

Original Article

Long-chain omega-3 fatty acids in aneurysmal subarachnoid hemorrhage: A randomized pilot trial of pharmaconutrition

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Abstract

Background: Functional recovery after aneurysmal subarachnoid hemorrhage (SAH) remains a significant problem. We tested a novel therapeutic approach with long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) to assess the safety and feasibility of an effectiveness trial.

Methods: We conducted a multicentre, parallel, randomized, open-label pilot trial. Patients admitted within 72 hours after SAH with modified Fisher scale scores of 3 or 4 who were selected for scheduled aneurysm clipping were allocated to receive either n-3 PUFA treatment (parenteral perioperative: 5 days; oral: 8 weeks) plus usual care or usual care alone. Exploratory outcome measures included major postoperative intracranial bleeding complications (PIBCs), cerebral infarction caused by delayed cerebral ischemia, shunt-dependent hydrocephalus, and consent rate. The computed tomography evaluator was blinded to the group assignment.

Results: Forty-one patients were randomized, but one patient had to be excluded after allocation. Twenty patients remained for intention to treat analysis in each trial arm. No PIBCs (95% confidence interval [CI]: 0.00 to 0.16) or other unexpected harm were observed in the intervention group (IG). No patient suspended the intervention due to side effects. There was a trend towards improvements in all benefit-related outcomes in the IG. The overall consent rate was 0.91 (95% CI: 0.78 to 0.96), and there was no consent withdrawal.

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Conclusions: Although the balance between the benefit and harm of the intervention appears highly favourable, further testing on SAH patients is required. We recommend proceeding with amendments in a dose-finding trial to determine the optimal duration of parenteral treatment.

Key Words: Omega-3 fatty acids, pharmaconutrition, pharmaconutrients, randomized pilot trial, subarachnoid hemorrhage

INTRODUCTION

Functional recovery after aneurysmal subarachnoid hemorrhage (SAH) remains a significant problem.^[1] Despite advances in therapeutics, no efficacious pharmaceutical intervention has emerged in the last few decades.^[19] A more integrative approach seems to be needed to successfully treat such a complex disease.^[31,63] Novel therapeutic strategies should have the capability to address not only delayed cerebral ischemia (DCI), but also shunt-dependent hydrocephalus (SDH), medical complications and long-term functional sequelae.^[1,39,47,67,68,72]

For over 30 years, the long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been linked to major health benefits.^[4,28] However, the current nutritional imbalance favouring n-6 PUFAs is an important counteracting factor.^[28,59] Evidence of the clinical efficacy of n-3 PUFAs is reasonably strong in some inflammatory diseases and in cardiovascular disease and mood disorders.^[10,13,14,21,38,70,71] Preliminary data on their clinical usefulness in cognition and age-related cognitive decline are also available.^[61,78] Their role in preventing DCI after SAH have also been examined, but in very few clinical studies.^[3,44,76,77] The accretion of DHA in neural tissue is essential for optimal neuronal function and may also support neurovascular unit integrity.^[5,22,37] Growing preclinical evidence supports that DHA plays a critical role in neuroprotection as well as in neurogenesis and synaptogenesis.^[6,26,46,51] N-3 PUFAs are precursors of diverse endogenous lipid mediators which modulate cellular and molecular events involved in crucial steps of neuroinflammation (inhibiting nuclear factor-kappaB [NF-κB] and microglial activation), oxidative stress (activating nuclear factor E2-related factor 2 [Nrf2]), microcirculatory dysfunction (decreasing vasoconstriction, leukocyte infiltration and platelet aggregation) and neuronal survival (downregulating apoptosis and parthanatos).^[7,40,48,56,60,73,79] The activity of the cytoprotective enzyme heme oxygenase-1 (HO-1) is also upregulated.^[53,79] On the other hand, there is preliminary evidence indicating significant loss of brain DHA in SAH patients.^[32,49] Theoretically, the cumulative burden of DHA loss after SAH may reduce the endogenous capacity for neuroprotection in the short-term, rendering the brain

more vulnerable to focal injury and diffuse atrophy.^[8,50,62,65] The promising role of n-3 PUFAs in recovering brain homeostasis after SAH is well supported by preclinical evidence, and thus a translational approach is warranted. An efficient pharmaconutrition therapy seems to be the most suitable strategy for this purpose.^[2,34,73]

Although fish oil (FO) in different clinical formulations has a high safety profile, no clinical trial of EPA plus DHA in SAH had previously been reported.^[24,71] Thus, we conducted a pilot study aimed at obtaining preliminary data regarding the safety and the feasibility of an effectiveness trial.^[17,42]

MATERIALS AND METHODS

The study protocol was approved by a central medical ethics committee at each participating centre and by the government regulatory agency, “Instituto de Salud Pública de Chile” (www.ispch.cl), Santiago, Chile. All the procedures were performed in accordance with the major codes and declarations relevant to research involving human subjects, including the Declaration of Helsinki of 1975, as revised in 2000.

Study design

This study was a pragmatic, multicentre, open-label, randomized pilot clinical trial of 2 parallel groups (allocation ratio of 1:1).^[17,81] N-3 PUFAs treatment (5 days of a parenteral perioperative regimen followed by almost 8 weeks of oral treatment) plus usual care was compared with usual care alone in surgically treated SAH patients.

Patients and participating centres

The study was conducted at two public health care centres in Chile between March 2013 and October 2016. The “Instituto de Neurocirugía Asenjo” (INCA [www.institutodeneurocirugia.cl]) in Santiago is the main reference centre for neurosurgery in Chile. The “Hospital Regional Libertador Bernardo O’ Higgins” (HRLBO [www.nuevo.hospitalrancagua.cl]) has a unique neurosurgical service in the VI Region of the country. The annual caseload of surgically treated SAH patients at INCA and HRLBO is approximately 100 and 35, respectively.

Patients were 18–69 years of age with a modified Fisher grade 3 or 4 SAH (short axis of cisternal blood on computed tomography [CT] ≥ 4 mm) who were

admitted to the emergency department during the first 72 hours after the initial bleeding episode. Eligible patients had a ruptured intracranial aneurysm of the anterior circulation demonstrated by computed tomographic angiography (CTA) or digital subtraction angiography (DSA). Patients were required to be scheduled for surgical clipping no earlier than 5 hours after CTA/DSA diagnosis. The patient's clinical condition at admission or following medical stabilization or external ventricular drainage (EVD) placement was required to be World Federation of Neurological Surgeons (WFNS) grades 1–4. Eligible patients could not have severe unstable acute or chronic systemic disturbances, nor could they have received antiplatelets, anticoagulants or valproic acid during the 3 weeks before SAH. A history of allergy to iodine contrast media, fish or eggs; cerebral sequelae visible on admission CT; and refusal of informed consent were additional exclusion criteria. Legal representatives of eligible patients were approached by a researcher or attending physician to provide informed consent. Patients had to be stable (hemodynamic: 70 < mean arterial pressure (MAP) < 130 mm Hg; 90 < systolic blood pressure (SBP) < 180 mm Hg and headache < 6 in the visual analogue scale [VAS]) at the intensive or intermediate care unit (ICU or IMCU) before randomization.

Randomization

Patients were randomized to treatment groups using sequentially numbered opaque sealed envelopes (SNOSEs) according to the literature.^[15] The randomized sequence was created with permuted blocks whose sizes (4 and 6) were unknown until the end of the trial. The randomization process was performed by members of the medical society of the VI Region who were not involved in any other part of the trial or in the treatment of neurosurgical patients.

Allocation concealment

The SNOSEs were placed and stapled within transparent plastic envelopes and filed in numerical order in a folder. This folder was stored in a safe with an access code and nursing surveillance to prevent any further manipulation.

Implementation

The determination to enrol and allocate patients to treatment groups was made by an attending neurosurgeon who had to confirm this decision with one of the principal investigators. The SNOSEs were opened according to specific instructions, and the procedure was witnessed by two professionals. The allocation card containing the transcribed data was kept attached to each individual case report form for future audit.

Intervention^[30]

A bimodal regimen of pharmaconutrition with n-3 PUFAs and controlled dietary supplementation of n-6 PUFAs was implemented as follows:

Parenteral treatment

A single daily dose of 100 ml of the FO-based lipid emulsion (FOLE), Omegaven 10% (Fresenius-Kabi Germany), was administered intravenously for 5 consecutive days under nursing surveillance at the ICU/IMCU. The first dose of FOLE had to be fully administered before beginning anaesthesia for aneurysm surgery and was continued thereafter every 24 hours. The infusion was performed by an infusion pump at 25 ml/hour via a central or peripheral venous line. However, after recruiting 22 subjects, we amended the protocol to increase the infusion rate to the maximum of 0.5 ml/kg body weight (BW)/hour, thus shortening the infusion time. Patients with a BW less than 50 kg were required to receive an adjusted dose of 0.2 g FO/kg BW/day. Concurrent administration of FOLE with iodinated contrast media was avoided. Flushing the residual volume of FOLE (12 ml) in the infusion line at the end of the procedure was not part of the protocol. This nursing procedure should always be performed to ensure full administration of the selected dose. Detailed information regarding FOLE is published elsewhere.^[71]

Oral treatment

Four high-concentration FO capsules, Omacor 1000 mg (Ferrer Chile/Spain - Pronova BioPharma Norway), each containing 460 mg of EPA and 380 mg of DHA as ethyl esters, were given daily beginning the day after the last dose of FOLE until the 60th day after SAH. The daily dose was fractionated twice a day and given orally with the main meals under nursing assistant surveillance at the ICU/IMCU or the neurosurgical ward. SAH patients in poor clinical condition and those with swallowing disturbances received the oil contained in the capsules by an emulsion via a nasogastric tube. Although flushing the nasogastric tube with 20 ml of water after use is a regular nursing procedure, it was not protocolized. Capsules were given to the patient by a relative during outpatient care.

Basal diet

The dietary provision of n-6 PUFAs (by regular diet or enteral formula) did not exceed 9 g daily, and no additional n-3 PUFAs were given during the hospital stay. Thus, the total n-6/n-3 PUFA supplementation ratio did not exceed 2.5/1. Clinical dieticians supervised compliance with these instructions. Patients and their relatives received written nutritional recommendations to be followed during outpatient care: fish and seafood consumption had to be avoided during the treatment. Thereafter, patients were required to begin eating two portions of fatty fish twice weekly until the third month after SAH.^[25] Foods with high n-6 PUFAs content (mayonnaise, margarine, spreads and dressings and vegetable oils [except olive and canola]) had to be avoided until the third month after SAH.

Potential drug interactions

Statins, acetylsalicylic acid, and selective inhibitors of non-steroidal anti-inflammatory drugs (NSAIDs) had to be avoided for 30, 60, and 90 days respectively, as they theoretically have the potential to modify the effects of the intervention.^[56,58,75]

Supplemental information regarding the intervention

A parenteral perioperative regimen may significantly shorten the time to an effective treatment, and surgically treated SAH patients may receive further clinical benefits.^[18,23,35,45,64,71] There seem to be clinically significant differences in the incorporation and washout of n-3 PUFAs in diverse blood lipid fractions (pools) between oral and parenteral administration.^[23,80] There are also differences in the bioavailability among different types of oral formulations, and disturbed gastrointestinal function in critically ill patients may further reduce the bioavailability of drugs and nutrients.^[16,52] Thus, the current intervention may be insufficient to maintain the most adequate fatty acids profile during the first few weeks of oral treatment (which may coincide with the period of DCI), particularly in poor clinical grade SAH patients.

Usual care

The standard of care of SAH patients during the study period was based on the Explicit Health Guarantee (GES 2007) statement published by the Chilean Ministry of Public Health (www.minsal.cl). The most relevant aspects are summarized as follows: medical stabilization at an ICU/IMCU, 60 mg of oral nimodipine every 4 hours, and no other drug or procedure to prevent DCI. DCI treatment had to be carried out with normovolemic hypertension. Aneurysm occlusion had to be performed within 24 hours after diagnosis. The surgical technique used in both centres included tailored craniotomy, clipping of the aneurysm, and basal cistern opening/irrigation with frequent lamina terminalis fenestration (LTF). EVD was used prior to the aneurysm surgery in cases of neurological deterioration caused by acute hydrocephalus. A definitive shunt was installed in those cases with unequivocal evidence of chronic hydrocephalus. Non-scheduled aneurysm surgery and endovascular treatment (aneurysm coiling and angioplasty for treating DCI) were conducted only at INCA during the study period.

Outcome measures

In addition to recently recommended clinical endpoints, we added exploratory outcome measures to test new hypotheses and rule out major bleeding complications associated with high morbimortality rates.^[39,43,55,69] The harm-related outcome measures were surgical evacuation of PIBCs and PIBCs visible on any postoperative CT scan. PIBCs were defined as non-laminar (>10 mm in thickness) juxta-dural collections and/or intracerebral hematomas (>10 ml in volume) that were not visible

on the admission CT scan. Primary outcome measures related to benefit were the Glasgow Outcome Scale Extended (GOSE) score at 90 days and cerebral infarction caused by DCI. GOSE scores were dichotomized to identify patients with poor functional outcome (score 1 to 4: dead, vegetative or severely disabled).^[74] Secondary outcome measures were combined cerebral infarction caused by DCI, neurological deterioration attributed to DCI and SDH at 90 days after SAH. Cerebral infarction and neurological deterioration caused by DCI were defined according to specific criteria established by international consensus and published elsewhere.^[69] Combined cerebral infarction was included as a severity measure of DCI.^[43] The feasibility of patient recruitment was assessed by the consent rate.

Clinical and radiological assessment

Clinical safety monitoring was provided to all patients during the hospital stay by a dedicated investigator. Two experienced neurosurgeons/neurointensivists established the diagnosis of neurological deterioration attributed to DCI. A neurologist/neurosurgeon conducted the interview (in person or by telephone with the patient or their relative) to assess GOSE and determine the diagnosis of SDH at 90 days. A single experienced neuroradiologist evaluated and compared the admission and follow-up CT scans obtained at 24 to 48 hours postoperatively or when clinically indicated. An additional cerebral CT scan was always obtained approximately 5 to 6 weeks after SAH to establish the definitive diagnosis of cerebral infarction and to rule out a more insidious development of chronic hydrocephalus.^[47,69] The amount of intraventricular hemorrhage (IVH) detected by CT was graded according to a semi-quantitative scale published elsewhere.^[29] The neuroradiologist was the only trial investigator unaware of the treatment group assignments.

Data lost

Unavailable data from patients lost to follow-up were exclusively assigned to the benefit-related outcomes, according to the following criteria: In case of death, patients were regarded as having cerebral infarction and clinical deterioration due to DCI and poor clinical outcome. In case of lost to follow-up at three months, patients were regarded as having poor clinical outcome when the last available score in the GCS was <11.^[66] SDH was assigned when these patients were seen for the last time with an EVD or a definitive shunt due to SAH.

Statistics

We choose a sample size of 50 subjects, as this number would provide clinically relevant information while maintaining ethical requirements.^[18,42] Descriptive statistics were tabulated for each characteristic. For each outcome measure, the results including expressions of uncertainty (95% confidence intervals [CI]) were obtained.^[17] The results were analyzed by randomized

groups according to the intention to treat analysis. A formal statistical analysis comparing the outcomes of trial arms was not performed, as pilot studies are not intended to determine the efficacy of the intervention.^[17] Statistical calculations were performed using the web-based, open source OpenEpi programme, version 3.01. The consent rate was calculated by dividing the number of enrolled patients by the number of those enrolled plus those who were approached and refused to participate.

RESULTS

Early stopping^[17]

Patient enrolment was stopped before reaching the intended recruitment of 50 subjects, as the planned interval for trial execution of 3 years was exceeded. The main rationale behind this decision (made by the principal investigators) was that an adverse event rate of interest equal to zero in the IG group would provide almost the same estimate for 20 as for 25 subjects.^[11] Patient recruitment became increasingly difficult during the trial execution, as a growing number of SAH patients were operated on at a non-scheduled basis at INCA, thus restricting the chance to carry out perioperative intervention. Despite this variable local clinical context, we preferred not to modify our restrictive eligibility criteria based on safety concerns.^[18]

Flow of participants

A flow diagram of the participants through each phase of the trial is depicted in Figure 1. Only 4 patients

refused to participate in the trial, resulting in an overall consent rate of 91% (HRLBO: 82%, INCA: 100%). There was no consent withdrawal after randomization. Patient recruitment was evenly distributed between the trial arms. One patient was excluded at INCA after allocation to the IG, as the hemorrhage was due to hypertension and not due to a paraclinoid aneurysm as initially diagnosed. One patient in the control group (CG) died 2 days postoperatively due to cardiopulmonary complications and two patients were lost to follow-up at three months (one in each trial arm). The outcomes of these three patients were assigned as described in the methods section.

Demographic and clinical characteristics

The demographic and clinical characteristics of both groups are shown in Table 1. The mean parenteral FO dose was 0.14 g FO/kg BW/day (0.10 – 0.19) based on the average BW of intervened patients. The average time interval between SAH and the start of intervention was 30 hours (8 – 75). Most patients received pharmacological deep vein thrombosis (DVT) prophylaxis with unfractionated heparin or low molecular weight heparin, no earlier than 24 h after aneurysm surgery.

Outcomes

Benefit- and harm-related outcomes are shown in Table 2. There were no PIBCs in the IG, even though two patients had major risk factors. One patient erroneously received 40 mg of enoxaparin preoperatively, and the other one experienced a significant decrease in platelet counts to 88×10^3 postoperatively. Furthermore, 7 additional

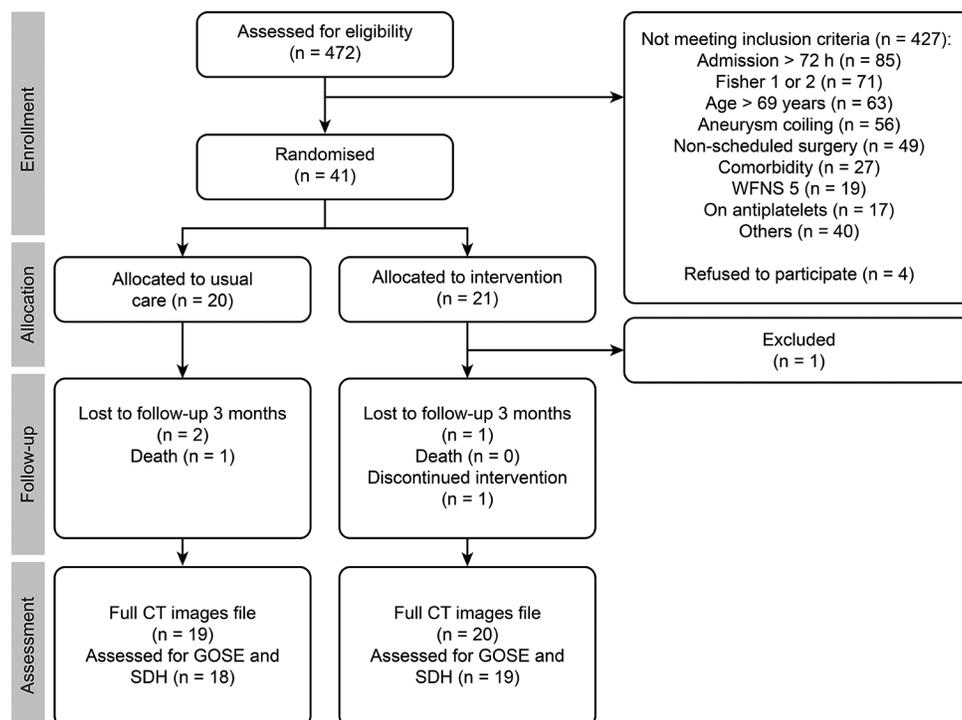


Figure 1: Flow diagram of the participants through the clinical trial phases

cranial surgeries were performed in 5 patients at different time points (days) after the start of the intervention: one intracranial pressure (ICP) monitor placement (1), one decompressive craniectomy (2), one EVD (7), three ventriculoperitoneal shunts (VPSs) (36, 41, 48) and one non-ruptured aneurysm (22). Aneurysm rebleeding occurred in one patient in the IG (INCA 11) who was enrolled despite having had clear exclusion criteria (a previous posterior fossa surgery and an indwelling VPS). A minor amount of FOLE was extravasated in the subcutaneous tissue of the forearm in 2 patients causing a faint and transient cutaneous erythema. No patient suspended the intervention due to side effects, and there were no complaints about gastrointestinal disturbances.

Table 1: Baseline and clinical characteristics of 40 patients

Characteristics	Control group (n=20)	Intervention group (n=20)
Female sex - no. (%)	13 (65)	12 (60)
Age - median years (range)	48 (20-67)	53 (22-68)
BW kg - median (range)	72 (55-110)	71 (52-97)
WFNS scale - no. (%)		
WFNS 1	6 (30)	8 (40)
WFNS 2	7 (35)	8 (40)
WFNS 3	1 (5)	1 (5)
WFNS 4	6 (30)	3 (15)
GCS on admission <11 - no (%)	3 (15)	2 (10)
Mod. Fisher grade - no. (%)		
Fisher 3	9 (45)	7 (35)
Fisher 4	11 (55)	13 (65)
IVH score* - median (range)	4.9 (1-8)	3.53 (1-11)
SIRS on admission - no. (%)	8 (40)	8 (40)
EVD - no of patients. (%)	5 (25)	2 (10)
LTF - no of patients. (%)	13 (65)	17 (85)
Aneurysm coiling - no. (%)	1 (5)	1 (5)
Angioplasty for DCI	5 (25)	4 (20)
DVT prophylaxis - no. (%)	17 (85)	19 (95)
Smoking	17	12

*IVH grading scale: 0, no blood; 1, sedimentation of blood in the posterior part; 2, partly filled with blood; or 3, completely filled with blood in each of the four ventricles. The total amount of IVH (sum score) was the total of the four scores and ranged from 0 to 12

Table 2: Primary and secondary outcomes

Outcome measures	No. of patients (%); [95% confidence interval]	
	Control group (n=20)	Intervention group (n=20)
Surgical evacuation of PIBCs	0 (0); [0.00 to 0.16]	0 (0); [0.00 to 0.16]
PIBCs on CT scan	1 (5); [0.00 to 0.23]	0 (0); [0.00 to 0.16]
Poor clinical outcome	9* (45); [0.25 to 0.65]	7# (35); [0.18 to 0.56]
Cerebral infarction by DCI	8* (40); [0.21 to 0.61]	5 (25); [0.11 to 0.46]
Combined cerebral infarction by DCI	2* (10); [0.02 to 0.30]	0 (0); [0.00 to 0.16]
Clinical deterioration by DCI	10* (50); [0.29 to 0.70]	5 (25); [0.11 to 0.46]
SDH	7 (35); [0.18 to 0.56]	3 (15); [0.05 to 0.36]

*1 case assigned due to death. #1 case assigned due to lost to follow-up

Protocol violations^[9]

Protocol violations concerning admission criteria occurred in two cases (the aforementioned patient and another patient on antiplatelets and allocated to the CG). The therapeutic strategy was changed to endovascular treatment after allocation in two patients (one in each trial arm). The intervention was discontinued after one dose of FOLE in the endovascularly treated patient due to safety concerns, but this case was also included in the intention to treat analysis. Furthermore, the recruitment of one patient was not approved by the principal investigators, and one dose of FOLE was administered at a greater infusion rate than the maximum recommended.

DISCUSSION

The intervention did not increase the occurrence of PIBCs and there was no evidence of unexpected harm. Although these results parallel those gathered in other clinical fields, further testing on SAH patients is required.^[24,33,71]

A bimodal regimen of pharmaconutrition offers significant clinical advantages to provide n-3 PUFAs after SAH. Parenteral treatment can be efficiently delivered regardless of the patient's clinical condition and oral treatment can be easily extended to address novel therapeutic targets.^[1,8,23,57,62]

The n-3 PUFAs dosage was supported by clinical evidence. The therapeutic dose range of FOLE (0.1 – 0.2 g FO/kg BW/day) is significantly lower than that of regular lipid emulsions.^[27] Thus, 100 ml of FOLE is suitable for patients with a BW of 50 to 100 kg. The total daily dose of EPA + DHA provided by capsules (3360 mg) is also therapeutic, exceeding the currently recommended dose for treating mood disorders.^[20,21] Although the optimal n-6/n-3 PUFAs supplementation ratio has not yet been established, a ratio below 4/1 is associated with several health benefits.^[59]

There was a lower occurrence of DCI-related outcomes and SDH in the IG than in the CG. Nevertheless, it would be erroneous to draw any conclusion about this from a

small pilot trial that, consequently, was underpowered to detect any meaningful difference. The rate of consent was unexpectedly high in both centres. If the main drivers of acceptance were the less invasive nature of the intervention and the good reputation of n-3 PUFAs, the current trial should be widely replicable.^[36] The feasibility of implementing the intervention in clinical settings and our findings may also foster other translational approaches for neuroprotection.^[6,41,79]

Our pilot trial had several limitations. We did not define formal progression criteria to decide whether to proceed with a future definitive randomized controlled trial (RCT).^[17] Open-label treatment undoubtedly increases the risk of acknowledgement bias. However, the use of less subjective harm-related outcome measures and blinding the CT evaluator may have counteracted this shortcoming. Additionally, blood lipid pools of n-3 PUFAs were not measured.

To date, four clinical studies have investigated the role of n-3 PUFAs in SAH. Three of these studies reported an improved clinical outcome when oral n-3 PUFAs were compared with usual care.^[44,76,77] A prospective observational study found an association between injury severity and increases in serum free fatty acid (FFA) levels; the authors also found that an increased n-6/n-3 FFA ratio was associated with DCI.^[3]

Although the balance between the benefit and harm of the intervention appears highly favourable, there are scarce data regarding the isolated use of FOLE as a specific treatment.^[23,71] Thus, we recommend proceeding with amendments in a double-blind, placebo-controlled, dose-finding trial (phase II b RCT) to estimate the dose-response relationships for the efficacy and safety of an extended parenteral treatment.^[54] Three parenteral regimes of different duration should be tested. Considering the duration of the DCI period and the findings of a recent and relevant clinical study, the extension of parenteral treatments should range from 5 to 14 days.^[23] Selecting a parenteral FO dose according to body weight in the middle of the therapeutic range would be more accurate. Including other groups of SAH patients and a postoperative intervention may increase the generalizability of our findings and the recruitment rate. However, care should be taken not to jeopardize an early intervention strategy. Ideally, the incorporation of n-3 PUFAs in diverse blood lipid pools should be measure at different time points during the intervention. This would provide valuable data regarding effective dose-response relationship, and some surrogate markers of brain DHA accretion.^[12,23,50,65] A health-related quality of life index would also be required as a secondary outcome measure to assess the effects of the intervention on functional domains. Such a large-scale RCT does not seem feasible in our setting, unless significant improvements

in technical resources and in the performance of the research centres can be achieved.

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Conflicts of interest

There are no conflicts of interest.

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