BMJ Open

Cerebral and cognitive modifications in retired professional soccer players: TC-FOOT protocol, a transverse analytic study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-060459
Article Type:	Protocol
Date Submitted by the Author:	21-Dec-2021
Complete List of Authors:	Kepka, Sabrina; University Hospital Centre Strasbourg, Emergency department Lersy, François; University Hospital Centre Strasbourg, Imaging department Godet, Julien; Public health Unit, University hospital of Strasbourg Blanc, Frederic; University Hospital of Strasbourg Bilger, Mathias; University Hospital Centre Strasbourg, Neuropsychology department Botzung, Anne; University Hospital Centre Strasbourg, Geriatrics and Neurology Departments, Research and Resources Memory Center (CM2R) Kleitz, Catherine; University Hospital Centre Strasbourg, Neuropsychology department Merignac, Jeanne; University Hospital Centre Strasbourg, Geriatrics and Neurology Departments, Research and Resources Memory Center Ohrant, Emmanuel; Fédération Française de Football Garnier, franck; School of Osteopathy, College COS Strasbourg Pietra, François; Racing club Strasbourg Noblet, Vincent; ICube, UMR 7357, University of Strasbourg-C Deck, Caroline; ICube, UMR 7357, University of Strasbourg Willinger, Remy; Université de Strasbourg Kremer , Stéphane ; Université de Strasbourg
Keywords:	Magnetic resonance imaging < RADIOLOGY & IMAGING, SPORTS MEDICINE, Delirium & cognitive disorders < PSYCHIATRY

Cerebral and cognitive modifications in retired professional soccer players: TC-FOOT protocol, a transverse analytic study.

Sabrina Kepka[1, 2], François Lersy[3], Julien Godet [2,4], Frédéric Blanc[2,5], Matthias Bilger[6], Anne Botzung[5], Catherine Kleitz[6], Jeanne Merignac[5], Emmanuel Ohrant[7], Franck Garnier[8,9], François Pietra[10], Vincent Noblet[2], Caroline Deck[2], Rémy Willinger[2], Stéphane Kremer[2, 3]

[1]Emergency department, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[2]ICube, UMR 7357, University of Strasbourg-CNRS, Strasbourg, France

[3]Imaging department 2, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France

[4]Public Health department, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[5]Geriatrics and Neurology Departments, Research and Resources Memory Center (CM2R), Strasbourg University Hospital, Strasbourg,

[6]Neuropsychology department, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France

[7]Fédération Française de Football (FFF), Paris, France

[8]School of Osteopathy, College COS Strasbourg, Strasbourg, France

[9]Medical Sport Center of Strasbourg (CMSM), Strasbourg, France

[10]Racing Club Strasbourg, Strasbourg, France

Corresponding Author:

Sabrina Garnier-Kepka

Permanent address:

Emergency department

CHRU Strasbourg – Nouvel hôpital civil

1 place de l'hôpital, 67091 Strasbourg

France

Tel: +33 3 69 55 13 35

Fax: +33 3 69 55 18 56

Email: sabrina.garnier-kepka@chru-strasbourg.fr

ABSTRACT

Introduction

Soccer is the most popular sport in the world. This contact sport carries the risk of exposure to repeated head impacts in the form of subconcussions, defined as minimal brain injuries following head impact, with no symptom of concussion. Whilst it has been suggested that exposure to repetitive subconcussive events can result in long-term neurophysiological modifications, and the later development of chronic traumatic encephalopathy, the consequences of these repeated impacts remain controversial and largely unexplored in the context of soccer players.

Methods and analysis

This is a prospective, single center, exposure/nonexposure, transverse study assessing the onset of MRI and neuropsychological abnormalities in professional retired soccer players exposed to subconcussive impacts, compared to high-level athletes not exposed to head impacts. The primary outcome corresponds to the identification of modifications found by advanced MRI techniques (diffusion tensor, cerebral perfusion, fMRI, cerebral volumetry and cortical thickness, spectroscopy, susceptibility imaging). Secondary outcomes are the results of the neuropsychological tests: number of errors and time to complete tests. We hypothesize that repeated subconcussive impacts could lead to morphological lesions and impact on soccer players' cognitive skills in the long term.

Ethics and dissemination

Ethics approval has been obtained and the study was approved by the Comité de Protection des Personnes (CPP) N° 2021-A01169-32. Study findings will be disseminated by publication in a high-impact international journal. Results will be presented at national and international imaging meetings.

Registration

This trial is registered with Clinical Trials Registry NCT04903015.

Key words

Soccer

subconcussions

MRI

Neuropsychological assessment

Article summary

Strengths and limitations of this study

- To the best of our knowledge, this is the first prospective study to evaluate both the cerebral and cognitive modifications following repetitive subconcussive impacts in male professional soccer players at the end of their careers, or in retirement, a population which is understudied in the literature.

- The major strength of this study is its combinatorial approach: the onset of abnormalities will be evaluated by a combination of advanced Magnetic Resonance Imaging (MRI) techniques (brain volumetry and cortical thickness, diffusion tensor, magnetic susceptibility, spectroscopy, functional MRI (fMRI), and cerebral perfusion), using specific modalities of analysis, by a team specialized in the biomechanics of head injury.

- The neuropsychological work-up will be based on validated tests in the field of head injuries, with a proven sensitivity for assessing those functions known to be frequently impaired by repetitive subconcussive impacts e.g., processing speed, working memory, sustained attention and executive functions, and episodic memory.

- Although the single center study design may be seen as a limitation, players in different soccer teams from different towns will be recruited, in order to represent a large panel of soccer players with intensive practice, whilst ensuring a similar imaging protocol for all participants.

- This study is limited in its generalisability to both sexes, as only male soccer players are considered.

Word count : 3768

INTRODUCTION

Soccer, one of the most popular sports in the world, exposes the player to cerebral concussions, or mild traumatic brain injuries, which represent 5.8–22% of all soccer-related injuries [1]. An expert working group from the French Ministry of Sports has defined concussion as a brain injury caused by a direct or indirect transmission of kinetic energy to the head, resulting in an immediate and transient dysfunction of the brain [2]. It is characterised by at least one of the following features: loss of consciousness, loss of memory, altered mental status, and neurological signs and symptoms which cannot be explained by another cause [2].

In addition, soccer players are exposed to repetitive subconcussive impacts, notably by soccer heading. This is a skill specific to soccer, with an average of 6 to 12 headings occurring per game per player [3]. These events may be defined as minimal brain injuries following head impact, but with no symptom characteristic of concussion [4]. Whilst concussions are associated with neurological impairment [4], the consequences of subconcussion remain unclear, notably for professional soccer players [5]. It has been suggested that subconcussive impacts could increase the risk of traumatic chronic encephalopathy, a neurodegenerative disease affecting subjects who have undergone head impacts over a number of years [6]. Clinically, subjects present memory impairment, cognitive impairment, anxiety and depressive symptoms, and psychiatric problems [7]. The diagnosis is confirmed upon anatomopathological examination of brain tissue, by the presence of phosphorylated tau protein deposits distributed throughout the brain, with a tendency to occur in clusters at the sulcal depths of the cortex [6,8–10]. However, it is currently impossible to confirm the diagnosis *in vivo*.

Conventional imaging techniques such as computed tomography (CT) or MRI only show morphological cerebral abnormalities (intra- or pericerebral hemorrhages, diffuse axonal lesions) in cases of severe head injury. These morphological techniques do not usually identify an abnormality in cases of mild concusssion, even when repetitive. On the other hand, advanced MRI techniques (brain volumetry and cortical thickness, diffusion tensor, magnetic susceptibility, spectroscopy, functional MRI, and cerebral

perfusion) have made it possible to detect subtle brain abnormalities in athletes or former athletes exposed to repetitive subconcussive impacts, including in the absence of proven concussion [11–14]. Several of the aforementioned techniques have demonstrated a loss of both white and grey matter in athletes who are subjected to repeated asymptomatic head impacts, which is likely to be amplified by the association with subconcussive impacts [12,15,16]. According to magnetic susceptibility studies, the loss of substance can be associated with hemorrhagic lesions and inflammation [17]. Moreover, a study using fMRI techniques revealed a prolonged alteration in resting state functional connectivity in American football players over the course of a football season [11]. This suggests that repetitive impacts may have a cumulative effect on modifications to functional connectivity in the brain networks of athletes. In former American football players, fMRI has also demonstrated a cerebral reorganisation during memory tasks, including hyperactivation in compensatory zones. It is suspected that these abnormalities are related to later development of chronic traumatic encephalopathy [9,14,18].

In American football players, the number of years of practice and the degree of exposure to physical impacts linked to the player's position has been shown to increase the risk of developing traumatic chronic encephalopathy [19]. In soccer players, a recent systematic review revealed a higher risk of mortality from motor neuron disease than in the general population [20]. However, previous research revealed that head impacts in soccer had no effect on blood-based biomarkers for structural brain damage, such as serum neurofilament light or tau [21]. A limited number of studies have carried out cognitive assessments of soccer players, and have reported contradictory results [5,22,23]. Whilst the study of Matser *et al.* suggested that soccer may adversely affect some aspects of cognitive functioning [23], no association was found between exposure and cognitive performance in a recent cross-sectional study specifically evaluating the effects of soccer heading [5], in agreement with the results of Straume-Naesheim *et al*. [22]. However, this study did not have a follow-up period, and soccer players were relatively young (average age of 24.6 ± 4.5 years), meaning that any cumulative effects of heading across a player's career may not have been detected. Indeed, a systematic review has highlighted the lack of studies evaluating the long-term effect of heading on cognitive performance [25]. A further study assessing the effect of soccer heading on diffusion tension images and cognitive function found no

significant difference in terms of microstructural features and cognitive performance between non-athlete participants unexposed to heading and amateur soccer players with high exposure [26]. However, the players enrolled in this study were, again, of relatively young age (25.5 ± 7.2), which limits its long-term relevance. Moreover, to date, none of these studies have employed all neuroimaging modalities in a complementary fashion. A recent systematic review by Snowden *et al.* determined that there is not enough evidence to conclude on the effects of subconcussion in soccer players, and that further studies are required to better evaluate the impact of repetitive subconcussive injuries [27].

Objectives

The objective of this study is therefore to evaluate, using advanced MRI techniques, the microstructural consequences and the onset of any cognitive impairment in professional soccer players at the end of their careers who have experienced repetitive subconcussive impacts. The primary objective is to identify and describe any cerebral abnormalities observed by MRI in professional soccer players exposed to repeated subconcussive impacts, and to compare these results to those of high-level athletes who are not exposed to head impacts. The secondary objectives are to assess the cognitive impact of repeated subconcussive impacts *via* a neuropsychological evaluation, and to evaluate the relationship between the microstructural anomalies demonstrated by MRI and cognitive impairment.

We hypothesize that these subconcussive impacts could lead to morphological lesions, and impact soccer players' cognitive skills over the long term.

METHODS AND ANALYSIS

Study design and setting

This is a monocentric, transversal, exposure/non-exposure study assessing the relationship between exposure to repetitive subconcussive impacts during professional soccer play and the onset of cerebral and cognitive abnormalities. This study will take place at Strasbourg University Hospital, Strasbourg, France, a public university hospital, over a study period of 24 months, with an estimated start date of February 2022.

Patient and public involvement

Patients were not involved in the conception of this study.

Eligibility criteria

Two groups of participants will be established: a group of professional soccer players exposed to repetitive subconcussive impacts, and a control group of non-exposed high-level athletes.

Inclusion criteria common to both groups are: (1) male, (2) aged 32–55 years old, (3) affiliated to the French social security system, and (4) able to give written informed consent. Non-inclusion criteria common to both groups are: (1) a history of concussion including the presence of one or more of the following signs or symptoms after a head injury: a period of confusion or disorientation, a period of loss of consciousness of 30 minutes or less, post-traumatic amnesia not exceeding 24 hours; (2) history of neurological or psychiatric disorder; (3) known cerebral abnormality diagnosed by an imaging exam (CT or MRI); (4) history of severe brain/head injury; (5) contraindication to MRI (claustrophobia, implanted material not compatible with MRI, refusal to be informed of any abnormalities discovered by MRI); (6) history of regular or occasional drug use: active smoker or non-smoker for less than 1 year, excessive consumption of alcohol (>20 g alcohol per day, evaluated with the following formula: [8 x percentage of alcohol x volume (cL))/100]), whether current or in the past; (7) use of medication targeting the central nervous system in the 2 weeks preceding inclusion in the study; (8) prior history of

severe hypertension, diabetes, chronic heart disease, progressive or disabling disease; (9) incapacity to give informed consent or under a legal protection order; (10) refusal to participate in the study.

Inclusion criteria specific to the exposed group are: (1) professional soccer players at the end of their career, or recently retired; (2) playing or having played in clubs in the French Ligue 1 and Ligue 2; (3) previous exposure to repetitive subconcussive impacts; (4) no history of severe head injury or cerebral lesion, (5) playing or having played a position which is not goalkeeper. Inclusion criteria specific to the control group are: (1) high-level athletes who have never regularly played a sport exposing them to repetitive mild head injuries (e.g., rugby, basketball, handball, American football, hockey, combat sports).

Recruitment

The group of professional soccer players at the end of their careers will be recruited from clubs playing in French Ligue 1 and Ligue 2, with the assistance of club physicians, under the supervision of the medical director of the French Federation of Soccer (FFF). The control group of high-level athletes not exposed to head injuries will be recruited *via* the Medical Sport Center of Strasbourg (CMSM) located in Strasbourg, France, which is a referent center for the care of high-level athletes, and by the regional delegations of the French federations of various sports (tennis, athletics, swimming). Information leaflets will be displayed within the center and on club premises. Recruitment will take place during the entire 24-month period of the study.

Participant timeline and follow-up

An initial information visit will take place by phone call, to verify that the subject meets the eligibility criteria. Approximately one week after this information visit, a second visit will take place at the Radiology department of Strasbourg University Hospital. During this visit, an investigating physician will collect written informed consent. Following this, the neuropsychological evaluations and MRI tests will be carried out.

A follow-up visit will be organized by an investigating physician, in order to communicate the results of the examinations to participants. The results can fall into three categories: normal work-up, FLAIR sequence MRI abnormality, and/or demonstration of cognitive impairment. This visit will be carried out by telephone call in the case of normal results, and by an in-person visit at Strasbourg University Hospital in the case of abnormal results.

Outcomes

The primary outcome of this study is the identification of cerebral modifications, using advanced MRI techniques, namely: diffusion tensor imaging, cerebral perfusion, fMRI, cerebral volumetry and cortical thickness, magnetic resonance spectroscopy (MRS), and susceptibility imaging. Secondary outcomes of this study are the results of the neuropsychological tests: performance in a targeted battery of neuropsychological tasks as evaluated by the number of errors and time to complete tests.

Data collection, storage and verification

Socio-demographic and clinical data

Socio-demographic and clinical characteristics of patients will be recorded by an investigating physician: age, education level, medical history including cardiovascular risk factors, neurological history, history of COVID-19, type of sport practiced, and the modalities of practice. For the group of soccer players, the position played will also be recorded.

Neuropsychological assessment

For the neuropsychological assessment we have decided to employ a series of validated tests, that have shown sensitivity in the field of head injuries, in order to assess the functions frequently impaired by subconcussive impacts, i.e. processing speed, working memory, sustained attention and executive functions, and episodic memory. This assessment will last approximately 2 hours, and will be carried out by specialised neuropsychologists, ensuring the quality of the data.

The following tests were chosen:

1. Montreal Cognitive Assessment scale: an overall efficiency assessment test (= 10 min) [28]
2. RL RI 16 items (a French adaptation of the Free and Cued Selective Reminding Test) to assess the functioning of verbal episodic memory (encoding = 10 min) [29]
3. Copy of Rey's complex figure assessing visual-constructive abilities and planning (= 5 min) [30]
4. Auditory-verbal and visuospatial spans to assess short-term working memory (Wechsler scale Memory form III) (= 5 min) [31]
5. Trail Making Test A and B, assessing treatment speed and mental flexibility (= 5 min) [32]
6. The Brixton Test, a spatial anticipation task that also evaluates executive functioning (= 15 min) [32]
7. RL RI 16 items to assess the functioning of verbal episodic memory (delayed recall at 20 min) [29]
8. Memory reproduction of Rey's complex figure in order to assess episodic visual memory (delayed recall at 20 min) [30]
9. Phonological and semantic fluency tests for executive functions (letter P and Animals; 2 minutes per test, 4 minutes total) [32]
10. Computerized attention tests will also be used to measure reaction times and executive aspects. Subtests: Phasic Alert, Split Attention and Incompatibility version 2.3.1. This is a computerized battery of tests, using no paper questionnaires (= 15 min) [33]
11. Social cognition test "Reading the Mind in the Eyes", to assess ability to read the emotions of others which, in turn, is related to performance in team problem-solving tasks (= 10 min) [34]

In addition to the aforementioned tests, two further questionnaires will be carried out:

1. A self-administered questionnaire, BRIEF-A (Behavior Rating Inventory of Executive Function – Adult Version), evaluating executive functions (<10 minutes) [35]
2. Questioning on recurrent symptoms such as headaches (number of years the subject has experienced headaches, since when, frequency), fatigability, sleep disturbance, dizziness, blurry vision, photophobia/phonophobia.

MRI

Acquisition

MRIs will be carried out on 3 Tesla MRI scanners.

The following sequences will be acquired:

1. 3D T1 gradient echo (GRE): anatomy, registration, cerebral, white and grey matter volume, cortical thickness;
2. Multi-echo 3D T2 GRE: quantitative susceptibility mapping (QSM), iron overload quantification;
3. 3D fluid-attenuated inversion recovery (FLAIR);
4. Continuous arterial spin labeling (ASL) 3D: cerebral perfusion;
5. Resting-state fMRI: functional connectivity;
6. 64-direction DTI (b=1000 and 2500): alterations in white matter and its microstructure, anatomic connectivity;
7. Monovoxel spectroscopy of the mesencephalus with short echo time (TE).

Any contraindications will be verified before performing MRI. The patient should preferably be wearing a hospital gown without snaps for safety reasons. The patient's head should be placed so as to acquire DTI and 3D ASL sequences without having to tilt the views in the axis of the petrous bones (angles at 0° and good right/left symmetry of the acquisitions).

The images will be acquired with the examination room door closed. Checks will be carried out prior to image acquisition, to ensure that there is sufficient disc space in the database, and the image quality will be checked during the acquisition. If artifacts are present, the sequences will be repeated after correction.

The sequences should be oriented respecting the three spatial planes (Figure 1).

- The T1 sagittal sequence should cover the entire encephalon;

- The 3D T1, 3D FLAIR and the multiecho T2 GRE sequences should cover the entire encephalon;
- The DTI, fMRI, and 3D ASL sequences should be oriented strictly axial (angles at 0°). All of the encephalon should be covered.

The monovoxel spectroscopy should be placed on the brain stem (Figure 2). The voxel should be placed on the posterior two-thirds of the brain stem and cover the entire height.

Image processing and analysis

Each of the imaging modalities will be analysed independently, at different levels of analysis: 1) overall analysis conducted on all white matter and all grey matter, 2) regional analysis on a limited number of regions of interest (cerebral anatomic structures, cortical regions), and 3) focal analysis (or voxel-based) on the entire brain.

Cerebral atrophy will be studied by analysing the 3D T1 GRE morphological sequences, globally, using the SIENAX method implanted in the FSL library (University of Oxford) [36]. Regional analysis (volume of anatomic structures and mean cortical thickness of certain cortical regions) will be carried out using FREESURFER software [37], and focal analysis will be carried out using the Voxel-Based Morphometry (VBM) approach [38], using the implantation available in the Statistical Parametric Mapping (SPM) software (Wellcome Centre for Human Neuroimaging).

The completeness of the white matter will be investigated by analysing the tensor diffusion MRI sequences, globally and regionally, using segmentations obtained from morphological MRI (see preceding paragraph), and focally using the Tract-Based Spatial Statistics (TBSS) method implanted in the FSL library [9].

Vascular damage will be assessed *via* the study of global and regional cerebral perfusion in ASL, as well as by regional analysis of microhemorrhages and of iron deposits revealed by the QSM sequences.

These analyses will also be carried out using the segmentations obtained from morphological MRI. It should be noted that focal analysis of these modalities cannot be performed given the high level of anisotropy in terms of the spatial resolution of these sequences.

Finally, the impact on cerebral functional connectivity will be investigated *via* the analysis of resting-state fMRI. Connectivity matrices will be calculated for each individual based on cortical fragmentation obtained from morphological MRI, then compared between the two groups. The connectivity defects within a limited number of key regions (to be defined depending on the results obtained on the analysis of the above-mentioned methods) will also be studied. All of these analyses will be performed with the CONN toolbox [39].

Statistical analysis

Analysis of this unmatched exposure/non-exposure study will include a descriptive analysis of the different variables collected. For each continuous variable, we will calculate the statistical distribution parameters (means, medians, quartiles, percentiles of interest, range) as well as the dispersion parameters (standard deviation, variance, interquartile range, confidence interval of the main values). Qualitative variables will be described using the number of members and proportion of each category.

We will then explore whether a relationship exists between the different evaluation criteria and the participants' sociodemographic or clinical variables, so as to identify the presence of any confounding factors that may influence the measurement of the relationship between exposure and the onset of MRI abnormalities.

Generalized linear models will be used to assess differences between the two groups, exposed and unexposed, in terms of the cerebral modifications identified by MRI, as well as to assess the correlations between these imaging results and the neuropsychological test results. Confounding variables identified in univariate analysis (participant age, etc.) will be considered as potential adjustment variables. Given the number of subjects in this study, only a limited number of adjustment variables (one or two) will be

used, in order to ensure a sufficient power. The estimations of adjusted odds ratios and their confidence intervals will be calculated using regression models.

The effects exposure to heading will be estimated on the resulting a posteriori distributions (obtained by Markov chain Monte-Carlo methods). Differences between the two groups will be considered statistically significant if the probability that the difference is positive exceeds 0.95, or if the probability that the OR > 1 exceeds 0.95. All analyses will be carried out using R software.

Sample size and power calculations

The sample size was calculated based on the primary outcome, using the proportion of subjects presenting at least one MRI alteration. The size of the sample was calculated within an unmatched case–control study model (one case for one control subject) – setting the type-I error at 5%, power at 80% and assuming that 50% of the exposed cases will present at least one MRI alteration. The calculations were performed with EpiData software. A total of 40 subjects per group (Kesley formula) or 39 subjects (Fleiss formula) will allow us to demonstrate ORs of 4 or higher (or 0.25 or lower) between the two groups.

Missing, unused, or invalid data

After data entry and verification, the missing or invalid data will be analyzed in order to determine whether there is a non-random distribution, or not. After verification that there is no relationship between these data and the judgement criteria, the corresponding subjects will be excluded from the analysis.

Study management

Strasbourg University Hospital is the Sponsor of this study. The study will be overseen by the Department of Clinical Research and Innovation (DRCI) of Strasbourg University Hospital, in collaboration with the team of investigating physicians. A Data Monitoring Committee is not required for this study, as data collection takes place over the course of a single visit.

DISCUSSION

To the best of our knowledge, the present study will be the first prospective study to evaluate both cerebral and cognitive modifications in professional retired soccer players who have been exposed to subconcussive impacts. The primary limitation of this study is its monocentric nature. However, players in different soccer teams from different towns will be recruited, in order to represent a large panel of soccer players with intensive practice. Furthermore, it allows to pursue a similar imaging protocol for all participants, to ensure comparability of sequences between the groups, thus increasing the internal validity of the study. Another limitation is the focus on male soccer players, preventing the generalisability of the study to both sexes. Although it has been found that male players are more frequently exposed to heading than female players [24], studies suggest that subconcussion and its consequences are also relevant to female players [40]. Indeed, female soccer players seem to be at an elevated risk of concussion due to increased ball-to head impact [41].

However, despite these limitations, this study presents several strengths. To date, no previous study focusing on the development of chronic traumatic encephalopathy in athletes exposed to repetitive subconcussive head impacts has employed a combination of these different neuroimaging modalities with the aim of detecting subtle brain abnormalities [11–14]. This combinatorial approach is the major strength of this study, in addition to the complementary neuropsychological work-up, which will allow the evaluation of cognitive modifications in this population. By focussing on participants at the end of their professional careers, we hope to gain a more global vision of the cumulative effects of heading over the entire course of a footballer's career. Previous studies have featured populations with relatively young average ages, where the cumulative effects of heading impacts may not yet be detectable.

A further strength of this project is its highly collaborative nature. This project will be carried out in collaboration with research teams of the ICube laboratory (IMAGeS and MMB), who have specialized in the biomechanics of head injury for the past 20 years [42–44]. This team has acquired a good knowledge of single head injuries and has recently opened its research to the topic of repeated concussions. This protocol has been devised in collaboration with the French Football Federation, and

this partnership will also ensure the feasibility of the study in terms of recruitment of professional soccer players.

We expect that this study will provide a better understanding of the long-term effects of repetitive subconcussive impacts on the cerebral structures of professional male soccer players, as well as the functional and cognitive consequences of these impacts. This could ultimately contribute to the establishment of suitable, scientifically-founded follow-up and preventive measures in this population, which do not yet exist, and the extension of these measures to all those who practice this sport, especially children.

ETHICS AND DISSEMINATION

Ethical approvals, data and safety monitoring

Ethics approval has been obtained: the study was approved by the Comité de Protection des Personnes (CPP) N° 2021-A01169-32. A declaration of conformity to the Commission Nationale de l'Informatique et des Libertés (CNIL) was obtained (agreement number 2208067v0). This trial is registered with Clinical Trials Registry, NCT04903015. Any important modifications to the protocol will be submitted for approval to the CPP. If approved, updated versions of all trial documents will be provided to all persons involved in the study. Written informed consent will be obtained for each participant prior to enrolment, and each signature will be personally dated by the participant. The consent form will be securely retained by the investigator of the study. All participants will be informed that their personal study-related data will be used by the principal investigator in accordance with the local data protection law. Although no adverse events are expected in this trial (no contrast agent will be employed, and no medicinal product administered), any adverse events will be reported *via* the French Ministry of Health's Adverse Health Event Reporting Portal. Only the investigating team will have access to the trial dataset.

Dissemination

The findings of this study will be disseminated by publication in an international journal and in presentations at international conferences of neuroradiology. We plan to work in collaboration with the Medical Writer of Strasbourg University Hospital concerning the drafting and edition of this article. Participants will be notified of the results of their examinations by a follow-up visit with an investigating physician. Participants, the FFF, and regional delegations will be sent the final publication reporting study results.

REFERENCES

1 Levy ML, Kasasbeh AS, Baird LC, *et al.* Concussions in soccer: a current understanding. *World Neurosurg* 2012;**78**:535–44. doi:10.1016/j.wneu.2011.10.032

2 Decq P, Brauge D, Calmat A, *et al.* Diagnosis clinical criteria of sport related concussion: Toward an operational criteria definition in France. *Neurochirurgie* 2021;**67**:222–30. doi:10.1016/j.neuchi.2020.11.010

3 Spiotta AM, Bartsch AJ, Benzel EC. Heading in soccer: dangerous play? *Neurosurgery* 2012;**70**:1–11; discussion 11. doi:10.1227/NEU.0b013e31823021b2

4 Gysland SM, Mihalik JP, Register-Mihalik JK, *et al.* The relationship between subconcussive impacts and concussion history on clinical measures of neurologic function in collegiate football players. *Ann Biomed Eng* 2012;**40**:14–22. doi:10.1007/s10439-011-0421-3

5 Rodrigues AC, Lima MDM, de Souza LC, *et al.* No Evidence of Association Between Soccer Heading and Cognitive Performance in Professional Soccer Players: Cross-Sectional Results. *Front Neurol* 2019;**10**:209. doi:10.3389/fneur.2019.00209

6 McKee AC, Alosco ML, Huber BR. Repetitive Head Impacts and Chronic Traumatic Encephalopathy. *Neurosurg Clin N Am* 2016;**27**:529–35. doi:10.1016/j.nec.2016.05.009

7 Stern RA, Daneshvar DH, Baugh CM, *et al.* Clinical presentation of chronic traumatic encephalopathy. *Neurology* 2013;**81**:1122–9. doi:10.1212/WNL.0b013e3182a55f7f

8 Ling H, Morris HR, Neal JW, *et al.* Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. *Acta Neuropathol* 2017;**133**:337–52. doi:10.1007/s00401-017-1680-3

9 Grinberg LT, Anghinah R, Nascimento CF, *et al.* Chronic Traumatic Encephalopathy Presenting as Alzheimer's Disease in a Retired Soccer Player. *J Alzheimers Dis* 2016;**54**:169–74. doi:10.3233/JAD-160312

10 Mez J, Daneshvar DH, Kiernan PT, *et al.* Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. *JAMA* 2017;**318**:360–70. doi:10.1001/jama.2017.8334

11 Abbas K, Shenk TE, Poole VN, *et al.* Effects of repetitive sub-concussive brain injury on the functional connectivity of Default Mode Network in high school football athletes. *Dev Neuropsychol* 2015;**40**:51–6. doi:10.1080/87565641.2014.990455

12 Koerte IK, Ertl-Wagner B, Reiser M, *et al.* White matter integrity in the brains of professional soccer players without a symptomatic concussion. *JAMA* 2012;**308**:1859–61. doi:10.1001/jama.2012.13735

13 Manley G, Gardner AJ, Schneider KJ, *et al.* A systematic review of potential long-term effects of sport-related concussion. *Br J Sports Med* 2017;**51**:969–77. doi:10.1136/bjsports-2017-097791

14 Ng TS, Lin AP, Koerte IK, *et al.* Neuroimaging in repetitive brain trauma. *Alzheimers Res Ther* 2014;**6**:10. doi:10.1186/alzrt239

15 Coughlin JM, Wang Y, Munro CA, *et al.* Neuroinflammation and brain atrophy in former NFL players: An in vivo multimodal imaging pilot study. *Neurobiol Dis* 2015;**74**:58–65. doi:10.1016/j.nbd.2014.10.019

16 Hart J, Kraut MA, Womack KB, *et al.* Neuroimaging of cognitive dysfunction and depression in aging retired National Football League players: a cross-sectional study. *JAMA Neurol* 2013;**70**:326–35. doi:10.1001/2013.jamaneurol.340

17 Casson IR, Viano DC, Haacke EM, *et al.* Is There Chronic Brain Damage in Retired NFL Players? Neuroradiology, Neuropsychology, and Neurology Examinations of 45 Retired Players. *Sports Health* 2014;**6**:384–95. doi:10.1177/1941738114540270

18 Lin AP, Ramadan S, Stern RA, *et al.* Changes in the neurochemistry of athletes with repetitive brain trauma: preliminary results using localized correlated spectroscopy. *Alzheimers Res Ther* 2015;**7**:13. doi:10.1186/s13195-015-0094-5

19 Baugh CM, Kiernan PT, Kroshus E, *et al.* Frequency of head-impact-related outcomes by position in NCAA division I collegiate football players. *J Neurotrauma* 2015;**32**:314–26. doi:10.1089/neu.2014.3582

20 Morales JS, Valenzuela PL, Saco-Ledo G, *et al.* Mortality Risk from Neurodegenerative Disease in Sports Associated with Repetitive Head Impacts: Preliminary Findings from a Systematic Review and Meta-Analysis. *Sports Med* Published Online First: 21 October 2021. doi:10.1007/s40279-021-01580-0

21 Sandmo SB, Filipcik P, Cente M, *et al.* Neurofilament light and tau in serum after head-impact exposure in soccer. *Brain Inj* 2020;**34**:602–9. doi:10.1080/02699052.2020.1725129

22 Straume-Naesheim TM, Andersen TE, Dvorak J, *et al.* Effects of heading exposure and previous concussions on neuropsychological performance among Norwegian elite footballers. *Br J Sports Med* 2005;**39 Suppl 1**:i70-77. doi:10.1136/bjsm.2005.019646

23 Matser JT, Kessels AG, Jordan BD, *et al.* Chronic traumatic brain injury in professional soccer players. *Neurology* 1998;**51**:791–6. doi:10.1212/wnl.51.3.791

24 Sandmo SB, Andersen TE, Koerte IK, *et al.* Head impact exposure in youth football-Are current interventions hitting the target? *Scand J Med Sci Sports* 2020;**30**:193–8. doi:10.1111/sms.13562

25 McCunn R, Beaudouin F, Stewart K, *et al.* Heading in Football: Incidence, Biomechanical Characteristics and the Association with Acute Cognitive Function-A Three-Part Systematic Review. *Sports Med* 2021;**51**:2147–63. doi:10.1007/s40279-021-01492-z

26 Strauss SB, Fleysher R, Ifrah C, *et al.* Framing potential for adverse effects of repetitive subconcussive impacts in soccer in the context of athlete and non-athlete controls. *Brain Imaging Behav* 2021;**15**:882–95. doi:10.1007/s11682-020-00297-4

27 Snowden T, Reid H, Kennedy S, *et al.* Heading in the Right Direction: A Critical Review of Studies Examining the Effects of Heading in Soccer Players. *J Neurotrauma* 2021;**38**:169–88. doi:10.1089/neu.2020.7130

28 Nasreddine ZS, Phillips NA, Bédirian V, *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;**53**:695–9. doi:10.1111/j.1532-5415.2005.53221.x

29 Van der Linden M, GREMEM. *L'évaluation des troubles de la mémoire*. De Boeck Supérieur 2004.

30 Meyers JE, Meyers KR. Rey complex figure test under four different administration procedures. *The Clinical Neuropsychologist* 1995;**9**:63–7. doi:10.1080/13854049508402059

31 Bright P, Hale E, Gooch VJ, *et al.* The National Adult Reading Test: restandardisation against the Wechsler Adult Intelligence Scale-Fourth edition. *Neuropsychol Rehabil* 2018;**28**:1019–27. doi:10.1080/09602011.2016.1231121

32 Godefroy O, GREFEX. *Fonctions exécutives et pathologies neurologiques et psychiatriques*. De Boeck Supérieur 2012.

33 Zimmermann P, Fimm B. *Testbatterie zur Aufmerksamkeitsprüfung - Version 2.2: (TAP) ; [Handbuch]*. Psytest https://www.psytest.net/de/testbatterien/tap/bestellen

34 Baron-Cohen S, Wheelwright S, Hill J, *et al.* The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry* 2001;**42**:241–51.

35 Løvstad M, Sigurdardottir S, Andersson S, *et al.* Behavior Rating Inventory of Executive Function Adult Version in Patients with Neurological and Neuropsychiatric Conditions: Symptom Levels and Relationship to Emotional Distress. *J Int Neuropsychol Soc* 2016;**22**:682–94. doi:10.1017/S135561771600031X

36 Smith SM, Zhang Y, Jenkinson M, *et al.* Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002;**17**:479–89. doi:10.1006/nimg.2002.1040

37 Fischl B, Salat DH, Busa E, *et al.* Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;**33**:341–55. doi:10.1016/s0896-6273(02)00569-x

38 Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000;**11**:805–21. doi:10.1006/nimg.2000.0582

39 Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* 2012;**2**:125–41. doi:10.1089/brain.2012.0073

40 Myer GD, Barber Foss K, Thomas S, *et al.* Altered brain microstructure in association with repetitive subconcussive head impacts and the potential protective effect of jugular vein compression: a longitudinal study of female soccer athletes. *Br J Sports Med* 2019;**53**:1539–51. doi:10.1136/bjsports-2018-099571

41 Blyth RJ, Alcock M, Tumilty DS. Why are female soccer players experiencing a concussion more often than their male counterparts? A scoping review. *Phys Ther Sport* 2021;**52**:54–68. doi:10.1016/j.ptsp.2021.08.001

42 Deck C, Bourdet N, Meyer F, *et al.* Protection performance of bicycle helmets. *J Safety Res* 2019;**71**:67–77. doi:10.1016/j.jsr.2019.09.003

43 Sahoo D, Robbe C, Deck C, *et al.* Head injury assessment of non-lethal projectile impacts: A combined experimental/computational method. *Injury* 2016;**47**:2424–41. doi:10.1016/j.injury.2016.09.004

44 Bliven E, Rouhier A, Tsai S, *et al.* Evaluation of a novel bicycle helmet concept in oblique impact testing. *Accid Anal Prev* 2019;**124**:58–65. doi:10.1016/j.aap.2018.12.017

AUTHORS' CONTRIBUTIONS

SK, FL, FB, SK conceived the idea of the study. SK, FL, JG, FB, MB, AB, CK, JM, EO, FP, FG, CD, VN, RW and SK formed the working group that wrote the study protocol. JG performed statistical analysis for the sample size calculation. All co-authors have approved the protocol and will participate in the study.

ACKNOWLEDGEMENTS

Manuscript edition was provided by Dr. Kate Dunning (HUS).

FUNDING STATEMENT

This work was supported by Strasbourg University Hospital (HUS), Philipps and GE Healthcare. Philipps and GE Healthcare were not involved in the conception or design of this study protocol.

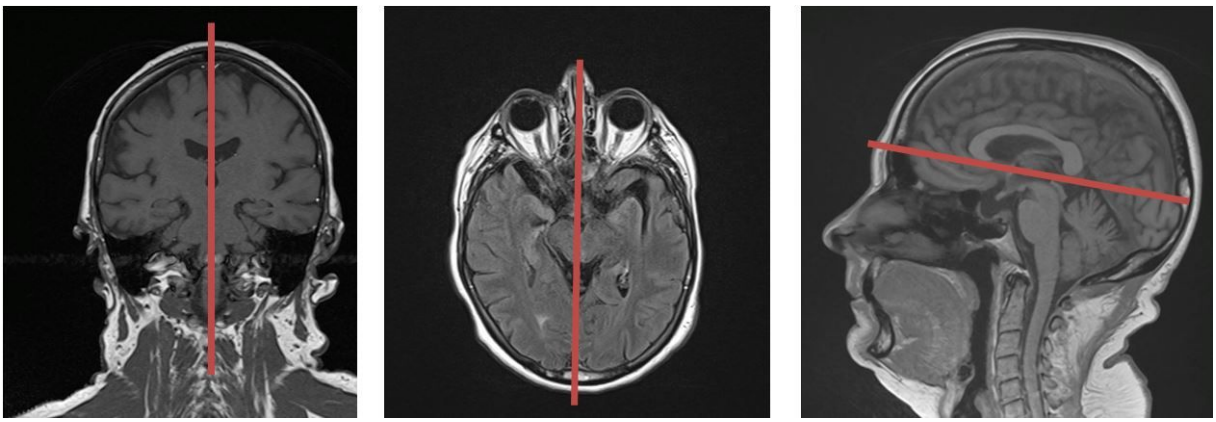
COMPETING INTERESTS STATEMENT

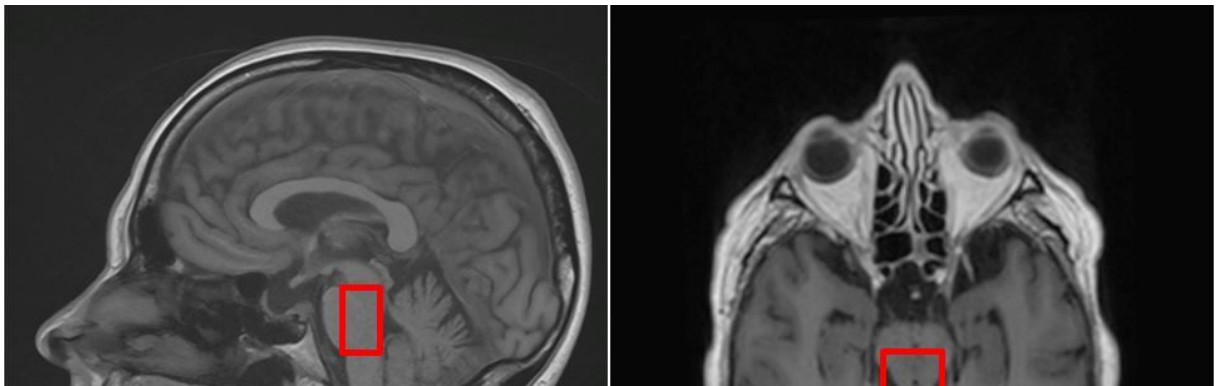
No author declared competing interests concerning this study.

FIGURE LEGENDS

Figure 1. Human brain in the three spatial planes: coronal, horizontal, and sagittal sections. From left to right: coronal T1-weighted, axial FLAIR, and sagittal T1-weighted images.

Figure 2. Short echo time monovoxel 1H MR spectroscopy of the brainstem. With a width of 20 mm, the voxel is placed on the two posterior thirds of the pons and must cover its entire height.





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2, 16
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	/
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 22
	5b	Name and contact information for the trial sponsor	15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15, 22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	/

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4–6
	6b	Explanation for choice of comparators	N/A
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7–8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9–12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	/
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8–9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9–13

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**N/A – all study data is collected at the enrolment visit.**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**9, 11**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**13–14**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**14**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**14**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**15**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N/A**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**16–17**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**N/A**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**16**

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16–17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16–17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	/
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Cerebral and cognitive modifications in retired professional soccer players: TC-FOOT protocol, a transverse analytic study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-060459.R1
Article Type:	Protocol
Date Submitted by the Author:	20-Sep-2022
Complete List of Authors:	Kepka, Sabrina; University Hospital Centre Strasbourg, Emergency department Lersy, François; University Hospital Centre Strasbourg, Imaging department Godet, Julien; Public health Unit, University hospital of Strasbourg Blanc, Frederic; University Hospital of Strasbourg Bilger, Mathias; University Hospital Centre Strasbourg, Neuropsychology department Botzung, Anne; University Hospital Centre Strasbourg, Geriatrics and Neurology Departments, Research and Resources Memory Center (CM2R) Kleitz, Catherine; University Hospital Centre Strasbourg, Neuropsychology department Merignac, Jeanne; University Hospital Centre Strasbourg, Geriatrics and Neurology Departments, Research and Resources Memory Center Ohrant, Emmanuel; Fédération Française de Football Garnier, franck; School of Osteopathy, College COS Strasbourg Pietra, François; Racing club Strasbourg Noblet, Vincent; ICube, UMR 7357, University of Strasbourg-C Deck, Caroline; ICube, UMR 7357, University of Strasbourg Willinger, Remy; Université de Strasbourg Kremer , Stéphane ; Université de Strasbourg
Primary Subject Heading:	Radiology and imaging
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Magnetic resonance imaging < RADIOLOGY & IMAGING, SPORTS MEDICINE, Delirium & cognitive disorders < PSYCHIATRY

Cerebral and cognitive modifications in retired professional soccer players: TC-FOOT protocol, a transverse analytic study.

Sabrina Kepka[1, 2], François Lersy[3], Julien Godet [2,4], Frédéric Blanc[2,5], Matthias Bilger[6], Anne Botzung[5], Catherine Kleitz[6], Jeanne Merignac[5], Emmanuel Ohrant[7], Franck Garnier[8,9], François Pietra[10], Vincent Noblet[2], Caroline Deck[2], Rémy Willinger[2], Stéphane Kremer[2, 3]

[1]Emergency department, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[2]ICube, UMR 7357, University of Strasbourg-CNRS, Strasbourg, France

[3]Imaging department 2, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France

[4]Public Health department, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

[5]Geriatrics and Neurology Departments, Research and Resources Memory Center (CM2R), Strasbourg University Hospital, Strasbourg,

[6]Neuropsychology department, Hôpitaux Universitaires de Strasbourg, Hôpital de Hautepierre, Strasbourg, France

[7]Fédération Française de Football (FFF), Paris, France

[8]School of Osteopathy, College COS Strasbourg, Strasbourg, France

[9]Medical Sport Center of Strasbourg (CMSM), Strasbourg, France

[10]Racing Club Strasbourg, Strasbourg, France

Corresponding Author:
Sabrina Garnier-Kepka

Permanent address:
Emergency department
CHRU Strasbourg – Nouvel hôpital civil
1 place de l'hôpital, 67091 Strasbourg
France
Tel: +33 3 69 55 13 35
Fax: +33 3 69 55 18 56
Email: sabrinakepka@yahoo.fr

ABSTRACT

Introduction

Soccer is the most popular sport in the world. This contact sport carries the risk of exposure to repeated head impacts in the form of subconcussions, defined as minimal brain injuries following head impact, with no symptom of concussion. Whilst it has been suggested that exposure to repetitive subconcussive events can result in long-term neurophysiological modifications, and the later development of chronic traumatic encephalopathy, the consequences of these repeated impacts remain controversial and largely unexplored in the context of soccer players.

Methods and analysis

This is a prospective, single center, exposure/nonexposure, transverse study assessing the MRI and neuropsychological abnormalities in professional retired soccer players exposed to subconcussive impacts, compared to high-level athletes not exposed to head impacts. The primary outcome corresponds to the results of MRI by advanced MRI techniques (diffusion tensor, cerebral perfusion, fMRI, cerebral volumetry and cortical thickness, spectroscopy, susceptibility imaging). Secondary outcomes are the results of the neuropsychological tests: number of errors and time to complete tests. We hypothesize that repeated subconcussive impacts could lead to morphological lesions and impact on soccer players' cognitive skills in the long term.

Ethics and dissemination

Ethics approval has been obtained and the study was approved by the Comité de Protection des Personnes (CPP) N° 2021-A01169-32. Study findings will be disseminated by publication in a high-impact international journal. Results will be presented at national and international imaging meetings.

Registration

This trial is registered with Clinical Trials Registry NCT04903015.

Key words

Soccer

Subconcussions

MRI

Neuropsychological assessment

Article summary

Strengths and limitations of this study

- To the best of our knowledge, this is the first prospective study to evaluate both the cerebral and cognitive modifications following repetitive subconcussive impacts in male professional soccer players at the end of their careers, or in retirement, a population which is understudied in the literature.

- The major strength of this study is its combinatorial approach: the abnormalities will be evaluated by a combination of advanced Magnetic Resonance Imaging (MRI) techniques (brain volumetry and cortical thickness, diffusion tensor, magnetic susceptibility, spectroscopy, functional MRI (fMRI), and cerebral perfusion), using specific modalities of analysis, by a team specialized in the biomechanics of head injury.

- The neuropsychological work-up will be based on validated tests in the field of head injuries, with a proven sensitivity for assessing those functions known to be frequently impaired by repetitive subconcussive impacts e.g., processing speed, working memory, sustained attention and executive functions, and episodic memory.

- Although the single center study design may be seen as a limitation, players in different soccer teams from different towns will be recruited, in order to represent a large panel of soccer players with intensive practice, whilst ensuring a similar imaging protocol for all participants.

- This study is limited in its generalisability to both sexes, as only male soccer players are considered.

Word count : 4457

INTRODUCTION

Soccer, one of the most popular sports in the world, exposes the player to cerebral concussions, or mild traumatic brain injuries, which represent 5.8–22% of all soccer-related injuries [1]. An expert working group from the French Ministry of Sports has defined concussion as a brain injury caused by a direct or indirect transmission of kinetic energy to the head, resulting in an immediate and transient dysfunction of the brain [2]. It is characterised by at least one of the following features: loss of consciousness, loss of memory, altered mental status, and neurological signs and symptoms which cannot be explained by another cause [2]. The Consensus statement from the Concussion in Sport Group defined sport related concussion as a traumatic brain injury induced by biomechanical forces caused by a direct blow to the head, face, neck or elsewhere on the body with an impulsive force transmitted to the head, resulting in the rapid onset of short-lived impairment of neurological function that resolves spontaneously, a range of clinical signs that may or may not involve loss of consciousness and cannot be explained by drug, alcohol, or medication use, other injuries or other comorbidities [3].

Subconcussion is a cranial impact that does not result in known or diagnosed concussion on clinical grounds [4]. It can occur with rapid acceleration-deceleration of the body notably when the brain is free to move within the cranium creating a “slosh” phenomenon [5]. Whilst concussions are associated with neurological impairment [6], the consequences of subconcussion remain unclear, notably for professional soccer players [7]. Soccer headings is a skill specific to soccer, with an average of 6 to 12 headings occurring per game per player, exposing soccer player to repetitive subconcussions [8]. Repetitive exposure to subconcussion could increase its effect with accruing sufficient anatomical and/or physiological damage, explaining that these injuries are potentially expressed later in life [4]. Indeed, it has been suggested that subconcussive impacts could increase the risk of traumatic chronic encephalopathy, a neurodegenerative disease affecting subjects who have undergone head impacts over a number of years [9]. Clinically, subjects have memory impairment, cognitive impairment, anxiety and depressive symptoms, and psychiatric problems [10]. The diagnosis is confirmed upon

anatomopathological examination of brain tissue, by the presence of phosphorylated tau protein deposits distributed throughout the brain, with a tendency to occur in clusters at the sulcal depths of the cortex [9,11–13]. However, it is currently impossible to confirm the diagnosis *in vivo*. The link between traumatic chronic encephalopathy and repetitive subconcussive impact remains controversial as other studies have not corroborated that subconcussive impacts can be responsible for MRI abnormalities and cognitive impairments.

Conventional imaging techniques such as computed tomography (CT) or MRI only show morphological cerebral abnormalities (intra- or pericerebral hemorrhages, diffuse axonal lesions) in cases of severe head injury. These morphological techniques do not usually identify an abnormality in cases of mild concusssion, even when repetitive. On the other hand, advanced MRI techniques (brain volumetry and cortical thickness, diffusion tensor, magnetic susceptibility, spectroscopy, functional MRI, and cerebral perfusion) have made it possible to detect subtle brain abnormalities in athletes or former athletes exposed to repetitive subconcussive impacts, including in the absence of proven concussion [14–17]. Several of the aforementioned techniques have demonstrated a loss of both white and grey matter in athletes who are subjected to repeated subconcussive impacts [15,18,19]. According to magnetic susceptibility studies, the loss of substance can be associated with hemorrhagic lesions and inflammation [20]. Moreover, a study using fMRI techniques revealed a prolonged alteration in resting state functional connectivity in American football players over the course of a football season [14]. This suggests that repetitive impacts could have a cumulative effect on modifications to functional connectivity in the brain networks of athletes. In former American football players, fMRI has also demonstrated a cerebral reorganisation during memory tasks, including hyperactivation in compensatory zones. It is suspected that these abnormalities are related to later development of chronic traumatic encephalopathy [12,17,21].

In American football players, the number of years of practice and the degree of exposure to physical impacts linked to the player's position has been shown to increase the risk of developing traumatic chronic encephalopathy [22]. In soccer players, a recent systematic review revealed a higher risk of

mortality from motor neuron disease than in the general population [23]. However, previous research revealed that head impacts in soccer had no effect on blood-based biomarkers for structural brain damage, such as serum neurofilament light or tau [24]. A limited number of studies have carried out cognitive assessments of soccer players, and have reported contradictory results [7,25,26]. Whilst the study of Matser *et al.* suggested that soccer may adversely affect some aspects of cognitive functioning [26], no association was found between exposure and cognitive performance in a recent cross-sectional study specifically evaluating the effects of soccer heading [7], in agreement with the results of Straume-Naesheim *et al*. [25]. However, this study did not have a follow-up period, and soccer players were relatively young (average age of 24.6 ± 4.5 years), meaning that any cumulative effects of heading across a player's career. Indeed, a systematic review has highlighted the lack of studies evaluating the long-term effect of heading on cognitive performance [27]. A further study assessing the effect of soccer heading on diffusion tension images and cognitive function found no significant difference in terms of microstructural features and cognitive performance between non-athlete participants unexposed to heading and amateur soccer players with high exposure [28]. However, the players enrolled in this study were, again, of relatively young age (25.5 ± 7.2), which could limit its long-term relevance. Moreover, to date, none of these studies have employed all neuroimaging modalities in a complementary fashion. A recent systematic review by Snowden *et al.* determined that there is not enough evidence to conclude on the effects of subconcussion in soccer players, and that further studies are required to better evaluate the impact of repetitive subconcussive injuries [29].

Objectives

The objective of this study is therefore to evaluate, using advanced MRI techniques, the potential microstructural abnormalities and the cognitive impairment in professional soccer players at the end of their careers who have experienced repetitive subconcussive impacts. The primary objective is to identify cerebral abnormalities observed by MRI in professional soccer players exposed to repeated subconcussive impacts, and to compare these results to those of high-level athletes who are not exposed to head impacts. The secondary objectives are to compare the cognitive performances in a group of professional soccer player exposed to repeated subconcussive impacts with non-exposed high-level

athletes and to evaluate the relationship between the microstructural anomalies demonstrated by MRI and cognitive impairment.

METHODS AND ANALYSIS

Study design and setting

This is a monocentric, transversal, exposure/non-exposure study assessing the relationship between exposure to repetitive subconcussive impacts during professional soccer play and the presence of cerebral and cognitive abnormalities. This study will take place at Strasbourg University Hospital, Strasbourg, France, a public university hospital, over a study period of 24 months, with a study start date of January 7, 2022 and will end on January 8, 2024.

Patient and public involvement

Patients were not involved in the conception of this study.

Eligibility criteria

Two groups of participants will be established: a group of professional soccer players exposed to repetitive subconcussive impacts, and a control group of non-exposed high-level athletes.

Inclusion criteria common to both groups are: (1) male, (2) aged 32–55 years old

Inclusion criteria specific to the exposed group are: (1) professional soccer players at the end of their career (2) playing in clubs in the French Ligue 1 and Ligue 2; (3) exposed to repetitive subconcussive impact; (4) no history of severe head injury or cerebral lesion.

Inclusion criteria specific to the control group are: (1) high-level athletes who have never regularly played a sport exposing them to repetitive subconcussive impact and who have no history of head injury (e.g., rugby, basketball, handball, American football, hockey, combat sports). Professional tennis players or former players will be preferentially recruited.

Non-inclusion criteria common to both groups are: (1) refusal to particate in the study; (2) refusal to be informed of abnormalities on MRI; (3) incapacity to give informed consent or under a legal protection order; (4) history of sport related concussion defined as a traumatic brain injury induced by biomechanical forces caused by a direct blow to the head, face, neck or elsewhere on the body with an

impulsive force transmitted to the head, resulting in the rapid onset of short-lived impairment of neurological function that resolves spontaneously, with a range of clinical signs and symptoms that may or may not involve loss of consciousness and cannot be explained by drug, alcohol, or medication use, other injuries or other comorbidities ; (5) history of severe brain/head injury; (6) history of neurological or psychiatric disorder according to the patient's declaration; (7) known cerebral abnormality diagnosed by an imaging exam (CT or MRI); (8) history of regular or occasional drug use: active smoker or non-smoker for less than 1 year, excessive consumption of alcohol (>20 g alcohol per day, evaluated with the following formula: [8 x percentage of alcohol x volume (cL))/100]), whether current or in the past; (9) use of medication targeting the central nervous system in the 2 weeks preceding inclusion in the study; (10) prior history of severe hypertension, diabetes, chronic heart disease, progressive or disabling disease; (11) contraindication to MRI (claustrophobia, implanted material not compatible with MRI, refusal to be informed of any abnormalities discovered by MRI)

If a psychiatric disorder is suspected at the time of assessment, the participant will be automatically excluded.

Recruitment

The group of professional soccer players at the end of their careers will be recruited from clubs playing in French Ligue 1 and Ligue 2, with the assistance of club physicians, under the supervision of the medical director of the French Federation of Soccer (FFF). The control group of high-level athletes not exposed to head injuries will be recruited *via* the Medical Sport Center of Strasbourg (CMSM) located in Strasbourg, France, which is a referent center for the care of high-level athletes, and by the regional delegations of the French federations of various sports (tennis, athletics, swimming). Information leaflets will be displayed within the center and on club premises. Recruitment will take place during the entire 24-month period of the study.

Participant timeline and follow-up

An initial information visit will take place by phone call, to verify that the subject meets the eligibility criteria. Approximately one week after this information visit, a second visit will take place at the Radiology department of Strasbourg University Hospital. During this visit, an investigating physician will collect written informed consent. Following this, the neuropsychological evaluations and MRI tests will be carried out.

A follow-up visit will be organized by an investigating physician, in order to communicate the results of the examinations to participants. The results can fall into three categories: normal work-up, FLAIR sequence MRI abnormality, and/or demonstration of cognitive impairment. This visit will be carried out by telephone call in the case of normal results, and by an in-person visit at Strasbourg University Hospital in the case of abnormal results.

Outcomes

The primary outcome of this study is the results of brain MRI in professional soccer players exposed to repeated subconcussive impact potentially related to chronic traumatic encephalopathy, compared to high-level athletes who are not exposed to head injuries, using advanced MRI techniques, namely: diffusion tensor imaging, cerebral perfusion, fMRI, cerebral volumetry and cortical thickness, magnetic resonance spectroscopy (MRS), and susceptibility imaging. The main evaluation criterion corresponds to the quantitative MRI modifications (professional soccer player compared with control group).

Secondary outcomes of this study are the results of the neuropsychological tests:

-performance in a targeted battery of neuropsychological tasks as evaluated by the number of errors and time to complete tests

-Questioning on recurrent symptoms such as headaches (number of years the subject has experienced headaches, since when, frequency), fatigability, sleep disturbance, dizziness, blurry vision, photophobia/phonophobia.

Data collection, storage and verification

Socio-demographic and clinical data

Socio-demographic and clinical characteristics of patients will be recorded by an investigating physician: age, education level, medical history including cardiovascular risk factors, neurological history, history of COVID-19, type of sport practiced, and the modalities of practice. For the group of soccer players, the position played will also be recorded.

Neuropsychological assessment

For the neuropsychological assessment we have decided to employ a series of validated tests, that have shown sensitivity in the field of head injuries, in order to assess the functions frequently impaired by subconcussive impacts, i.e. processing speed, working memory, sustained attention and executive functions, and episodic memory. This assessment will last approximately 2 hours, and will be carried out by specialised neuropsychologists, ensuring the quality of the data.

The following tests were chosen:

1. Montreal Cognitive Assessment scale: an overall efficiency assessment test (= 10 min) [30]
2. RL RI 16 items (a French adaptation of the Free and Cued Selective Reminding Test) to assess the functioning of verbal episodic memory (encoding = 10 min) [31]
3. Copy of Rey's complex figure assessing visual-constructive abilities and planning (= 5 min) [32]
4. Auditory-verbal and visuospatial spans to assess short-term working memory (Wechsler scale Memory form III) (= 5 min) [33]
5. Trail Making Test A and B, assessing treatment speed and mental flexibility (= 5 min) [34]
6. The Brixton Test, a spatial anticipation task that also evaluates executive functioning (= 15 min) [34]
7. RL RI 16 items to assess the functioning of verbal episodic memory (delayed recall at 20 min) [31]
8. Memory reproduction of Rey's complex figure in order to assess episodic visual memory (delayed recall at 20 min) [32]

9. Phonological and semantic fluency tests for executive functions (letter P and Animals; 2 minutes per test, 4 minutes total) [34]
10. Computerized attention tests will also be used to measure reaction times and executive aspects. Subtests: Phasic Alert, Split Attention and Incompatibility version 2.3.1. This is a computerized battery of tests, using no paper questionnaires (= 15 min) [35]
11. Social cognition test "Reading the Mind in the Eyes", to assess ability to read the emotions of others which, in turn, is related to performance in team problem-solving tasks (= 10 min) [36]

In addition to the aforementioned tests, two further questionnaires will be carried out:

1. A self-administered questionnaire, BRIEF-A (Behavior Rating Inventory of Executive Function – Adult Version), evaluating executive functions (<10 minutes) [37]
2. Questioning on recurrent symptoms such as headaches (number of years the subject has experienced headaches, since when, frequency), fatigability, sleep disturbance, dizziness, blurry vision, photophobia/phonophobia.

MRI

Acquisition

MRIs will be carried out on 3 Tesla MRI scanners.

The following sequences will be acquired:

1. 3D T1 gradient echo (GRE): anatomy, registration, cerebral, white and grey matter volume, cortical thickness;
2. Multi-echo 3D T2 GRE: quantitative susceptibility mapping (QSM), iron overload quantification;
3. 3D fluid-attenuated inversion recovery (FLAIR);
4. Continuous arterial spin labeling (ASL) 3D: cerebral perfusion;
5. Resting-state fMRI: functional connectivity;

6. 64-direction DTI (b=1000 and 2500): alterations in white matter and its microstructure, anatomic connectivity;
7. Monovoxel spectroscopy of the mesencephalus with short echo time (TE).

Any contraindications will be verified before performing MRI. The patient should preferably be wearing a hospital gown without snaps for safety reasons. The patient's head should be placed so as to acquire DTI and 3D ASL sequences without having to tilt the views in the axis of the petrous bones (angles at 0° and good right/left symmetry of the acquisitions).

The images will be acquired with the examination room door closed. Checks will be carried out prior to image acquisition, to ensure that there is sufficient disc space in the database, and the image quality will be checked during the acquisition. If artifacts are present, the sequences will be repeated after correction.

The sequences should be oriented respecting the three spatial planes (Figure 1).

- The T1 sagittal sequence should cover the entire encephalon;
- The 3D T1, 3D FLAIR and the multiecho T2 GRE sequences should cover the entire encephalon;
- The DTI, fMRI, and 3D ASL sequences should be oriented strictly axial (angles at 0°). All of the encephalon should be covered.

The monovoxel spectroscopy should be placed on the brain stem (Figure 2). The voxel should be placed on the posterior two-thirds of the brain stem and cover the entire height.

Image processing and analysis

Each of the imaging modalities will be analysed independently, at different levels of analysis: 1) overall analysis conducted on all white matter and all grey matter, 2) regional analysis on a limited number of regions of interest (cerebral anatomic structures, cortical regions), and 3) focal analysis (or voxel-based) on the entire brain.

Cerebral atrophy will be studied by analysing the 3D T1 GRE morphological sequences, globally, using the SIENAX method implanted in the FSL library (University of Oxford) [38]. Regional analysis (volume of anatomic structures and mean cortical thickness of certain cortical regions) will be carried out using FREESURFER software [39], and focal analysis will be carried out using the Voxel-Based Morphometry (VBM) approach [40], using the implantation available in the Statistical Parametric Mapping (SPM) software (Wellcome Centre for Human Neuroimaging).

The completeness of the white matter will be investigated by analysing the tensor diffusion MRI sequences, globally and regionally, using segmentations obtained from morphological MRI (see preceding paragraph), and focally using the Tract-Based Spatial Statistics (TBSS) method implanted in the FSL library.

Vascular damage will be assessed *via* the study of global and regional cerebral perfusion in ASL, as well as by regional analysis of microhemorrhages and of iron deposits revealed by the QSM sequences. These analyses will also be carried out using the segmentations obtained from morphological MRI. It should be noted that focal analysis of these modalities cannot be performed given the high level of anisotropy in terms of the spatial resolution of these sequences.

Finally, the impact on cerebral functional connectivity will be investigated *via* the analysis of resting-state fMRI. Connectivity matrices will be calculated for each individual based on cortical fragmentation obtained from morphological MRI, then compared between the two groups. The connectivity defects within a limited number of key regions (to be defined depending on the results obtained on the analysis of the above-mentioned methods) will also be studied. All of these analyses will be performed with the CONN toolbox [41].

Statistical analysis

Analysis of this unmatched exposure/non-exposure study will include a descriptive analysis of the different variables collected. Neuropsychological results will be compared with normative data by education level and age adapted to the country.

For each continuous variable, we will calculate the statistical distribution parameters (means, medians, quartiles, percentiles of interest, range) as well as the dispersion parameters (standard deviation, variance, interquartile range, confidence interval of the main values). Qualitative variables will be described using the number of members and proportion of each category.

We will then explore whether a relationship exists between the different evaluation criteria and the participants' sociodemographic or clinical variables (age, education level, medical history including cardiovascular risk factors, neurological history, history of COVID-19, type of sport practiced, and the modalities of practice), so as to identify the presence of any confounding factors that may influence the measurement of the relationship between exposure and the MRI abnormalities.

Generalized linear models will be used to assess differences between the two groups, exposed and unexposed, in terms of the cerebral modifications identified by MRI, as well as to assess the correlations between these imaging results and the neuropsychological test results. Confounding variables identified in univariate analysis (participant age, etc.) will be considered as potential adjustment variables. Given the number of subjects in this study, only a limited number of adjustment variables (one or two) will be used, in order to ensure a sufficient power. The estimations of adjusted odds ratios and their confidence intervals will be calculated using regression models.

The effects exposure to heading will be estimated on the resulting a posteriori distributions (obtained by Markov chain Monte-Carlo methods). Differences between the two groups will be considered statistically significant if the probability that the difference is positive exceeds 0.95, or if the probability that the OR > 1 exceeds 0.95. All analyses will be carried out using R software.

Sample size and power calculations

The sample size was calculated based on the primary outcome, using the proportion of subjects presenting at least one MRI alteration. The size of the sample was calculated within an unmatched case–control study model (one case for one control subject) – setting the type-I error at 5%, power at 80% and assuming that 50% of the exposed cases will present at least one MRI alteration. The calculations were performed with EpiData software. A total of 40 subjects per group (Kesley formula) or 39 subjects (Fleiss formula) will allow us to demonstrate ORs of 4 or higher (or 0.25 or lower) between the two groups.

Missing, unused, or invalid data

After data entry and verification, the missing or invalid data will be analyzed in order to determine whether there is a non-random distribution, or not. After verification that there is no relationship between these data and the judgement criteria, the corresponding subjects will be excluded from the analysis.

Study management

Strasbourg University Hospital is the Sponsor of this study. The study will be overseen by the Department of Clinical Research and Innovation (DRCI) of Strasbourg University Hospital, in collaboration with the team of investigating physicians. A Data Monitoring Committee is not required for this study, as data collection takes place over the course of a single visit.

DISCUSSION

Cerebral and cognitive impairments due to repetitive subconcussive impacts in soccer remains unknown as studies provided contradictory results. Indeed, no differences were verified in brain structure on MRI between soccer players and controls in the study of Jordan et al [42]. But, men in the soccer group were young, with a mean age of 24.8 years. Concerning biomarkers of brain injury, if some studies revealed that S-100B serum levels increase after heading [43], other studies found no difference with control group [44]. Finally, studies evaluating effect on brain function revealed contradictory findings. Some studies revealed that an increasing number of headings and concussions were associated negatively with

cognitive functioning [45-46] whereas other studies found no relationship between either cumulative head injury or cumulative heading and cognitive functioning but were conducted among young soccer players [47-48].

To the best of our knowledge, the present study will be the first prospective study to evaluate both cerebral and cognitive modifications in professional retired soccer players who have been exposed to subconcussive impacts.

The current study had some limitations. The primary limitation is its monocentric nature. However, players in different soccer teams from different towns will be recruited, in order to represent a large panel of soccer players with intensive practice. Furthermore, it allows to pursue a similar imaging protocol for all participants, to ensure comparability of sequences between the groups, thus increasing the internal validity of the study. Another limitation is the focus on male soccer players, preventing the generalisability of the study to both sexes. Although it has been found that male players are more frequently exposed to heading than female players [24], studies suggest that subconcussion and its consequences are also relevant to female players [49]. Indeed, female soccer players seem to be at an elevated risk of concussion due to increased ball-to head impact [50].

However, despite these limitations, this study presents several strengths. To date, no previous study focusing on cerebral modifications in athletes exposed to repetitive subconcussive head impacts has employed a combination of these different neuroimaging modalities with the aim of detecting subtle brain abnormalities [14–17]. This combinatorial approach is the major strength of this study, in addition to the complementary neuropsychological work-up, which will allow the evaluation of cognitive modifications in this population. By focussing on participants at the end of their professional careers, we hope to gain a more global vision of the cumulative effects of heading over the entire course of a footballer's career. Previous studies have featured populations with relatively young average ages, where the cumulative effects of heading impacts may not yet be detectable.

A further strength of this project is its highly collaborative nature. This project will be carried out in collaboration with research teams of the ICube laboratory (IMAGeS and MMB), who have specialized in the biomechanics of head injury for the past 20 years [51-53]. This team has acquired a good knowledge of single head injuries and has recently opened its research to the topic of repeated concussions. This protocol has been devised in collaboration with the French Football Federation, and this partnership will also ensure the feasibility of the study in terms of recruitment of professional soccer players.

We expect that this study will provide interesting data about cerebral structures and cognitive function of professional male soccer players exposed to subconcussive impacts. This could ultimately contribute to the establishment of suitable, scientifically-founded follow-up and preventive measures in this population, which do not yet exist, and the extension of these measures to all those who practice this sport, especially children.

ETHICS AND DISSEMINATION

Ethical approvals, data and safety monitoring

Ethics approval has been obtained: the study was approved by the Comité de Protection des Personnes (CPP) N° 2021-A01169-32. A declaration of conformity to the Commission Nationale de l'Informatique et des Libertés (CNIL) was obtained (agreement number 2208067v0). This trial is registered with Clinical Trials Registry, NCT04903015. Any important modifications to the protocol will be submitted for approval to the CPP. If approved, updated versions of all trial documents will be provided to all persons involved in the study. Written informed consent will be obtained for each participant prior to enrolment, and each signature will be personally dated by the participant. The consent form will be securely retained by the investigator of the study. All participants will be informed that their personal study-related data will be used by the principal investigator in accordance with the local data protection law. Although no adverse events are expected in this trial (no contrast agent will be employed, and no medicinal product administered), any adverse events will be reported *via* the French Ministry of Health's Adverse Health Event Reporting Portal. Only the investigating team will have access to the trial dataset.

Dissemination

The findings of this study will be disseminated by publication in an international journal and in presentations at international conferences of neuroradiology. We plan to work in collaboration with the Medical Writer of Strasbourg University Hospital concerning the drafting and edition of this article. Participants will be notified of the results of their examinations by a follow-up visit with an investigating physician. Participants, the FFF, and regional delegations will be sent the final publication reporting study results.

CONTRIBUTORSHIP STATEMENT

SK, FL, FB, SK conceived the idea of the study. SK, FL, JG, FB, MB, AB, CK, JM, EO, FP, FG, CD, VN, RW and SK formed the working group that wrote the study protocol. JG performed statistical analysis for the sample size calculation. All co-authors have approved the protocol and will participate in the study.

COMPETING INTERESTS STATEMENT

No author declared competing interests concerning this study.

FUNDING STATEMENT

This work was supported by Strasbourg University Hospital (HUS), Philipps and GE Healthcare; Grant Number PRI 2020-HUS N°8077. Philipps and GE Healthcare were not involved in the conception or design of this study protocol.

REFERENCES

1. Levy ML, Kasasbeh AS, Baird LC, *et al.* Concussions in soccer: a current understanding. *World Neurosurg* 2012;**78**:535–44. doi:10.1016/j.wneu.2011.10.032
2. Decq P, Brauge D, Calmat A, *et al.* Diagnosis clinical criteria of sport related concussion: Toward an operational criteria definition in France. *Neurochirurgie* 2021;**67**:222–30. doi:10.1016/j.neuchi.2020.11.010
3. McCrory P, Meeuwisse W, Dvořák J, Aubry M, Bailes J, Broglio S, Cantu RC, Cassidy D, Echemendia RJ, Castellani RJ, Davis GA, Ellenbogen R, Emery C, Engebretsen L, Feddermann-Demont N, Giza CC, Guskiewicz KM, Herring S, Iverson GL, Johnston KM, Kissick J, Kutcher J, Leddy JJ, Maddocks D, Makdissi M, Manley GT, McCrea M, Meehan WP, Nagahiro S, Patricios J, Putukian M, Schneider KJ, Sills A, Tator CH, Turner M, Vos PE. Consensus statement on concussion in sport-the 5th international conference on concussion in

sport held in Berlin, October 2016. *Br J Sports Med*. 2017;**51**:838-847. doi 10.3171/2013.7.JNS121822

4. Bailes JE, Petraglia AL, Omalu BI, Nauman E, Talavage T. Role of subconcussion in repetitive mild traumatic brain injury. *J Neurosurg*. 2013;**119**:1235-45. doi: 10.3171/2013.7.JNS121822.
5. Smith DW, Bailes JE, Fisher JA, Robles J, Turner RC, Mills JD: Internal jugular vein compression mitigates traumatic ax- onal injury in rat model by reducing intracranial slosh effect. ***Neurosurgery*** 2012***;70:***740–746, 2012 doi: 10.1227/NEU.0b013e318235b991.
6. Gysland SM, Mihalik JP, Register-Mihalik JK, *et al.* The relationship between subconcussive impacts and concussion history on clinical measures of neurologic function in collegiate football players. *Ann Biomed Eng* 2012;**40**:14–22. doi:10.1007/s10439-011-0421-3
7. Rodrigues AC, Lima MDM, de Souza LC, *et al.* No Evidence of Association Between Soccer Heading and Cognitive Performance in Professional Soccer Players: Cross-Sectional Results. *Front Neurol* 2019;**10**:209. doi:10.3389/fneur.2019.00209
8. Spiotta AM, Bartsch AJ, Benzel EC. Heading in soccer: dangerous play? *Neurosurgery* 2012;**70**:1–11; discussion 11. doi:10.1227/NEU.0b013e31823021b2
9. McKee AC, Alosco ML, Huber BR. Repetitive Head Impacts and Chronic Traumatic Encephalopathy. *Neurosurg Clin N Am* 2016;**27**:529–35. doi:10.1016/j.nec.2016.05.009
10. Stern RA, Daneshvar DH, Baugh CM, *et al.* Clinical presentation of chronic traumatic encephalopathy. *Neurology* 2013;**81**:1122–9. doi:10.1212/WNL.0b013e3182a55f7f
11. Ling H, Morris HR, Neal JW, *et al.* Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. *Acta Neuropathol* 2017;**133**:337–52. doi:10.1007/s00401-017-1680-3
12. Grinberg LT, Anghinah R, Nascimento CF, *et al.* Chronic Traumatic Encephalopathy Presenting as Alzheimer's Disease in a Retired Soccer Player. *J Alzheimers Dis* 2016;**54**:169–74. doi:10.3233/JAD-160312
13. Mez J, Daneshvar DH, Kiernan PT, *et al.* Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. *JAMA* 2017;**318**:360–70. doi:10.1001/jama.2017.8334

14. Abbas K, Shenk TE, Poole VN, *et al.* Effects of repetitive sub-concussive brain injury on the functional connectivity of Default Mode Network in high school football athletes. *Dev Neuropsychol* 2015;**40**:51–6. doi:10.1080/87565641.2014.990455

15. Koerte IK, Ertl-Wagner B, Reiser M, *et al.* White matter integrity in the brains of professional soccer players without a symptomatic concussion. *JAMA* 2012;**308**:1859–61. doi:10.1001/jama.2012.13735

16. Manley G, Gardner AJ, Schneider KJ, *et al.* A systematic review of potential long-term effects of sport-related concussion. *Br J Sports Med* 2017;**51**:969–77. doi:10.1136/bjsports-2017-097791

17. Ng TS, Lin AP, Koerte IK, *et al.* Neuroimaging in repetitive brain trauma. *Alzheimers Res Ther* 2014;**6**:10. doi:10.1186/alzrt239

18. Coughlin JM, Wang Y, Munro CA, *et al.* Neuroinflammation and brain atrophy in former NFL players: An in vivo multimodal imaging pilot study. *Neurobiol Dis* 2015;**74**:58–65. doi:10.1016/j.nbd.2014.10.019

19. Hart J, Kraut MA, Womack KB, *et al.* Neuroimaging of cognitive dysfunction and depression in aging retired National Football League players: a cross-sectional study. *JAMA Neurol* 2013;**70**:326–35. doi:10.1001/2013.jamaneurol.340

20. Casson IR, Viano DC, Haacke EM, *et al.* Is There Chronic Brain Damage in Retired NFL Players? Neuroradiology, Neuropsychology, and Neurology Examinations of 45 Retired Players. *Sports Health* 2014;**6**:384–95. doi:10.1177/1941738114540270

21. Lin AP, Ramadan S, Stern RA, *et al.* Changes in the neurochemistry of athletes with repetitive brain trauma: preliminary results using localized correlated spectroscopy. *Alzheimers Res Ther* 2015;**7**:13. doi:10.1186/s13195-015-0094-5

22. Baugh CM, Kiernan PT, Kroshus E, *et al.* Frequency of head-impact-related outcomes by position in NCAA division I collegiate football players. *J Neurotrauma* 2015;**32**:314–26. doi:10.1089/neu.2014.3582

23. Morales JS, Valenzuela PL, Saco-Ledo G, *et al.* Mortality Risk from Neurodegenerative Disease in Sports Associated with Repetitive Head Impacts: Preliminary Findings from a

Systematic Review and Meta-Analysis. *Sports Med* Published Online First: 21 October 2021. doi:10.1007/s40279-021-01580-0

24. Sandmo SB, Filipcik P, Cente M, *et al.* Neurofilament light and tau in serum after head-impact exposure in soccer. *Brain Inj* 2020;**34**:602–9. doi:10.1080/02699052.2020.1725129
25. Straume-Naesheim TM, Andersen TE, Dvorak J, *et al.* Effects of heading exposure and previous concussions on neuropsychological performance among Norwegian elite footballers. *Br J Sports Med* 2005;**39 Suppl 1**:i70-77. doi:10.1136/bjsm.2005.019646
26. Matser JT, Kessels AG, Jordan BD, *et al.* Chronic traumatic brain injury in professional soccer players. *Neurology* 1998;**51**:791–6. doi:10.1212/wnl.51.3.791
27. McCunn R, Beaudouin F, Stewart K, *et al.* Heading in Football: Incidence, Biomechanical Characteristics and the Association with Acute Cognitive Function-A Three-Part Systematic Review. *Sports Med* 2021;**51**:2147–63. doi:10.1007/s40279-021-01492-z
28. Strauss SB, Fleysher R, Ifrah C, *et al.* Framing potential for adverse effects of repetitive subconcussive impacts in soccer in the context of athlete and non-athlete controls. *Brain Imaging Behav* 2021;**15**:882–95. doi:10.1007/s11682-020-00297-4
29. Snowden T, Reid H, Kennedy S, *et al.* Heading in the Right Direction: A Critical Review of Studies Examining the Effects of Heading in Soccer Players. *J Neurotrauma* 2021;**38**:169–88. doi:10.1089/neu.2020.7130
30. Nasreddine ZS, Phillips NA, Bédirian V, *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005;**53**:695–9. doi:10.1111/j.1532-5415.2005.53221.x
31. Van der Linden M, GREMEM. *L'évaluation des troubles de la mémoire*. De Boeck Supérieur 2004.
32. Meyers JE, Meyers KR. Rey complex figure test under four different administration procedures. *The Clinical Neuropsychologist* 1995;**9**:63–7. doi:10.1080/13854049508402059
33. Bright P, Hale E, Gooch VJ, *et al.* The National Adult Reading Test: restandardisation against the Wechsler Adult Intelligence Scale-Fourth edition. *Neuropsychol Rehabil* 2018;**28**:1019–27. doi:10.1080/09602011.2016.1231121

34. Godefroy O, GREFEX. *Fonctions exécutives et pathologies neurologiques et psychiatriques*. De Boeck Supérieur 2012.

35. Zimmermann P, Fimm B. *Testbatterie zur Aufmerksamkeitsprüfung - Version 2.2: (TAP) ; [Handbuch]*. Psytest https://www.psytest.net/de/testbatterien/tap/bestellen

36. Baron-Cohen S, Wheelwright S, Hill J, *et al.* The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry* 2001;**42**:241–51.

37. Løvstad M, Sigurdardottir S, Andersson S, *et al.* Behavior Rating Inventory of Executive Function Adult Version in Patients with Neurological and Neuropsychiatric Conditions: Symptom Levels and Relationship to Emotional Distress. *J Int Neuropsychol Soc* 2016;**22**:682–94. doi:10.1017/S135561771600031X

38. Smith SM, Zhang Y, Jenkinson M, *et al.* Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002;**17**:479–89. doi:10.1006/nimg.2002.1040

39. Fischl B, Salat DH, Busa E, *et al.* Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;**33**:341–55. doi:10.1016/s0896-6273(02)00569-x

40. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage* 2000;**11**:805–21. doi:10.1006/nimg.2000.0582

41. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect* 2012;**2**:125–41. doi:10.1089/brain.2012.0073

42. Jordan SE, Green GA, Galanty HL, Mandelbaum BR, Jabour BA. Acute and chronic brain injury in United States National Team soccer players. *Am J Sports Med* 1996;**24**:205–10. doi: 10.1177/036354659602400216.

43. Mussack T, Dvorak J, Graf-Baumann T, Jochum M. Serum S-100B protein levels in young amateur soccer players after controlled heading and normal exercise. *Eur J Med Res* 2003;**8**:457–64.
44. Zetterberg H, Jonsson M, Rasulzada A, Popa C, Styrud E, Hietala MA, et al. No neurochemical evidence for brain injury caused by heading in soccer. *Br J Sports Med* 2007 ;**41**:574–7. doi:10.1136/bjsm.2007.037143
45. Downs DS, Abwender D. Neuropsychological impairment in soccer athletes. *J Sports Med Phys Fitness* 2002;**42**:103–7.
46. Matser JT, Kessels AG, Jordan BD, Lezak MD, Troost J. Chronic traumatic brain injury in professional soccer players. *Neurology* (1998) **51**:791–6. doi:10.1212/WNL.51.3.791
47. Stephens R, Rutherford A, Potter D, Fernie G. Neuropsychological impairment as a consequence of football (soccer) play and football heading: a preliminary analysis and report on school students (13-16 years). *Child Neuropsychol* 2005;**11**:513–26. doi:10.1080/092970490959629
48. Straume-Naesheim TM, Andersen TE, Dvorak J, Bahr R. Effects of heading exposure and previous concussions on neuropsychological performance among Norwegian elite footballers. *Br J Sports Med* 2005 ;**39**(Suppl I):i70–7. doi:10.1136/bjsm.2005.019646
49. Myer GD, Barber Foss K, Thomas S, *et al.* Altered brain microstructure in association with repetitive subconcussive head impacts and the potential protective effect of jugular vein compression: a longitudinal study of female soccer athletes. *Br J Sports Med* 2019;**53**:1539–51. doi:10.1136/bjsports-2018-099571
50. Blyth RJ, Alcock M, Tumilty DS. Why are female soccer players experiencing a concussion more often than their male counterparts? A scoping review. *Phys Ther Sport* 2021;**52**:54–68. doi:10.1016/j.ptsp.2021.08.001
51. Deck C, Bourdet N, Meyer F, *et al.* Protection performance of bicycle helmets. *J Safety Res* 2019;**71**:67–77. doi:10.1016/j.jsr.2019.09.003

52. Sahoo D, Robbe C, Deck C, *et al.* Head injury assessment of non-lethal projectile impacts: A combined experimental/computational method. *Injury* 2016;**47**:2424–41. doi:10.1016/j.injury.2016.09.004

53. Bliven E, Rouhier A, Tsai S, *et al.* Evaluation of a novel bicycle helmet concept in oblique impact testing. *Accid Anal Prev* 2019;**124**:58–65. doi:10.1016/j.aap.2018.12.017

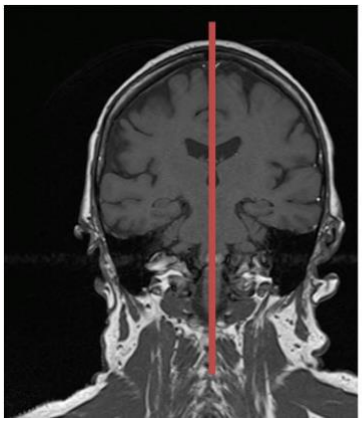
ACKNOWLEDGEMENTS

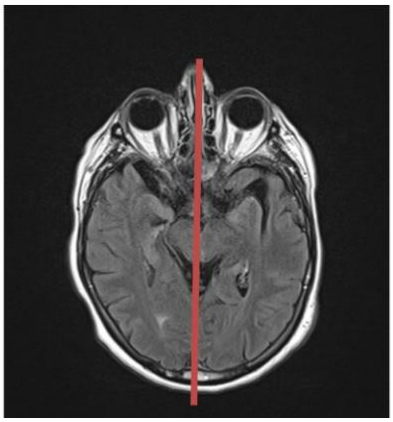
Manuscript edition was provided by Dr. Kate Dunning (HUS).

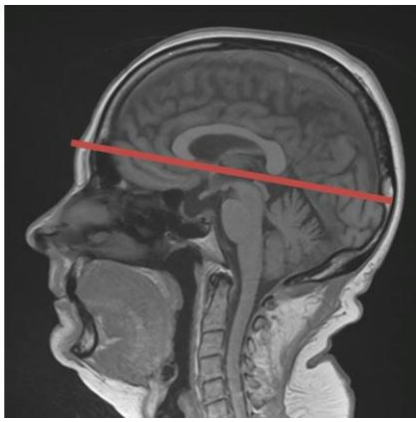
FIGURE LEGENDS

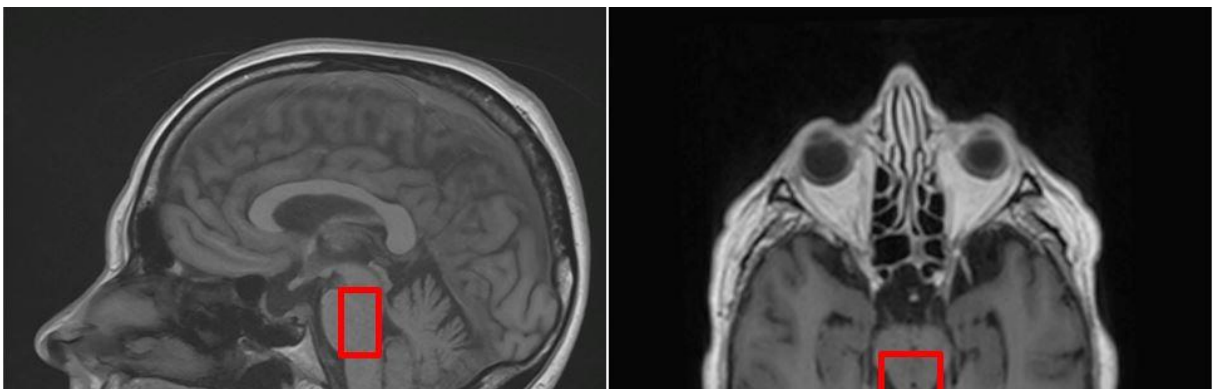
Figure 1. Human brain in the three spatial planes: coronal, horizontal, and sagittal sections. From left to right: coronal T1-weighted, axial FLAIR, and sagittal T1-weighted images.

Figure 2. Short echo time monovoxel 1H MR spectroscopy of the brainstem. With a width of 20 mm, the voxel is placed on the two posterior thirds of the pons and must cover its entire height.









SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2, 16
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	/
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 22
	5b	Name and contact information for the trial sponsor	15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15, 22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	/

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4–6
	6b	Explanation for choice of comparators	N/A
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7–8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9–12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	/
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8–9

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9–13

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**N/A – all study data is collected at the enrolment visit.**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**9, 11**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**13–14**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**14**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**14**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**15**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N/A**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**16–17**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**N/A**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**16**

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16–17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16–17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	17
	31b	Authorship eligibility guidelines and any intended use of professional writers	17
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	17
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	/
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.