 compared to day 0 (p=0.001 for both). However, no significant difference between the fish oil and control group was detected (p=0.74).

Subgroup analysis of the control and fish oil groups was performed. No significant change was detected in the hipoxillin concentration over time in the control group (p=0.68).

Group	Coefficient	P-value	95% CI	
Control	0.86	0.32	-0.82	2.53
Fish oil	0	0	0	0

Table 3.4.25: Comparison of Hipoxillin over time in the control and fish oil groups

3.4.3.2 Max SOFA score and fish oil in AA metabolome

Mixed effects linear regression showed a significant association between fish oil and max SOFA score in these patients. There was a significant difference in the max SOFA scores of the two groups. The max SOFA score in FO group ($p<0.001$) was significantly lower than in the control.

Max SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.90	0.000	-3.80	-2.00

Table 3.4.26: Effect of fish oil on max SOFA score

3.4.3.3 Delta SOFA score and fish oil

Linear regression showed a significant association between fish oil and delta SOFA score in these patients. There was a significant difference in the delta SOFA scores of the two groups. The delta sofa was significantly lower in FO group ($p<0.001$).

Delta SOFA	Coefficient	P-value	95% CI	
Fish oil	-2.60	0.000	-3.19	-2.01

Table 3.4.27: Effect of fish oil on delta SOFA score

4. Discussion

This single-centre, phase II, randomised controlled trial investigated the effect of parenteral omega-3 in septic patients admitted to ICU. This study demonstrated an increase in the levels of anti-inflammatory mediators and reduction in the pro-inflammatory mediators in the FO group as compared to the control group. It also showed a beneficial effect on organ dysfunction and 28-day mortality.

4.1 Safety of parenteral fish oil

Administration of omega-3 in this study was well tolerated and was found to be safe in critically ill septic patients. This finding has been supported by the literature (76, 99). No side effects were experienced apart from one report of a 'fish-like' taste in a patient's mouth. Omega-3 was discontinued in one patient due to coagulopathy although it was thought to be secondary to consumption due to sepsis and heparin. No adverse effects secondary to omega-3 were reported in any patient. The product information regarding Omegaven states that the infusion can cause prolonged bleeding time and an inhibited platelet aggregation. Therefore, the manufacturer advised that Omegaven should be administered with caution to patients requiring anticoagulant therapy. However, several studies have reported that administration of omega-3 fatty acids was not associated with an increased risk of excess bleeding (100, 101).

The manufacturer of OmegavenTM (Fresenius Kabi) does not recommend fish oil based emulsions as a nutrition monotherapy due to theoretical concerns that fish oils may cause oxidative stress. The OmegavenTM is, however, enriched with the antioxidant α-tocopherol to counteract any oxidative risk. The other risk of using fish oil as monotherapy is the development of essential fatty acid deficiency, which (102) typically occurs when <1%–2% of total calories are provided from essential fatty acids. Studies have not, however, supported this notion (103). All patients in our study received nutrition (enteral or parenteral) as directed by the dieticians and intensivists, depending on their condition and gut function. OmegavenTM was given as monotherapy for attenuating the effects of inflammation rather than providing nutrition. Its calorific content is, in fact, negligible (112 kcal/100ml).

4.2 Dose and route of omega-3 administration

In this study, Omegaven provided parenteral DHA/EPA at 0.054-0.12 g/kg/day (equating to 0.2 g/kg/day of fish oil). Omegaven was used as this was the only product available which was pure omega-3 to avoid bias introduced by effect of other constituents. A study by Heller and colleagues demonstrated that doses of 0.1-0.2 g/kg/day were needed to significantly improve rates of survival by reducing length of ICU stay and antibiotic demand (68). Wohlmuth et al retrospectively reviewed 42 patients with abdominal sepsis who received 10g intravenous fish oil and compared them to historic controls. They failed to demonstrate any clinical benefit in the fish oil group (104). However, this study has been criticised for significant pharmacological and statistical errors (105). This is specifically regards to selection bias, inadequate

sample size, under dosing of fish oil, infusion rate being too fast, and questionable propensity adjustment. Studies have demonstrated that lung injury was aggravated with regard to oxygenation index, shunt fraction, pulmonary vascular resistance and compliance with a lipid infusion at the rate of 0.21 0r 0.22 g/kg/hour (106, 107). In the current study, Omegaven was given at the rate of 0.5ml/kg/hour and did not cause any of these potential problems.

Different routes of administration of omega-3 have been used in various studies including parenteral and enteral. There are no standard recommended doses of omega-3 that will produce beneficial effects (66). In literature, different nomenclature is used i.e. fish oil, omega-3, EPA and DHA interchangeably thus adding confusion to interpretation of results. The studies also used different formulations with varying proportions of EPA and DHA. DHA generates protectins, D-series resolvins and maresins, whereas EPA generates E-series resolvins (108). Consequently, the effects on the inflammatory cascade and patient outcome varied. All these factors could explain the heterogeneous results of various studies. In our study, omega-3 was given parenterally so we can safely exclude that the effects of omega-3 were not due to reduced absorption as may be the case in enteral feeding or reduced bioavailability of free n-3 fatty acids (e.g. through lipid remodeling in the liver) (109).

The infusion of omega-3 was continued for 14 days. The length of stay of patients on ICU was variable from an average of 8 to 12 days. To maximise the effects of omega-3 a time period of 14 days was selected although the manufacturer advised that it could be given for a maximum of 4 weeks. Unfortunately, there is no evidence regarding the pharmacokinetics of omega-3.

4.3 Effect of pathogen type on outcome

There were 3 patients in the control group with gram positive sepsis while there were 7 in the FO group. There were 10 patients in the control group with gram negative sepsis and there were 4 in the treatment arm. There was heterogeneity between both the groups but this was not statistically significant. The inflammatory mediator concentrations, organ dysfunction and pathogen type were analysed using logistic regression but no significant association was demonstrated.

4.4 Comparison of this study to literature

There was extensive variation in the results of studies analysing n-3 and the reasons were multi-factorial. Several studies have investigated the effect of combined n-3 and immune-modulating diets (arginine, glutamine). Meta-analysis conducted by Marik et al in high risk surgical patients have demonstrated reduced risk of infections, wound complications and length of stay in hospital (LOS) (110). Since these studies have included nutrients in addition to n-3, the beneficial results cannot be attributed only to n-3. Mayer et al randomised 21 septic patients in ICU to receive either n-3 or n-6 infusion for 5 days. Within 2 days of administration of n-3, the n-3/n-6 ratio was reversed. Also, the concentration of pro-inflammatory cytokines was increased in n-6 group and decreased in n-3 group. However, the effects of n-3 were reversed after the infusion was stopped (83). Sungertekin et al demonstrated that fish oil based fat emulsions might have anti-inflammatory and hepatoprotective effects in hyper inflammation caused by sepsis (111). On the contrary, Friesecke et al found that

omega 3 did not affect inflammation or clinical outcome (80).

Wohlmuth et al performed a retrospective propensity-matched cohort study (42 patients) investigating the effects of n-3 supplementation on organ failure (assessed by SOFA score) in patients with septic shock from abdominal infection. Omega-3 was given as an enteral nutrition for a maximum of 7 days. No beneficial effects of n-3 supplementation were noted on organ function, duration of mechanical ventilation, renal replacement therapy or ICU length of stay. This study was flawed with multiple pharmacological and statistical problems including no dose adjustment to patient weight, rapid infusion (over 30-60 minutes) leading to fat overload, selection bias (retrospective analysis and comparison to historic controls), inadequate sample size and questionable propensity adjustment. This nullified their conclusion of the role of FO on patient outcomes in sepsis (104, 105).

Heller et al recognised a dose-dependent reduction in mortality predicted from SAPS II score after using Omegaven in a heterogeneous group of patients including post-surgical, septic and trauma patients (68). The effects of n-3 were significant when administered in doses between 0.1 and 0.2 g/kg/day. Patients in our study received n-3 according to their body weight, 0.05 g n-3/kg body weight/hour that equals to 0.2 g/kg/d of n-3.

4.5 Effect of omega-3 on cytokines in sepsis

This study demonstrated a significantly (p=0.035) higher concentration of cytokine IL-17, a pro-inflammatory cytokine in the C group. Also, other pro-inflammatory cytokines were higher in the C group as compared to FO group. However, the levels were not significant but trending towards significance. Although inflammation is body's response to various insults, inappropriate or excessive inflammatory response can be damaging and cause multiple organ dysfunction leading to septic shock which carries a mortality risk of more than 40% (112). A reduction in IL-17 and other pro-inflammatory cytokines in FO group could prevent the above process and improve morbidity and mortality. Also, the level of anti-inflammatory cytokine, IL-10 was higher in FO group when compared to control. This could also contribute to improvement in patient outcome and potential economic impact with reduction in cost of care. This is supported by our findings that max SOFA scores for IL-1RA, IL-6 and TNFR1 were significantly associated with their concentration. There was significant association between 28-day mortality and concentration of VCAM on day 1 (p=0.05) and day 5 (p=0.03). Similarly, significant association was observed between mortality and concentration of IL-17 on day 3 (p=0.02). ICAM and mortality were associated on day 1 (p=0.05) and day 5 (p=0.05). Concentrations of VCAM, ICAM and IL-17 on days 1, 3 and 5 were predictive of worsening organ dysfunction and subsequent mortality. These findings of different patterns of cytokine profiles may be reflected by different clinical severity as stated recently (113).

Our findings correspond with observations in sepsis induced in mice by Caecal ligation and puncture (CLP). Levels of pro-inflammatory cytokines and chemokines were

reduced when treated with anti-IL-17A (114). Freitas et al observed that IL-17R-deficient mice, subjected to CLP-induced non-severe sepsis, showed reduced neutrophil recruitment into the peritoneal cavity, spread of infection, and increased systemic inflammatory response. As a consequence, the mice showed an increased mortality rate. IL-17 improved the microbicidal activity of the migrating neutrophils by a NO dependent mechanism. Thus, IL-17 played an important role in host protection during sepsis (115). Dai et al analysed the effectiveness of IL-17 concentration for predicting severity in severe acute pancreatitis and advantages of removing IL-17 by continuous veno-venous hemofiltration. They concluded that earlier and higher serum IL-17 elevation predicted prolonged hospitalization, organ failure and death, possibly by disrupting gut barrier function. CVVH could remove inflammatory cytokines from serum, including IL-17 and IL-6, thereby reducing the inflammatory response and diminishing associated systemic complications (116).

Flierl et al examined the role of IL-17 in sepsis induced in mice by caecal ligation and puncture. An improvement in survival was demonstrated (10% to 60%) after neutralisation of IL-17 by two different antibodies. The study suggested that IL-17 could be a potential therapeutic target in sepsis (114).

Similarly, in our study, cytokine IL-17 was the best predictor of patient outcome and n-3 significantly affected its concentration. In future, it may be used as a marker of patient severity and to measure the effect of n-3 on the patient. Since no single biomarker can predict outcomes with 100% accuracy, it has been suggested that a combination of biomarkers may provide better results (113). As our study demonstrated significant association between 28-day mortality and IL-17 concentration, it may be proposed to

combine IL-17 concentration and Max-SOFA as biomarkers to predict patient outcome with good accuracy. Although, it is appealing to speculate that there is direct relationship between a particular cytokine concentration and pathophysiology of organ dysfunction, we believe one cannot place the full weight of disease severity on a single cytokine.

4.6 Effect of omega-3 on complement in sepsis

This study concluded that parenteral omega-3 reduced mortality by up to 50%. Progressive depletion of C3 was associated with poor outcome and may be used as a marker for clinical outcome.

20 patients with abdominal sepsis were analysed using C3 ELISA. There were 6 females and 14 males. All patients were C3 depleted before the treatment was commenced (less than 750 mcg/ml) except at two time points for two patients that reached “normal” C3 levels (86). This could be explained with the fact that they received blood transfusion. It has been demonstrated by numerous studies that, sepsis caused reduction of C3 level (86, 117). The patients in group 2 with improvement in C3 levels at time-point 2 had 50% lower mortality. However, group 1 in whom the levels of C3 continued to decline at time-point 2 had higher mortality. Also, patients in the C3 depleted group who received omega-3 showed better outcomes with no mortality.

Similar findings were noted by Jianen Ren et al who performed a study in 45 patients with severe abdominal sepsis. They received early goal-directed resuscitation, source control and antibiotics therapy. Acute physiology and chronic health evaluation II (APACHE II) and sepsis related organ failure assessment (SOFA) scores were used to evaluate the severity. Plasma levels of C3, C4, CRP, PCT, D-dimer and other parameters were measured at eight time-points. The 28-day mortality, length of stay, and postoperative complications were compared between the complement depletion and non-complement depletion groups. They concluded that C3 depletion was associated with coagulopathy and aggravated infection during sepsis resulting in poor prognosis.

When analysing patients according to the source of sepsis, gram negative sepsis was associated with declining C3 levels and one death. However, C3 levels increased in the gram positive sepsis with no deaths. Abe and colleagues (118) investigated 259 patients in ICU over a 8 year period. They examined the type of bacteremia, its pathophysiology, and clinical outcomes. They observed that gram negative bacteremia was significantly higher in septic shock patients as compared to patients with sepsis (43% vs 22%). Moreover, CRP, IL-6 and mortality were higher in gram negative septicaemia. Better understanding of the pathophysiology of different types of bacteremia will help predict clinical outcomes and management better. No trend of C3 behavior was detected in the group, where no or mixed pathogens were found. We did not observe a correlation between the white cell count and C3 level.

4.7 Effect of omega-3 on resolvins & protectins in sepsis

Concentration of 4 HDHA (0.01) and 17 HDHA (0.01) was significantly higher in the FO group. Concentration of other anti-inflammatory markers i.e. LXA4, PGD2, Hipoxillin, 14 HDHA, 12 HEPE, 13 HODE, 15 HEPE, 18 HEPE was higher in the FO group. There was significant association between FO, max SOFA and delta SOFA score in these patients. The max SOFA and delta SOFA scores in FO group were significantly lower than C group.

Moreover, omega-3 significantly decreased PGE2 (0.001) and TXB2 (0.02) over time in the FO group. Also, the concentration of other pro-inflammatory markers i.e. 5-HETE, 12-HETE, 15-HETE, LTB4, PGF2α were higher in the C group as compared to the FO group. There was a significant association between FO, max SOFA and delta SOFA scores in these patients. The max SOFA and delta SOFA scores in FO group were significantly lower than C group. Omega-3 improved organ dysfunction in these critically ill septic patients thus influencing clinical outcome.

Resolution of inflammation is not a passive process and involves active biochemical mediators. These were named as resolvins as they were first described during the resolution phase of acute inflammation (119-122). EPA derived compounds are designated as E-series resolvins, whereas the mediators derived from DHA were called D-series resolvins and (neuro) protectins (discovered initially in the brain). At the site of inflammation, the actions of neutrophils and production of pro-inflammatory cytokines such as TNFα and IL-1 is affected by activation of NF-kB. Resolvin E1 has been found to affect cell response by reducing the activity of NF-kB(123). Furthermore,

they are able to reduce the activity of proteases and reactive oxygen species (ROS), which leads to reduced tissue injury and oedema formation (124). RvE1 and Protectin D1 are mediators that have been effective in resolving animal models of airway inflammation and colitis(123, 125). Resolvins diminish the progression and enhance the resolution of inflammation via several different mechanisms. By attenuating the expression of adhesion molecules and stimulation of endothelial nitric oxide synthetase (NOS) they reduce neutrophil extravasation and invasion to inflammatory sites (126).

A special role of resolvins takes place during the resolution phase of inflammation. Resolvins increase local recruitment of monocytes to sites of inflammation by chemotaxis. They reduce neutrophil extravasation and invasion to inflammatory sites by the expression of adhesion molecules such as E-selectin, ICAM and VCAM and by decreasing IL-1 levels(127). The subsequent uptake of apoptotic neutrophils by macrophages is therefore accelerated. At the end of the inflammatory process, resolvins facilitate the removal of macrophages via lymphatic vessels (122). Results of experiments in animals indicate that they may bring new treatment options (123, 128, 129). These treatments include those for acute lung injury (ALI) and pneumonia (130) chronic airway inflammation (131), asthma (132) or peritonitis and sepsis (126). Until recently, few clinical studies analysing the role of resolvins in patients have been published. This is the only trial analysing the effect of omega-3 on resolvins and protectins in critically ill septic patients. This study has demonstrated significant alteration in the concentrations of various pro-resolving mediators resulting in clinical benefit. It is possible that resolvins may be able to prove beneficial by accelerating the resolution of inflammation (126, 130).

4.8 Effect of fish oil on organ dysfunction in sepsis

This study demonstrated a significant reduction in the development of morbidity by improving organ dysfunction (delta-SOFA, 2.2±2.2 vs. 1.0±1.5, C vs FO, p=0.005). Sepsis is a major burden on the healthcare system both with regards to patient outcome and financially. Therefore, any improvement in morbidity and mortality is beneficial.

Nine studies (summarised in table 4.1) have investigated the effects of fish oil on critically ill patients in ICU (66, 73, 78, 80, 82, 84, 104, 111, 133). Unfortunately, the studies demonstrate conflicting results, this may be due to heterogeneity in methodology, fish oil dosing and regime. Three studies did not give a weight adjusted dose leading to frequently under-dosing the fish oil therapy (66, 82, 104). Three studies, in addition to EPA, DHA and GLA, used a treatment consisting of antioxidant vitamins (OxepaTM; Abbott Nutrition, Ohio USA) as an enteral feed meaning the effects of fish oil alone could not be determined (73, 78, 133). Other studies have used arginine in combination with fish oil (77). These results need to be interpreted with caution as although arginine may have been found to be useful in elective surgical patients, it may be harmful to critically ill patients (134). Therefore, the negative results may not be due to fish oil containing arginine but due to arginine alone. The large phase III RCT (OMEGA) study was conducted to investigate a twice daily enteral supplement containing EPA, DHA, GLA, and antioxidants in patients with acute lung injury (ALI) (135). The trial demonstrated a lack of efficacy and was stopped prematurely after recruitment of 272 of the planned 1,000 patients. The study showed

no improvement in the outcomes of death at 60 days, ventilator-free days at day 28, or ICU-free days at day 28.

Author	Year	N	Study subjects	Initial severity	Intervention						Outcome
					Name	Weight adjusted dosing (y/n)	Route	Dose (DHA and EPA)	Duration (days)	Daily regimen	
Pontes-Arruda et al	2011	106	Sepsis	APACHE II median 19.5, SOFA median 5.5	Oxepa™	Y	Enteral	Median 0.11g/kg/d (6.65g FO/d)	<7	Continuous feed	Significantly reduced new organ failures
Khor et al	2011	28	Sepsis	APACHE II median 16.3-19.3	Omegaven™	N	IV	Mean 0.05-0.11g/kg/d (0.18+/-0.04g FOkg/d)	5	Infused over 6 hours every day	Significantly reduces procalcitonin, APACHE II on day 3,5,7
Grau-Carmona et al	2011	132	Sepsis and ALI or ARDS	APACHE II median 19, SOFA median 9	Oxepa™	Y	Enteral	0.09g/kg/d (6.65g FO/d)	As indicated, median 11	Continuous feed	Non significant trend towards reduced SOFA
Wohlmuth et al	2010	71	Abdominal sepsis	SAPS II median 37-40	Omegaven™	N	IV	0.12g/kg/d	<7	Infused over 30-60 minutes as bolus	No differences
Sungurtekin et al	2011	40	SIRS and sepsis	APACHE II median 19.5-20.5	Omegaven™	Y	IV	0.16-0.35g/kg/d (0.6g FO/kg/d)	7	Continuous feed	Significant reduction in day 7 TNF-α, IL-1 and IL-6
Friesecke et al	2008	160	SIRS and sepsis	SAPS II mean 49-54	Omegaven™	Y	IV	0.1g/kg/d	7	Continuous feed	No difference in inflammatory or clinical outcomes
Barbosa et al	2010	23	SIRS and sepsis	SOFA mean 8.6-9.5	Lipoplus™	Y	IV	0.09+/-0.02 g/kg/d	5	Continuous infusion	Significant reduction in IL-6, IL-10 and improved respiratory function

Pontes-Arruda et al	2006	103	Severe sepsis or shock	SOFA mean 8.6-8.8	Oxepa™	Y	Enteral	0.11g/kg/d (6.65g FO/d)	As indicated	Continuous feed	Improved oxygenation, reduced ventilator free days and new organ dysfunction
Mayer et al	2003	21	Sepsis	APACHE II 15.2-19.6	Omegaven™	N	IV	0.13-0.3g/kg/d (9.4-20.7g FO/d)	5	3x6 hour infusions per day	Enhanced proinflammatory cytokine profile in control group

Table 4.1: Studies investigating the effects of omega-3 fish oil in sepsis

4.9 Concerns regarding the detrimental effects of high EPA and DHA in sepsis

The study showed that there was a trend for improved survival outcomes with a low AA/(EPA+DHA) ratio, a finding that is supported by similar studies (84, 136). The mechanisms by which omega-3 produces this effect has already been discussed above. This is a combination of reducing the pro-inflammatory and increasing the anti-inflammatory response. Although, inflammation is the basis of numerous diseases, acute inflammation is an essential protective host response against the inciting agents. Therefore, any impairment of immune function may lead to secondary infections and delayed pathogen clearance.

4.9.1 Impaired immunological function

Some animal studies have shown negative results due to alteration of innate immunity to bacterial, viral and fungal pathogens. Others have demonstrated a variable response i.e. improve or impair host response following EPA and DHA supplementation depending on the pathogen type (137). Animal studies have shown delayed clearance of various organisms including influenza virus, mycobacterium tuberculosis and Salmonella enteritidis and increased bacterial load of Listeria monocytogenes as well as reduced wound healing (138-142). Other studies have demonstrated a pro-inflammatory response with EPA and DHA supplementation due to suppression of T cell activation and increased B cell activation. However, Virella et al demonstrated that omega-3 reduced the function of both T and B cells in humans (143). Fenton et al describe that manipulation of inflammation by omega-3 administration can influence the acute inflammatory response

to pathogens by changing the dynamics of inflammation to clear pathogens (137). However, the above findings of impairment of immunity were not demonstrated in this study as there were no secondary infections or delayed stay in ICU in the patients receiving omega-3.

4.10 Strengths of this study

4.10.1 Mode of administration of omega-3

The biggest strength of the study is in the methodology. Omega-3 was used in its purest form with no other additives. It was given parenterally so there was no doubt of effect of absorption of omega-3 if the gut barrier was affected due to sepsis in these unwell patients. It was administered within 12 hours of diagnosis of sepsis. The timing was of particular importance since the immunological effects after a single infusion fade within 24 hours (144-146). In addition, Omegaven was given according to the weight of the patient as it has shown by previous studies to demonstrate a clinical effect (68).

4.10.2 Single-centre trial

The patients were recruited from a single centre, therefore minimising the heterogeneity of the test population. Also, practices for the withdrawal of life support, which varies between centres, is also minimised by a single-centre study and therefore reduced bias. Although, single centre recruitment has advantages with regards to eliminating bias, the

recruitment of large numbers within a reasonable amount of time becomes difficult and may introduce 'investigator fatigue'.

4.11 Limitations of this study

There are several limitations of our study. This study included 60 patients, 30 in each group, which is a small size for the heterogeneous nature of the patient population admitted to a typical ICU. The sub-groups were too small to perform sub-group analysis. Omega-3 was given via parenteral route in the dose of 0.2 g/kg/d that is comparable to the dose used by Heller and colleagues to demonstrate clinical benefit (68). Also, n-3 was only given for a few hours a day in our patients, usually four hours. It is not known what the pharmacokinetic of Omegaven is and whether the level of n-3 falls below therapeutic levels in between doses. The manufacturer recommended that Omegaven should be given for a maximum of four weeks. All the patients in our trial received it for a maximum of 14 days or less as patients spent different periods of time in ICU depending on the severity of their clinical condition. The outcome measures were concentrations of various cytokines, resolvins, protectins, complement and organ dysfunction scores that are all objective. Also, intensivists who were independent of the study managed the medical treatment of all patients. Therefore, the theoretical risk of performance and assessment bias is minimal. This study was not blinded. Using another white emulsion as control is not straightforward. In the past, n-6 lipid emulsion has been used as a control in some studies. Omega-6 is a precursor of arachidonic acid, which is pro-inflammatory and will cause worsening of the clinical condition of patient (83). Use of n-6 has been criticised in septic patients and there is no other suitable white emulsion that can be used as control.

4.12 The future….

This study concluded that omega-3 is safe in critically ill septic patients. It was given as a once daily infusion based on the patient's weight instead of an infusion over 24 hours, there was no "trough effect" observed with this protocol. The infusion was given for a maximum of 14 days or less if patient left ICU earlier. Therefore, the "washout" effect remains unknown. May be, a future study should administer omega-3 during the entire stay on ICU. FO was given within 12 hours of admission to ICU with sepsis or onset of sepsis in ICU. This early administration has demonstrated beneficial effect. The question still remains unanswered is that whether patients would benefit more from an infusion prior to the onset of critical illness and ICU admission. Further studies may investigate the role of providing early parenteral FO to ward based patients who develop adverse signs of sepsis, perhaps incorporating the early warning score (EWS), to investigate if that prevents disease progression, organ dysfunction and requirement for ICU admission. There would, however, be logistical issues relating to costs and central line access in those patients without severely altered physiology.

The study was not powered to detect a significant difference in mortality. The advantages of using morbidity as a primary outcome has already been discussed, although mortality is frequently a preferred and more easily accepted outcome. Future studies should be powered to detect differences in mortality together with adequate subgroup analysis. However, this would probably require a multi-centre trial. The current trial was not blinded since there was no suitable placebo available. A future study should include an inert placebo. The results of this study certainly suggest that a multi-centre trial is warranted and may potentially answer some of the unanswered questions.

4.13 Conclusion

This study demonstrated that administration of n-3 was safe in critically ill septic patients in ICU. It was associated with significant reduction in pro-inflammatory cytokines. Cytokine IL-17 was the best predictor of patient outcome and its concentration was significantly affected by n-3. In future, it may be used as a marker of patient severity and to measure the effect of n-3 on the patient. Deficiency of C3 is associated with worse outcome of septic patients. This study has demonstrated that n-3 altered the concentrations of various pro-resolving mediators significantly resulting in clinical benefit. It is possible that resolvins may be able to prove beneficial by accelerating the resolution of inflammation.

The reason for high morbidity and mortality is the hyperinflammation causing multi-organ dysfunction. Therefore, there is need for a multi-centre randomised controlled trial investigating the role of n-3 on uncontrolled inflammation that is adequately powered, well conducted and aims to consolidate all the potential positive treatment effects. Also, studies need to define the optimal and safe dose range of n-3 to produce the desired clinical benefit. Since the population of ICU patients included in this trial is heterogeneous (source of sepsis, severity of sepsis) the results can be applied to a typical set of ICU.

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Appendix

1. Research Ethics Committee approval

South East Research Ethics Committee
South East Coast Strategic Health Authority
Preston Hall
Aylesford
Kent
ME20 7NJ

Telephone: 01622 713048
Facsimile: 01622 885966

23 November 2009

Mr Ashley Dennison
Consultant Pancreatic and Hepatobiliary surgeon
University Hospitals of Leicester NHS Trust
Leicester General Surgery
Gwendolen Road
Leicester
LE5 4PW

Dear Mr Dennison

Study Title:	**Randomised controlled trial of the effects of parenteral fish oil emulsion upon survival outcome of critically ill patients with sepsis in the intensive care unit**
REC reference number:	**09/H1102/111**
Protocol number:	**1**
EudraCT number:	**2009-016880-13**

The Research Ethics Committee reviewed the above application at the meeting held on 11 November 2009. Thank you for attending to discuss the study.

Ethical opinion

The committee stated that the process of consent in this study had been defined clearly. They went on to ask what could be done to maximise potential participants' capacity to consent.

You replied that the care team would often recognise when patients were deteriorating and were, therefore, likely to be admitted to the ITU. In these cases, the researchers would take the opportunity to speak to the potential participants before they were admitted to the ITU and gain consent at that point. It was stressed that the fish oils are most valuable if delivered early on in the process.

The committee asked what happens when the participants leave the ITU and their fish oil infusion stops.

You stated that you would discuss oil supplements with the participants and that the study would be able to provide oral omega 3 oil capsules for a limited period. It was suggested that

2. Omegaven details

1. NAME OF THE MEDICINAL PRODUCT

Omegaven emulsion for infusion

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

100 ml emulsion contain:

Highly refined fish oil	10.0 g
containing	
eicosapentaenoic acid (EPA)	1.25 - 2.82 g
docosahexaenoic acid (DHA)	1.44 - 3.09 g
myristic acid	0.1 - 0.6 g
palmitic acid	0.25 - 1.0 g
palmitoleic acid	0.3 - 0.9 g
stearic acid	0.05 - 0.2 g
oleic acid	0.6 - 1.3 g
linoleic acid	0.1 - 0.7 g
linolenic acid	≤0.2 g
octadecatetraenoic acid	0.05 - 0.65 g
eicosaenoic acid	0.05 - 0.3 g
arachidonic acid	0.1 - 0.4 g
docosaenoic acid	≤0.15 g
docosapentaenoic acid	0.15 - 0.45 g
dl-α-Tocopherol (as antioxidant)	0.015 - 0.0296 g

Glycerol	2.5 g
Purified egg phosphatide	1.2 g

Total energy:	470 kJ/100 ml = 112 kcal/100 ml
pH value:	7.5 to 8.7
Titration acidity:	< 1 mmol HCl/l
Osmolality:	308-376 mosm/kg

For excipients, see section 6.1.

3. PHARMACEUTICAL FORM

Emulsion for infusion
White homogenous emulsion

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Parenteral nutrition supplementation with long chain omega-3-fatty acids, especially eicosapentaenoic and docosahexaenoic acid, when oral or enteral nutrition is impossible, insufficient or contraindicated.

4.2 Posology and method of administration

Posology

Daily dose:
1 ml up to max. 2 ml Omegaven/kg body weight
= 0.1 g up to max. 0.2 g fish oil/kg body weight
= 70 ml up to max. 140 ml Omegaven for a patient with a body weight of 70 kg.

Maximum infusion rate:
The infusion rate should not exceed 0.5 ml Omegaven/kg body weight/hour corresponding to
0.05 g fish oil/kg body weight/hour.

The maximum infusion rate should be strictly adhered to, otherwise a severe increase in the serum triglyceride concentration can be observed.

Omegaven should be administered simultaneously with other fat emulsions. On the basis of a recommended total daily lipid intake of 1 - 2 g/kg body weight, the fish oil portion from Omegaven should constitute 10 - 20% of this intake.

Method of administration

For infusion via central or peripheral

vein. Containers should be shaken

before use.

When Omegaven is to be administered with other infusion solutions (eg amino acid solutions, cabohydrate solutions) via a common infusion line (by-pass, y-tube), the compatibility of the solutions/emulsions used must be ensured.

Duration of administration

The duration of administration should not exceed 4 weeks.

4.3 Contraindications

Severe haemorrhagic disorders.

Certain acute and life-threatening conditions such as:
- collapse and shock
- recent cardiac infarction
- stroke

- embolism
- undefined coma status

Due to lack of experience Omegaven should not be administered in patients with severe liver or renal insufficiency.
Omegaven should not be used in premature infants, newborns, infants and children due to limited experience.

General contra-indications for parenteral nutrition:
- hypokalaemia
- hyperhydration
- hypotonic dehydration
- unstable metabolism
- acidoses

Omegaven should not be administered to patients known to be allergic to fish or egg protein.

4.4 Special warnings and precautions for use

Omegaven should be given with caution to patients with an impaired lipid metabolism and uncontrolled diabetes mellitus.

The serum triglyceride level should be monitored daily. Checks of blood glucose profiles, acid base metabolism, serum electrolytes, fluid balance, blood count and bleeding time in patients treated with anticoagulants must be carried out regularly. The serum triglyceride concentration should not exceed 3 mmol/l during the infusion of fat emulsions.

4.5 Interaction with other medicinal products and other forms of interaction

The infusion of Omegaven can cause a prolonged bleeding time and an inhibited platelet aggregation. Therefore, Omegaven should be administered with caution to patients requiring anticoagulant therapy even with regard to a possible reduction of anticoagulants.

4.6 Pregnancy and lactation

There is no evidence on the safety of this medicine during pregnancy or breastfeeding. This medicine should not be used during pregnancy and breastfeeding.

4.7 Effects on ability to drive and use machines

Not applicable

4.8 Undesirable effects

The infusion of Omegaven can lead to a prolonged bleeding time and an inhibited platelet aggregation. In rare cases patients may experience a fishy taste.

- Undesirable effects observed during the administration of fat emulsions:
- slight rise in body temperature
- heat sensation and/or cold sensations
- chills
- flush or cyanosis

- lack of appetite, nausea, vomiting
- dyspnoea
- headache, pain in the chest, back and loins, bone-pain
- priapism (in very rare cases)
- increase or decrease in blood pressure
- anaphylactic reactions (e.g. erythema)

Possible signs of metabolic overload must be observed. The cause may be genetic (individually different metabolisms) and with respect to different previous illnesses with varying rapidity and following different doses, but has been observed mainly with the use of cottonseed oil emulsions.

- Metabolic overload might give the following symptoms:
- hepatomegaly with or without icterus
- a change or reduction of some coagulation parameters (e.g. bleeding time, coagulation time, prothrombin time, platelet count)
- splenomegaly
- anaemia, leucopenia, thrombocytopenia
- bleedings and tendency to bleed
- pathological liver function tests
- fever
- hyperlipidaemia
- headache, stomach pains, fatigue
- hyperglycemia.

Should these side-effects occur or should the triglyceride level during lipid infusion rise above 3 mmol/l, the lipid infusion should be stopped or, if necessary, continued at a reduced dosage.

4.9 Overdose

Overdose leading to fat overload syndrome may occur when the triglyceride level during lipid infusion rises above 3 mmol/l, acutely, as a result of too rapid infusion rate, or chronically at recommended rates of infusion in association with a change in the patient´s clinical condition
e.g. renal function impairment or infection.

Overdosage may lead to side-effects (see 4.8).

In these cases, the lipid infusion should be stopped or, if necessary, continued at a reduced dosage. The administration of fat also has to be stopped if a marked increase in blood glucose levels occur during infusion of Omegaven. A severe overdosage of Omegaven without simultaneous administration of a carbohydrate solution, may lead to metabolic acidosis.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Emulsion for parenteral nutrition ATC-Code: BO5BA
The long-chain omega-3 fatty acids in Omegaven are partly incorporated in plasma and tissue lipids. Docosahexaenoic acid is an important structural element in membrane phospholipids, while eicosapentaenoic acid is a precursor in the synthesis of a special class of eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other lipid mediators). Increased synthesis of these eicosapentaenoic acid-derived mediator substances may help promote antiaggregatory, and anti-inflammatory effects, and is associated with immunomodulatory effects.

The glycerol contained in Omegaven is designed for use in energy production via glycolysis or is re-esterified together with free fatty acids in the liver to form triglycerides.

Omegaven also contains egg phospholipids, which are hydrolysed or incorporated into the cell membranes, where they are essential for the maintenance of membrane integrity.

5.2 Pharmacokinetic properties

The lipid particles infused with Omegaven are similar in size and elimination to physiological chylomicrons. In healthy male volunteers, a triglyceride half-life for Omegaven of 54 minutes has been calculated.

5.3 Preclinical safety data

Preclinical data reveal no special hazard for humans based on conventional studies of acute and repeated dose toxicity, safety pharmacology and genotoxicity. Animal studies to evaluate the *reproductive* toxicity have not been conducted.

Sensitisation tests

In a test in guinea pigs (Maximisation test) Omegaven showed moderate dermal sensitisation. A systemic antigenicity test gave no indication of evidence of anaphylactic potential of Omegaven.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Sodium oleate, sodium hydroxide, water for injections

6.2 Incompatibilities

Incompatibilities may occur through the addition of polyvalent cations, e.g.

calcium, especially when combined with heparin.

6.3 Shelf-life

a) Shelf life of the medicinal product as packaged for sale:

18 months

b) Shelf life after dilution or reconstitution according to directions:

Chemical and physical in-use stability of mixtures containing Omegaven has been demonstrated for 24 hours at 25 ºC and data is available from the manufacturer. From a microbiological point of view, mixtures with fat emulsions or fat emulsions containing fat- soluble vitamins should be used immediately. If not used immediately, in-use storage time and conditions prior to use are the responsibility of the user. Only if compounding has taken place in controlled and validated aseptic conditions can storage conditions be based on the manufacturers stability data. From a microbiological point of view, mixtures compounded in uncontrolled and unvalidated conditions should normally be used within 24 hours, including the infusion time (see 6.6 for further information).

c) Shelf life after first opening the container:

Omegaven should be used with sterile transfer equipment immediately after opening. To be used immediately after breaking the vial seal.

6.4 Special precautions for storage

Do not store above 25 °C. Do not freeze.

6.5 Nature and content of container

Packs containing 10 glass vials with 50 or 100 ml emulsion

Glass bottles (type II,
colourless) Bromobutyl rubber
stoppers.

6.6 Instructions for use and handling, and disposal (if appropriate)

Containers should be shaken before use.
Use only if the emulsion is homogeneous and the container is undamaged.
Non-phthalate containing equipment should be used for administration wherever possible. Any portions of contents as well as mixtures remaining after use should be discarded.

Omegaven may be aseptically mixed with fat emulsions as well as fat-soluble vitamins. When simultaneously administered with other fat emulsions admixed or diluted before administration (see 6.2 and 6.3.for further information), the fish oil

portion from Omegaven should constitute 10-20% of the total daily lipid intake.

7. MARKETING AUTHORISATION HOLDER

Fresenius Kabi Deutschland
GmbH 61346 Bad Homburg
v.d.H. Germany
Telephone: +49 / 61 72 / 6 86 - 0

This Product Information is valid and approved only in countries within the European Union. The Product Information applicable in your country may differ from this version. For detailed information please refer to the local/national Fresenius Kabi affiliate.

3. Patient consent form

Consent Form: Patient

Trial Number: UHL 10838

Patient number:……...

Patient name:……

Title of Project:
Randomised controlled trial of the effects of parenteral fish oil emulsion upon survival outcome of critically ill patients with sepsis in the intensive care unit

Principal Investigator: **Mr Ashley Dennison** **Please initial box**

1. I confirm that I have read and understand the information sheet dated 20/03/2011, version 3 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from Departments of Surgery and Critical Care, Leicester General Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐
5. I agree to allow the researchers to inform my General Practitioner about my participation in the study ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Researcher	Date	Signature

One copy for patient; 1 copy for researcher and 1 copy to be kept in patient's hospital note

4. Patient information leaflet

Patient information leaflet
Version 5 13.07.2011

Randomised controlled trial of the effects of parenteral fish oil emulsion upon survival outcome of critically ill septic patients in intensive care unit

Trial Number: UHL-10838
Principle Investigator: Mr Ashley Dennison
Consultant General and Hepatopancreaticobiliary Surgery

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The purpose of this trial is to investigate the potential benefits of an intravenous infusion of omega-3 fatty acids, found in fish oils in patients with sepsis (severe infection) who are requiring treatment on the intensive care unit. Intravenous infusion is where a small plastic tube is placed in one of your veins (commonly known as a 'drip') and the solution or drug is passed into your veins through this tube.

Why have I been chosen?

You have been chosen because you are not well enough to receive treatment on the normal ward and a higher level of care and sophisticated monitoring is required. We wish to investigate the beneficial effects of fish oils on patients with severe infection requiring intensive or high dependency care.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

Omega 3 fatty acids and vegetarians

Omega 3 fatty acids are extracted from fish, if you are a vegetarian you may not wish to enrol in the study as you will receive a product produced from fish.

What will happen to me if I take part?

If you agree to take part in this trial, we will begin by reviewing your blood tests and your patient notes to check that it would be safe for you to enter the trial. Assuming everything is fine, we will then commence the infusion of omega-3 fish oil via the drip (a drip consisting of a bag of fluid that is attached to the plastic tube in your neck or your arm and slowly flows in). It is important to note however, that this is not part of routine treatment on the intensive care unit; in other words, patients not in clinical trials do not usually receive this treatment.

This trial is what is called a 'randomised' trial. In order to identify the effects of the fish oils, we need to compare the standard clinical care in conjunction with omega-3 fish oil infusion against the standard clinical care alone. Prior to omega-3 infusion commencement, you will have been randomly selected to receive the fish oil solution, or no fish oil infusion, much like tossing a coin. It is important to know that this will not affect the standard clinical care for your condition.

The omega 3 fish oil intravenous infusion will be for around 3-4 hours, depending on your weight, daily for a maximum of 14 days or till you are well enough to be discharged from the ITU/HDU, much like many other drugs given by the vein on the intensive care unit. Generally, patients in the intensive care unit will have daily and frequent blood tests as part of the routine clinical care. Research team will record these tests and will obtain extra blood samples specific for the above trial on days 0,1,2,3,5,7,10 and 14 of the omega 3 fish oil infusion or equivalent days if you are not receiving the omega-3 fish oil infusion. Should any of these routine results be abnormal, measures will be taken to correct it, which may include stopping the intravenous omega-3 fish oil. Separate blood samples will be taken for analysis at a later date to assess the effect of the **fish oil on your body (about 5 tea-spoons of blood).**

If you are chosen to receive fish oil we may also ask your permission to carry out a further blood test on days 21, 28 and 52 to see how much fish oil remains in the body when treatment had ceased. This is entirely voluntary.

We may also ask your permission to carry out an ultrasound of a blood vessel in your arm. This is totally painless and lasts about 5 minutes a day on 4 occasions. This will give us useful information on the blood vessels ability to prevent fluid leakage (as is often a consequence of severe infection).

Once you are well enough to be discharged back to a 'normal' ward from the intensive care unit the trial and the infusion will stop. If you wish to continue with fish oil therapy, appropriate dietary advice can be provided, aiming to maintain a potentially beneficial **omega-3/omega-6 ratio**. There is no firm evidence that oral supplements of Omega 3 fatty acid will benefit you directly.
The team looking after your care on the ward will continue with your routine medical care and decide whether you need any further treatment for your condition and when you will be well enough to be discharged home. A flow chart is given at the end of this booklet showing the pathway through the trial.

What do I have to do?

If after you have read this information and discuss the trial with one of our investigators, you wish to take part, then we will ask you to sign a consent form saying that you understand the potential benefits and risks of the trial. We will then take some blood tests and examine you to ensure that it is safe for you to enter the trial. If it is safe, you will receive the fish oil infusion for the rest of your treatment period on the intensive care unit.

What is being tested?

The substance that is being tested is omega-3 fish oil known as omega-3 fatty acids. These are fats found in fish oils. Our bodies cannot make these fats but they are essential to a balanced diet; therefore we must gain them from our diet. They are found in preparations like cod liver oil tablets. We can give higher doses of these fatty acids, which are potentially beneficial, through a drip. This lipid formulation contain 10% fish oil with a high percentage of w-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

What are the side effects of any treatment received when taking part?

There are very few side effects of the treatment in this trial. Omega-3 fatty acids have been shown to be a safe nutritional supplement, which is why preparations like cod liver oil are available in the supermarkets and pharmacies. The possible risks in this trial are that you may find that you are allergic to the omega-3 fatty acids. It also can have effects on other salt and sugar levels in your blood, which is why you will have daily blood tests. It is also important to monitor the fat levels in your blood to ensure that they do not rise to high, as this may affect some of the organs in your body including your liver. It may also make you feel a bit nauseous, may reduce you appetite and may affect your blood pressure. It may also cause some breathing problems and can cause priapism (painful erection) in males. All of these side effects are extremely uncommon.

What are the possible disadvantages and risks of taking part?

The potential risks of this trial are virtually identical to those that would occur should you not participate in this trial, aside from the potential side effects of omega-3 listed above, which are very uncommon. The insertion of the tube for the infusion into the veins also has some small risks including infection and bleeding, but you would have one of these tubes placed as part of your care on the intensive care unit.

What are the possible benefits of taking part?

Omega-3 fatty acids are components of the membranes surrounding the cells in the body and are important in controlling production of certain chemicals called cytokines. These cause inflammation (similar to when you bang your arm or get bitten by an insect and it becomes red, warm, painful and swollen). When you are unwell enough to require care on the intensive care unit your body produces an excess of these inflammatory cytokines, often contributing to how unwell you are. Omega-3 fish oils have been shown to have beneficial anti-inflammatory effects. They have also been shown to have other benefits in patients undergoing surgery, including reducing infection rates, reducing the need for antibiotics, reducing the need for re-operation, and reducing the risk of blood clots in the leg and lung (deep vein thrombosis (DVT) and pulmonary embolism). They have also been shown to reduce stay in hospital after operation by up to 7 days and to reduce the death rate following major surgery.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

What happens when the research study stops?

The study will stop when you are well enough to be discharged from the high dependency unit. Your medical care will then continue on one of the wards in the hospital. When you have recovered from your illness, you will be discharged home from hospital as per normal care. Upon discharge home, you will proceed with standard clinical management of your condition. If you wish to continue with fish oil therapy, knowing the lack of firm evidence that it will benefit you directly, a dietary advice can be provided aiming to increase the amount of omega 3 fatty acids in your body. We may contact you or your General Practitioner in future for follow up from the study.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms would be available to you.

Will my taking part in this study be kept confidential?

All information, which is collected about you during the course of the research will be kept strictly confidential. Any information about you, which leaves the hospital/surgery will have your name and address removed so that you cannot be recognised from it.

We would like to notify your own GP of your participation in this trial. This may be important for them to know should they alter any medication that you are on. We ask for your permission to do this.

What will happen to the results of the research study?

The results of the study will be fed back to you and the other participants. Should the treatment be beneficial, the results may be shown to other patients in your condition and treatment be offered to them. The results may also be reported in medical journal and at conference presentations to educate other medical professions. All information will be strictly confidential.

Who is organising and funding the research?

The Department of Surgery at the Leicester General Hospital are organising and funding the study with help from the pharmaceutical company, Fresenius Kabi.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises or facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits and that you have been given sufficient information on which to make an informed decision.

Contact for Further Information

If you would like any further information, please feel free to contact us. The contact details are given below.

Finally, thank you very much for reading this information. After you have read this information, if you would like to participate in the project, we will ask you to sign a consent form. We will give you a copy of the signed consent form and this information sheet to keep.

Mr. Ashley Dennison

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Flow-chart documenting progress through the trial:

Identified as suitable for trial on admission to ITU/HDU

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Detailed discussion with trial Investigator after being given Patient information sheet

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Participant agrees to enter trial and gives written consent, or if patient too unwell to give direct consent ascent will be sought from relative/next of kin (legal representative) or from professional legal representative

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Detailed history taken, participant examined and blood tests taken to confirm eligibility for trial

↓

Tube inserted into the vein (if not already done as routine on admission to ITU) and intravenous omega-3 fish oil solution started

↓

Blood tests on days 0,1,2,3,5,7,10 and 14

↓

Daily omega-3 fish oil infusion for a maximum of 14 days or till patient is well enough to be discharged from ITU/HDU

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Patient discharged back to hospital ward for continuing medical care