Effect of Pycnogenol® on Attention-Deficit Hyperactivity Disorder (ADHD) : study protocol for a randomized controlled trial

EFFECT OF PYCNOGENOL® ON ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD):

STUDY PROTOCOL FOR A RANDOMIZED CONTROLLED TRIAL.[1]

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[1] This paper is dedicated to the memory of our colleague Sandra Apers (°19/08/1972 - † 05/02/2017).

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Abstract

Background: Methylphenidate (MPH), the first choice medication for Attention-Deficit Hyperactivity Disorder (ADHD), is associated with serious adverse effects like arrhythmia. Evidence on the association of ADHD with immune and oxidant-antioxidant imbalances offers potential for antioxidant and/or immunomodulatory nutritional supplements as ADHD therapy. One small randomised trial in ADHD suggests, despite various limitations, therapeutic benefit from Pycnogenol®, a herbal, polyphenol rich extract.

Methods: This phase III trial is a 10 week randomized, double blind, placebo and active treatment controlled multicentre trial with three parallel treatment arms to compare the effect of Pycnogenol® to MPH and placebo on the behaviour of 144 paediatric ADHD and Attention-Deficit Disorder (ADD) patients. Evaluations of behaviour (measured by the ADHD-Rating Scale (primary endpoint) and the Social-Emotional Questionnaire (SEQ)), immunity (plasma cytokine and antibody levels, white blood cell counts and faecal microbial composition), oxidative stress (erythrocyte glutathione, plasma lipid soluble vitamins and malondialdehyde and urinary 8-OHdG levels, as well as antioxidant enzyme activity and gene expression), serum zinc and neuropeptide Y level, urinary catecholamines and physical complaints (Physical Complaints Questionnaire) will be performed in week 10 as compared to baseline. Acceptability evaluations will be based on adherence, drop outs and reports of adverse events. Dietary habits will be taken into account.

Discussion: This trial takes into account co-morbid behavioural and physical symptoms, as well as a broad range of innovative immune and oxidative biomarkers, expected to provide fundamental knowledge on ADHD aetiology and therapy. Research on microbiota in ADHD is novel. Moreover, the active control arm is rather unseen in research on nutritional supplements, but of great importance, as patients and parents are often concerned with side effects of MPH.

Trial registration: Clinical Trial Registry number NCT02700685 (registered 18 January 2016), EudraCT 2016-000215-32 (registered 4 October 2016).

Keywords

ADHD, ADD, behaviour, Pycnogenol®, antioxidant, polyphenols, oxidative stress, immunity, catecholamines

Background

Attention-Deficit Hyperactivity Disorder (ADHD) is a common neurocognitive behavioural disorder with childhood onset and core symptoms of hyperactivity, impulsivity and inattention [1]. ADHD has a worldwide prevalence of 5.9-7.1% and is associated with other psychiatric disorders, such as oppositional defiant disorder (ODD), autism and anxiety [2, 3].

Methylphenidate (MPH), the first choice medication for ADHD, is a central nervous system stimulant. It increases attentiveness and reduces hyperactivity and impulsivity by inhibition of dopamine reuptake in the striatum, without triggering its release. MPH is prescribed for chronic use to a large proportion of ADHD patients, but is linked to possible publication bias in reported efficacy [4-6]. In addition, parents are often disinclined to use MPH due to negative publicity and its frequent side effects, including serious side effects like arrhythmia, and, subsequently, non-adherence to therapy is high [4-7]. A recent review reports adverse effects like insomnia and decreased appetite in about 25% of patients using MPH [8]. Other therapeutic options are therefore warranted, at least for a subgroup of patients [4, 5, 7, 8].

ADHD is a complex and multifactorial disorder, influenced by both genetics and environment. Its exact pathophysiology remains however unclear. Dopaminergic dysfunction is for instance involved, but also associations with immune and oxidant-antioxidant imbalances exist [9, 10]. Various studies demonstrated for example increased levels of plasma malondialdehyde (MDA) and exhalant ethane (oxidative stress markers) and decreased activity of antioxidant enzymes such as glutathione peroxidase (GPX) and catalase (CAT) [11-14]. ADHD has also been hypothesised to be a

hypersensitivity disorder, with a disrupted immune regulation contributing to its aetiology [10]. I.e., ADHD has comorbidity with both Th1- and Th2-mediated disorders and several related genes have immune functions [9, 10, 15-18]. Ceylan *et al.* observed increased levels of adenosine deaminase, a marker of cellular immunity, and of the oxidative enzymes xanthine oxidase (XO) and nitric oxide synthase, and decreased levels of the antioxidant enzymes glutathione S-transferase and paraoxonase-1. These results indicate the involvement of oxidative changes and cellular immunity in ADHD [9].

Still, specific immune biomarkers other than antibodies have not been systematically studied in ADHD, despite growing evidence on associations in autism [19, 20]. In addition, immune and oxidative effects of both standard therapy and nutritional supplementation in ADHD is a neglected topic in research. Yet, immune and oxidative imbalances linked with ADHD offer potential for appropriate supplementation in ADHD therapy [21].

Due to its antioxidant and immunomodulatory properties, a commercially available standardised extract from French maritime pine (*Pinus pinaster*) bark with a high content of polyphenolic compounds (including phenolic acids and procyanidins), Pycnogenol®, was selected for this study [22-24]. One small randomised trial and few observational studies suggest its therapeutic benefit in ADHD. Still, this trial had some limitations (e.g. short supplementation period) and the mechanisms of action involved remain unclear [22, 25-29]. The efficacy, mechanism(s) of action and value of Pycnogenol® in ADHD as compared to MPH treatment thus remain to be confirmed.

Methods

Objective

To evaluate the effect of Pycnogenol® on ADHD and ADD behaviour and co-morbid physical and psychiatric symptoms, as well as on immunity, oxidative damage, antioxidant status and neurochemical parameters, as compared to placebo and MPH treatment.

Hypotheses

1. In ADHD therapy, Pycnogenol® is more effective than placebo and not less effective than MPH;
2. As compared to placebo and MPH, Pycnogenol® increases antioxidant levels, reduces oxidative damage, improves immune and neurochemical status and reduces co-morbid physical and psychiatric complaints;
3. The tolerability of Pycnogenol® is higher than that of MPH.

Design

This is a phase III, randomized, double blind, placebo and active product controlled, multicentre clinical trial with three parallel treatment arms to compare effects on ADHD and ADD behaviour between Pycnogenol®, MPH (Medikinet® Retard) and placebo, using the ADHD-Rating Scale (ADHD-RS) as a primary outcome measure. Secondary outcome measures are comorbid physical and psychiatric complaints (including side effects), oxidative stress, immunity, neurochemical parameters and tolerance of the intervention. Following screening and baseline assessments, 144 patients (6-12 years) will receive one of the three treatments for 10 weeks (see Table 2). Evaluations will be performed in week 5 and 10, as compared to baseline. Dietary habits will be taken into account.

Two visits with similar evaluations and sample collections will be conducted: at baseline and after 10 weeks. To analyse biomarkers of interest, 16 ml of venous blood will be collected at the start and the end of intervention, as well as urine. Faecal samples will be collected from participant subgroups (n = 60). Next to baseline and final evaluations, an extra evaluation of behaviour and physical symptoms will be conducted in week 5 by means of questionnaires. Two reminders will be sent in case

questionnaires are not received within one week after the required date. After every blood and urine collection and in case questionnaires are completed, participants receive two movie tickets.

Inclusion and randomisation

Recruitment starts in March 2017. The trial population will consist of ADHD and ADD patients recruited at the University Hospitals of Antwerp (UZA) and Ghent (UZ Ghent), and the Hospital Network Antwerp (ZNA). With an expected inclusion rate of 30-50 patients per year (10-20 participants in UZA, 15-20 in UZ Ghent and 5-10 in ZNA), about 3 years will be required for subject recruitment. Though, as compared to inclusion rate, a ten-fold higher diagnosis rate of ADHD and ADD is expected in these centres, in- and exclusion criteria of the proposed trial (e.g. regarding autism or the recent intake of supplements or medication) are expected to exclude at least half of all newly diagnosed patients, while a consent rate of 30% is expected, taking into account potential reluctance regarding the use of medication or supplements, as well as "risk" for placebo treatment [30, 31]. In addition, patients from random primary schools in Flanders will be invited for this trial by letters and diagnosed in one of the trial centres before inclusion. In case of slow recruitment, also "ZitStil" (information centre on ADHD/ADD), revalidation centres, independent child psychiatrists/paediatricians and other hospitals can be involved. Patients meeting eligibility criteria (Table 1) will be informed in detail and written consent of the legal representative to participate in the trial will be obtained prior to inclusion.

[Table 1 should be placed here]

Participants will be randomised, stratified by trial centre, to one of the three treatment arms (placebo, Pycnogenol® or Medikinet® Retard) by randomization.com randomisation software (original generator, different starting number across trial sites, and taking into account body weights below and above 30 kg; Figure 1). The number of patients per trial site is not limited. The involved physicians and hospital pharmacies will assure confidentiality by retaining the randomisation code at all times in a sealed envelope, only to be used in case of emergency or serious adverse events.

145 [Figure 1 should be placed here]

146 Treatment

147 Patients will receive all capsules required for the complete study at inclusion, at a dose based on
148 their body weight (1 or 2 oral capsules at breakfast):

149 • MPH (Medikinet® Retard, methylphenidate hydrochloride modified release): Patients with a body
150 weight < 30 kg will receive 20 mg/day, those with a body weight ≥ 30 kg 30 mg/day. Treatment
151 during the first week always contains 10 mg, increasing 10 mg per week to limit side effects.
152 • Pycnogenol®: Patients with a body weight < 30 kg will receive 20 mg/day, those with a body
153 weight ≥ 30 kg 40 mg/day, aiming at a daily dose of 1 mg/kg and taking into account formulation
154 issues [22]. Treatment during the first two weeks always contains 20 mg.
155 • Placebo: Placebo contains excipients only.

156 In case of adverse events, the investigator, principal caregiver and participant can decide to
157 discontinue the trial medication/supplement. However, no dose adjustment will be performed. Using
158 a standardized questionnaire, adverse events will be documented at week 5 and 10, taking into
159 account the patient's medical records as well. Also spontaneously reported adverse events will be
160 recorded. In case of a serious adverse event, the trial code will be broken and treatment
161 discontinued. In case 10% of participants experiences a potentially related serious adverse event, the
162 trial will be discontinued.

163 Pycnogenol® and placebo will be produced in capsules identical to Medikinet® Retard (Medice
164 GmbH). All treatments will be provided in identical jars, labelled with the subject's trial number and
165 week of intake. Compliance will be determined based on accountability of investigational products
166 and self-reported adherence.

167 Primary outcome

As the primary objective is to assess the efficacy of Pycnogenol® for improving ADHD and ADD behaviour as rated by teachers compared to placebo and Medikinet® Retard, the primary outcome is the summed ADHD score of the ADHD Rating Scale (ADHD-RS) as rated by teachers (Table 2). Teachers will fill out this questionnaire before the start of the intervention, and after 5 and 10 weeks.

Secondary outcomes

Secondary outcomes related to ADHD/ADD behaviour are:

- Summed ADHD score of the ADHD-RS, rated by parents.
- Summed ADHD score of the Social-Emotional Questionnaire (SEQ), rated by parents and teachers.
- Scores on ADHD subscales of the ADHD-RS and SEQ, rated by parents and teachers (hyperactivity, impulsivity and inattention).
- Percentage of responders rated by parents and teachers, defined as participants with a reduction of at least 20% of their baseline summed ADHD-RS score [32].

Other objectives are to evaluate the effect of Pycnogenol® compared to placebo and MPH on comorbid psychiatric and physical complaints, antioxidant levels, oxidative damage, immunity and neurotransmitters. Other secondary outcomes are therefore:

Psychiatric complaints

- Social behaviour problems subscale of the SEQ, rated by parents and teachers, to evaluate to what extent symptoms of ODD and CD are displayed [33].
- Anxiety subscale of the SEQ, rated by parents and teachers, to evaluate symptoms of general anxiety, social anxiety and anxiety-depression [33].

Physical complaints

- Physical and sleep complaints, including various potential side effects, measured by the Physical Complaints Questionnaire (PCQ) [34].

Antioxidant levels

- Erythrocyte glutathione (GSH) level, the most important intracellular antioxidant, analysed by HPLC with electrochemical detection [35].
- Lipid-soluble antioxidants: plasma vitamin E (α- and γ-tocopherol), vitamin A (β-carotene, retinol, retinyl palmitate) and co-enzyme Q10, analysed by HPLC with coulometric detection [36-39].
- Antioxidant enzyme activity (CAT, SOD and GPX) and total antioxidant status, analysed by ELISA [11, 40].
- Gene expression, quantified by RT-qPCR, focusing on networks counteracting oxidative stress (GPX, CAT, superoxide dismutase (SOD), XO) and stress-related proteins (Clusterin, Apolipoprotein J) [41, 42].
- Serum zinc level, analysed by AAS [43].

Oxidative damage

- Urinary 8-OHdG level, marker of oxidative DNA damage, corrected for urinary creatinine concentration, analysed by ELISA [44].
- Plasma malondialdehyde (MDA) level, marker of lipid peroxidation, analysed by HPLC with fluorescence detection [45].

Immunity

- Plasma cytokines for monocytes (IL-1β, IL-6, IL-8, IL-10, IL-12, TNF-α) and T-cells (IL-4, IL-5, IL-6, IL-10, IFN-γ) as well as antibody levels (IgA_{1-2}, IgG_{1-4}, IgE) by flow cytometry and ELISA, as markers of immune activation state and skewing [21, 46, 47].
- Identification of PBMCs like neutrophils, CD4, CD8 and B-cells and measurement of their functional responses (e.g. cytokine release) after stimulation, as a marker of immune activation state, skewing and responsivity [21, 46, 47].

- Intestinal microbial composition, assessed using extreme throughput multiplexed sequencing of 16S ribosomal RNA gene pools PCR-amplified from intestinal content samples [48, 49].

Neurochemistry

- Urinary catecholamines (dopamine, noradrenaline and adrenaline) and their metabolites, determined by HPLC with coulometric detection [50].
- Serum neuropeptide Y (NPY), analysed by ELISA [51].

The final objective is to investigate the acceptability of Pycnogenol® compared to Medikinet® Retard and placebo, based on the prevalence of side effects, treatment adherence (defined as >90% ingestion as scheduled) and proportion of drop-outs.

Dietary habits of participants such as consumption of vegetables, chocolate, fruit, etc. will be assessed by a food frequency questionnaire (FFQ) at the start and end of intervention [52, 53], to assess potential dietary adaptations during the study as well as baseline differences between treatment groups. The highest educational achievement of both parents will be determined as a proxy for socioeconomic status.

[Table 2 should be placed here]

Statistics

For the estimation of the required sample size, following assumptions were made:

- Patients improve 0,75 SD on the ADHD-RS summed ADHD score as rated by teachers if using Pycnogenol® for 10 weeks [22, 54], which corresponds to a 20% improvement with active treatment as compared to placebo
- Power of 80%, drop-out of 20%
- Two-sided testing, at a significance level of 0.05 with Bonferroni post-hoc testing correction

Based on these considerations, 48 patients per group will be necessary (n = 144 in total).

Data will be checked for outliers. Missing data will not be accounted for. The three groups will be compared with regard to baseline characteristics. A two-way ANOVA will be performed to investigate a potential interaction between treatment and weight. Change in ADHD-RS score as rated by teachers (primary outcome measure) will be compared between the three groups by means of a one-way ANOVA (categories: group, time; $\alpha = 0.05$) with post-hoc testing. Changes regarding secondary target variables will also be compared between the three groups, by one-way ANOVA with post-hoc analysis with multiple testing correction, Kruskall-Wallis or Fisher's exact test. Separate analyses for subgroups (e.g. based on gender, severity of ADHD, dietary habits, etc.) will be performed. Non-inferiority of Pycnogenol® as compared to Medikinet® Retard will be demonstrated when the difference in effect on ADHD-RS score is no more than 5 points *[55]*. This wide margin might be justified due to frequent side effects of MPH. Non-inferiority will only be accepted if supported by both intention-to-treat and per protocol analyses *[56, 57]*.

Ethics and registration

Ethical approval has been obtained in UZA (EC 15/35/365), ZNA (EC approval 4656) and UZ Ghent (2016/0969). The trial has been registered at Clinicaltrials.gov (NCT02700685) and EudraCT (2016-000215-32).

Trial management and research team: The University of Antwerp (Laboratory of Nutrition and Functional Food Science) is the sponsor of this trial, with NH being the coordinating investigator. As principle investigators, BC, DVW and HV will be primarily responsible for patient inclusion. NH and AV are responsible for the analysis of oxidative stress and neurological biomarkers and questionnaire results, as well as data management. HS is responsible for the analysis of immune biomarkers and genetics. No Data Monitoring Committee will be set up. BC, HV, DVW, NH and AV will discuss potential issues regarding e.g. subject recruitment.

For more information, see both the SPIRIT checklist and figure (supplementary material), with more detailed information on the execution of the trial, and scientific, ethical, and administrative elements.

Discussion

This randomised controlled trial addresses the potential of a herbal extract in ADHD by investigating its efficacy, mechanism of action and value as compared to standard treatment and placebo. Results can be partly compared to a previously conducted study [22, 28, 29]. A double blind design was chosen to avoid bias, due to the subjectivity of questionnaire responses. Behavioural assessment by teachers is preferred as primary objective due to the higher sensitivity of teachers' ratings [58, 59]. A 10-week treatment is considered long enough to see clear effects of both Pycnogenol® and Medikinet® Retard, though still minimizing the patient burden and thus maximising compliance [22]. The parallel design was therefore also chosen to reduce patient burden.

Pycnogenol® is a patented, proprietary powder extract made exclusively from French maritime pine bark by Horphag Research (Geneva, Switzerland). The extract is standardized to contain 70 ± 5% procyanidins. Pharmacological studies employing *in vitro*, animal and/or human models have found potent antioxidant activity, anti-inflammatory actions, improvement of endothelial function, etc. [60]. Pycnogenol® was selected for the present study based on previous research suggesting its therapeutic benefits in ADHD, though this trial had several limitations [22, 27-29]. Further research is needed to investigate its efficacy, mechanism of action and value, especially compared to MPH treatment. For example, dietary polyphenols and their metabolites exert prebiotic-like effects, stimulating the growth of intestinal microbiota, which play a fundamental role in immunity [48, 61, 62]. Also the Pycnogenol® dosage is based upon this previous clinical trial, using 1 mg/kg body weight [22]. In the present trial, due to practical reasons, 0.67-1.33 mg/kg body weight will be applied.

Despite being the first choice medication for ADHD, MPH is associated with various adverse effects (including serious adverse events), some of them frequently occurring, including irritability, insomnia, loss of appetite and headache [8]. Based on data from 70 human clinical studies on 5723 healthy subjects and patients, the overall frequency of adverse side effects due to Pycnogenol® is very low (1.8%) and unrelated to dose or duration of use. The majority of adverse effects observed is mild. Gastrointestinal discomfort, the most frequently occurring adverse effect, may be avoided by taking Pycnogenol® with or after meals. In children with ADHD, 2 of 41 Pycnogenol® supplemented participants experienced side effects (rise of slowness and moderate gastric discomfort). Pycnogenol® did not cause any significant changes in blood pressure or heart rate in 4 clinical studies (total n = 185). There have been no reports of serious adverse effects since its introduction into the European market around 1970 [49]. Safety trials demonstrated absence of mutagenic and teratogenic effects, no perinatal toxicity and no negative effects on fertility [63]. Therefore, the use of Pycnogenol® in children is considered to be safe.

The ADHD-RS is validated and internationally accepted, and consists of 9 inattention and 9 impulsivity and hyperactivity items based on the DSM, each marked out on a four-point rating scale [23]. The ADHD-RS allows comparison of results to those of previously performed trials [22, 54].

In addition to the ADHD-RS, the SEQ is used in this trial. Though this increases the number of questions on behaviour significantly (72 questions), the SEQ is a behaviour evaluation list to assess core symptoms of social-emotional problems, including frequently occurring psychiatric comorbidities of ADHD. Besides ADHD, three other clusters of social-emotional problems are incorporated in the SEQ (social behaviour problems, anxiety and autism), with items covering the core symptoms of these clusters according to DSM. The SEQ can be used for screening, diagnosis and treatment evaluation. Items are rated on a five-point scale [33].

The approved PCQ consists of 36 questions, of which 18 items are relevant with respect to specific physical and sleep complaints, including eight domains: (1) pain (e.g. headache), (2) unusual thirst or

perspiration, (3) eczema, (4) asthma or rhinitis, (5) skin problems (e.g. blotches in the face), (6) tiredness, (7) gastrointestinal problems and (8) sleep problems. Items, including various potential adverse effects, are rated on a five-point scale at baseline and after 5 and 10 weeks [34]. In addition, parents will be asked whether the participant experienced any illness during the trial, what illness, whether any medication was taken, and the type, dose and duration of medication intake.

The FFQ consists of 50 questions on different food groups to be rated on a nine-point scale by parents at the start and end of the intervention, to assess baseline dietary habits and potential adaptations during the study, as well as to relate potential differential effects of Pycnogenol® to dietary polyphenol intake [52, 53]. Insight in global dietary habits (e.g. whether or not the participant consumes fresh fruit on a daily basis) is therefore aimed for.

Patients and especially their parents are often worried about side effects of MPH, the standard medication for ADHD. It is therefore important to take into account side effects of Pycnogenol® and effects on co-morbid complaints. In addition, the behavioural effects of Pycnogenol® compared to placebo, but also compared to MPH, will be investigated. This active control is rather unseen in research on nutritional supplements, but of great importance. In one previous trial, the effect of Pycnogenol® was compared to MPH and placebo. However, neither MPH nor Pycnogenol® outperformed placebo, possibly due to the short treatment period of 3 weeks [27].

Most research on nutritional supplements or medication in ADHD predominantly assesses effects on ADHD behaviour. This trial however takes into account co-morbid behavioural and physical symptoms, such as ODD, anxiety and side effects, as well as a broad range of innovative immune, oxidative and neurochemical biomarkers. The analysis of gene expression and biomarkers can indicate genetic effects and biological processes involved in the mechanism of action of Pycnogenol® and possibly affecting ADHD symptom expression. Research on microbiota in ADHD in itself is novel, too. Results of this project will therefore increase insight in ADHD aetiology and (dietary) treatment options, which is highly desired by medical staff, parents and patients.

Trial status

Not yet recruiting as of February 2016.

List of abbreviations

8-OHdG	8-hydroxy-2-deoxyguanosine
AAS	Atomic Absorption Spectroscopy
ADD	Attention-Deficit Disorder
ADHD	Attention-Deficit Hyperactivity Disorder
ADHD-RS	ADHD-Rating Scale
ANOVA	Analysis Of Variance
CAT	Catalase
DSM	Diagnostic and Statistical Manual of Mental Disorders
ELISA	Enzyme-Linked ImmunoSorbent Assay
FFQ	Food Frequency Questionnaire
GPX	Glutathione Peroxidase
GSH	reduced glutathione
IFN	Interferon
IL	Interleukin
MAO	Monoamine oxidase
MDA	Malondialdehyde
MPH	Methylphenidate
NPY	Neuropeptide Y
PCQ	Physical Complaints Questionnaire
RT-qPCR	Real-Time quantitative Polymerase Chain Reaction

SEQ	Social-Emotional Questionnaire
SOD	Superoxide dismutase
TNF	Tumour Necrosis Factor
UZ Ghent	University Hospital Ghent
UZA	University Hospital Antwerp
XO	Xanthine oxidase
ZNA	Hospital Network Antwerp

Declarations

Ethics approval and consent to participate

Ethical approval has been obtained in the University Hospitals of Antwerp (UZA; EC 15/35/365) and Ghent (UZ Ghent; 2016/0969), as well as in Hospital Network Antwerp (ZNA; EC approval 4656). Written informed consent of the participant's legal representative to participate in the trial will be obtained before inclusion.

Consent for publication

Not applicable.

Availability of data and material

No data obtained yet.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NH, HS and AV initiated the study, in discussion with BC, DVW and HV. AV drafted the manuscript. NH, LP, HS, BC, DVW, TDB and HV critically reviewed the manuscript for final submission. All authors have read the final version and approve its submission.

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Tables and Figures

Table 1. Inclusion and exclusion criteria for patient selection.

Table 2: Investigations and data acquisition during the trial.

Figure 1. Design of the trial.

Supplementary Material

Supplementary Material1 (SPIRIT Checklist) and Supplementary Material2 (SPIRIT figure), both Word documents (.docx), with more detailed information on the execution of the trial, and scientific, ethical, and administrative elements.