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Impact of a gluten-free diet on quality of life in patients with axial spondyloarthritis: study protocol of a randomized double-blind placebo-controlled trial.

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Key words: spondyoarthritis; ankylosing spondylitis; gluten-free diet; gut microbiota.

Abstract

Introduction

Subclinical intestinal inflammation and gut dysbiosis have been reported in patients with spondyloarthritis (SpA). In common practice, rheumatologists are increasingly confronted with patients with inflammatory rheumatism who are on gluten-free diets (GFDs), despite the lack of reliable data from controlled studies. This study aims to determine the impact of a GFD on the quality of life of patients with axial SpA.

Methods and analysis

The GlutenSpA study is a 24-week, randomized, double-blinded, placebo-controlled, multicenter trial. Patients with axial SpA (n=200) will follow a 16-week GFD and be randomly assigned (1:1) to an experimental or control arm. In the experimental arm with receive at least 6 gluten-free breads per day + 200 g of gluten-free penne pasta per week + 6 rice flavor capsules per day. The control arm will receive at least 6 gluten-containing breads per day + 200 g of gluten-containing penne pasta per week + 6 vital gluten-containing capsules per day. The primary end-point is the variation in ASAS-HI questionnaire between S16 and baseline. A second open-label period of 8 weeks will follow the intervention period, during which the patient will be free to decide whether they will follow the GFD. The secondary outcomes comprise several patient-reported outcomes (SpA activity [BASDAI], fatigue [FACIT], depression [HAD], functional disability index [BASFI]), variations in BMI and HOMA index, and variations in the abundance and type of bacterial species found in the gut microbiota for a subgroup of patients (n=40). The data will be analyzed using the intention-to-treat principle.

Ethics and dissemination

The regional ethics committee (CPP Nord-ouest IV) has approved the study protocol v2 – March 10, 2019. (IDRCB 2018-A00309-46). The results of the trial will be submitted for publication in peer-reviewed journals.

ARTICLE SUMMARY

Strength and limitations:

GlutenSpA is the first randomized, double-blinded, placebo-controlled trial on the effects of a GFD on the quality of life of patients with axial SpA.

GlutenSpa could give highlights for understanding the links between symptoms, disease and diet.

It will provide new data on the microbiota of patients with SpA and aid in understanding the interaction between a GFD and the microbiota.

Background

Spondyloarthritis (SpA) is a chronic inflammatory rheumatism affecting the axial skeleton and especially the sacroiliac joints. Ankylosing spondylitis is the prototype disorder. In addition to axial involvement, several other impairments are common, including arthritis, dactylitis, enthesitis, uveitis, and chronic inflammatory bowel disease, defining several subgroups of SpA.

Since the 1990s, subclinical intestinal inflammation has been described in nearly 60% of patients with SpA (1) and is thought to be related to the disease activity (2-3). A more recent histological study of 65 patients with SpA confirmed the presence of gut inflammation in 42% of them, which was closely related to young age, male sex, SpA activity assessed by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and axial mobility assessed by BASMI (4). This gut inflammation, which can occur in macroscopically normal regions of the gut, is characterized in the acute phase by infiltrate comprising neutrophil polynuclear cells, which is gradually replaced by mononuclear cells and mediated by different types of immune cells (e.g., macrophages, dendritic cells, Th1, Th17, NK lymphocytes) (5). Fecal calprotectin, one of the markers commonly used to quantify gut inflammation in chronic inflammatory bowel disease (CIDI), could be increased in patients with SpA without gastrointestinal signs (6-7). However, the link with NSAID use remains controversial. Increased intestinal permeability due to gut inflammation could facilitate the passage of antigens and modulate the immune response. It is also enhanced by NSAIDs, the cornerstone of SpA treatment, as well as other treatments and diet.

The microbial environment, especially the gut microbiota, has up to 100,000 billion bacteria. High-throughput sequencing has identified tens of millions of bacterial genes attributed to a few thousand bacterial species that would protect the mucosal barrier from invasion by pathogens, metabolizing constituents of food in useful nutrients and contribute to immune system homeostasis. Over the past 10 years, the number of publications on the association between the gut microbiota and chronic pathologies has dramatically increased, first in intestinal diseases (clostridium colitis, celiac disease, CID), but also in other non-intestinal

pathologies, such as diabetes, herpetiform dermatitis, nephrotic syndrome, cardiovascular diseases, autism, and schizophrenia (8-9).

In chronic inflammatory rheumatism, whether it is SpA, psoriatic arthritis, or rheumatoid arthritis, there is dysbiosis (gut microbiota imbalance) (10) similar to chronic inflammatory bowel diseases (decreased microbial diversity, scarcity of Firmicutes with anti-inflammatory properties). Several studies in animal models have shown a link between the gut microbiota and joint inflammation, particularly in the transgenic HLAB27 rat (11). Two very small comparative studies have reported changes in the microbiota of patients with SpA. First, a study of the fecal microbiota in 25 children with a juvenile form of SpA found a decrease in Firmicutes and increase in Bifidobacterium compared to 13 control patients. Another study of gut biopsies in nine patients with SpA compared to nine controls reported changes in Firmicutes and Bacteroidogenes. However, these studies need to be confirmed (12-13).

The main mechanisms of action of a nutrient in inflammatory diseases have been detailed in a recent general review, particularly the direct role of food and nutrients (anti-oxidant effect, anti-inflammatory, immunomodulator, epigenetics, toxic) and the role of food on the gut microbiota (14). Diet clearly alters the microbiota, which has been shown for fasting (15) in mice, and probiotics or long-chain omega-3 fatty acids (16) in patients with CID.

The spectrum of gluten-related disorders has broadened and now includes celiac disease, non-celiac gluten sensitivity, and wheat allergy. Celiac disease is characterized by chronic enteral inflammation that causes malabsorption in genetically predisposed patients (HLA DQ2-DQ8), and alterations in the gut microbiota are thought to be involved in disease pathogenesis. This dysbiosis could be reduced by a gluten-free diet (GFD). In a study on the ileal microbiota of patients with celiac disease, decreased abundance in Bacteroidetes and Firmicutes was reported, as described in the microbiota of patients with SpA (17). Recently, a new clinical entity has emerged called non-celiac hypersensitivity to gluten, which is characterized by a heterogeneous clinical presentation combining intestinal and extra-intestinal signs occurring after gluten ingestion. The pathogenesis could be based on a direct toxic effect of gluten and possibly the gut microbiota (18). This entity could affect 5% of the population, but its diagnosis lacks validated criteria, and the effectiveness of a GFD varies.

In common practice, rheumatologists are increasingly confronted with patients with inflammatory rheumatism who are dieting despite the lack of reliable data from controlled studies. A survey presented in 2015 at the Annual Congress of the French Rheumatology Society reported that nearly a quarter of patients with inflammatory rheumatism (216 RA and 166 SpA) followed an eviction diet, 67.1% of which were on a GFD (19). To study the effect of a diet is difficult. The placebo effect or psychological factors may contribute to the response to

exclusion diets because patients know that their diet has changed. Patients with a strong belief in alternative treatments report more allergies and food intolerances than other patients and have more psychological facilities to modify their diet. This is important to consider in the design of diet studies, which require the use of randomized, double-blind, placebo trials to really answer the question. To the best of our knowledge, no studies to date have shown the effectiveness of a GFD in SpA.

Method/Design

Objectives

The primary objective is to determine the effects of a 16-week GFD versus placebo diet on quality of life as evaluated by the ASAS-HI questionnaire in a population of patients with axial SpA. The secondary objectives are to determine the effects of a 16-week GFD versus placebo diet on the activity of SpA, several patient-reported-outcomes (pain, fatigue, depression), and the gut microbiota. We will also determine the factors associated with the response to a GFD.

Study design

The GlutenSpA study is a 24-week, double-blinded, placebo-controlled, multicenter, randomized trial. The trial was approved by the French authorities (Comité de protection des Personnes Nord Ouest IV, protocol number: 2018-A00309-46). The study is being funded by the DGOS (regional multicentric PHRC 2017).

Eligibility criteria

Eligible participants must meet all of the following inclusion criteria: diagnosis of axial SpA defined by ASAS criteria for which the rheumatologist does not wish to change the treatment within 4 months of inclusion, stable treatment (NSAID and/or DMARD) for at least 3 months but no corticosteroid infiltration in the month prior to inclusion, and able to follow a GFD and to provide written informed consent and submit to the requirements of the study.

Patients will be excluded if they are on any diet at the time of inclusion or within 3 months prior to inclusion; have a history of celiac disease; received antibiotic treatment within 3 months of inclusion or are taking a probiotic; are pregnant, breastfeeding, or not covered by social security; or are minors or adults under the protection of the law or under the protection of justice. Furthermore, screening for serum anti-transglutaminase IgA or IgG will be done at the

screening visit. Patients with antibodies will not be included but referred to a gastroenterology specialist.

Recruitment

The Rheumatology departments of seven university hospitals (Clermont-Ferrand, Saint-Etienne, Lyon, Grenoble, Montpellier, Cochin APHP, Bordeaux) will participate in patient recruitment. Written informed consent is necessary for each patient to participate in this study. Thereafter, the participants will submit to an individual rheumatology evaluation and, if they meet the inclusion criteria, be offered enrollment in the study.

Intervention/Protocol

All patients (n=200) will be on a GFD for 16 weeks and randomly allocated into two intervention groups. A permuted-block randomization (i.e. random block sizes) will be conducted using a computer-generated random allocation (Stata software, version 15, StataCorp, College Station, US), with a 1:1 ratio allocation, ensuring complete randomness of the assignment of a patient to each randomized group. The experimental group (n=100) will be given at least 6 gluten-free breads per day (42 g), combined with 200 g (raw weight) of gluten-free pasta per week and 6 rice flour-containing capsules per day. The control group (n=100) will be given at least 6 gluten-containing breads per day (50 g), combined with 200 g (raw weight) of gluten-containing pasta per week and 6 vital gluten-containing capsules.

At baseline, the dietician will explain to the patients how to properly follow a GFD during a face-to-face interview. The patient's compliance to the GFD will be evaluated by the dietician at S2, S16, an S24 using an online 3-day alimentary questionnaire.

The bread and penne pasta were chosen for their visual resemblance and similar taste to maximize the blindness of the study. In order to reach the daily amount of gluten in a standard diet (estimated in France between 10 and 15 g/d) in the control arm, each day patients will have to ingest six capsules of vital gluten wheat made for the study. Vital gluten flour is an over-the-counter food supplement used to enrich a protein diet or as a base for making products, such as seitan. Capsules will be made using commercially available vital gluten and contain 0.35 g of gluten per capsule. The total amount of gluten in the control arm will be approximately 10.5 g/day.

After the 16-week GFD, patients will be offered to follow or not follow the GFD according to their own decision for an 8-week open-labelled follow-up period. The study duration for each patient is 24 weeks. The patient recruitment is expected to last 2 years (Figure 1).

Outcome measures

The primary outcome corresponds to the quality of life evaluated before and after the intervention (S16) as assessed by change in the ASAS-HI questionnaire. The secondary outcomes correspond to:

- SpA activity evaluated by the BASDAI questionnaire (J0, S16, S24)
- Fatigue assessed by the FACIT questionnaire (J0, S16, S24)
- Depression and anxiety assessed by the HAD questionnaire (J0, S16, S24)
- Functional disability assessed by the BASFI questionnaire (J0, S16, S24)
- Parameters of inflammation (VS and CRP) (J0, S16)
- Compliance with the GFD as evaluated by an interview with the dietician to assess the follow-up of the diet (S2, S16, S24)
- Digestive discomfort assessed by a weekly digestive discomfort questionnaire (S0 to S24)
- FIRST fibromyalgia questionnaire if present at J0 (J0, S16, S24)
- Weight and body mass index evaluated (J0, S16, S24
- Homeostasis Model Assessment (HOMA) variation: fasting blood glucose (mmol / l) x fasting insulin (µmol/l) /22.5 (J0, S16)
- Variations in the abundance and type of different bacterial species found in the intestinal microbiota for a subgroup of patients (n = 20 at Clermont-Ferrand and n = 20 at Bordeaux) (J0, S16)

Study visit

Information about the selection, recruitment, and evaluations carried out in each period is given in Table 1.

Table 1. Data collected at study visit.

STUDY PERIOD	SCREENING	ENROLLMENT BASELINE	INTERVENTION		FOLLOW-UP
WEEKS	**-2**	**0**	**2**	**16**	**24**
Informed consent	X				
Serum anti-transglutaminase IgA/IgG	X				
Eligibility criteria		X			
Randomization		X			
ASSESSMENTS					
Socio-demographic characteristics		X			
Medical history		X			
Concomitant medications		X		X	X
Physical examination		X		X	X
X-ray of the pelvis (if not available)		X			
Primary outcome: Quality of life (ASAS-HI)		X		X	X
Patient-reported outcome *		X		X	X
Dietician consultation		X	X	X	X
Laboratory samples		X		X	
Fecal samples: gut microbiota analysis		X		X	

Gut microbiota analysis

The microbiota will be analyzed in a subgroup of patients (n = 40) using stool samples collected at J0 and S16, frozen at -80°C, and then centralized according to the following procedure:
- extraction of the DNA contained in the feces

- 16S amplification (specific sequence for bacteria, highly conserved, allowing taxonomic classification)

- sequencing of amplicons

- bioinformatics analysis for taxonomic assignment of the obtained sequences.

Statistical analysis

Sample size

Sample size estimation is based on a comparison between randomized groups for the change in ASAS-HI questionnaire score. According to data described in literature, the smallest detectable and clinically relevant change is 3.0 (20). To take into account possible Hawthorne effect, a 2-points difference between randomization groups is expected. Considering a two-sided type I error at 5% and a statistical power equals 90%, an effect size of 0.5 (2-points difference for a standard deviation ranged between 3.5 and 4.5) can be highlighted for the ASAS-HI change score with 87 patients per group. It was proposed to include 200 patients (100 by group) to consider lost to follow-up.

Statistical analyses

Statistical analyses will be conducted using Stata software (version 15, StataCorp, College Station, US). A two-sided p-value of less than 0.05 will be considered to indicate statistical significance. Patients will be described at baseline and compared between randomized groups in regards to the following variables: compliance with eligibility criteria, demographic characteristics, center, seasonality, clinical characteristics, and medication. The comparability of the two arms at baseline will be assessed on the main characteristics of the participants and potential factors associated with the primary outcome. A possible difference between groups in any of these characteristics will be determined by both clinical and statistical considerations. The number of patients included and the inclusion curve will be presented by group.

The primary endpoint will be compared between groups by the Student's t-test, or the non-parametric Mann-Whitney test if assumptions of t-test are not met. The normality will be studied using Shapiro-Wilk test. The homoscedasticity will be analyzed with Fisher-Snedecor test. The results will be expressed using effect size and 95% confidence interval. Intention-to-treat analysis will be considered for the primary analysis. In order to prevent attrition bias, imputation of the missing data is planned. The statistical analysis plan also provides for an additional per-protocol analysis. Then, the analysis of the primary outcome will be completed by multivariable analysis using a linear mixed model to compare change in the ASAS-HI score between randomized groups taking into account: (1) the fixed effects covariates determined according to univariate results and to clinical relevance (gender, age, duration of disease, smoking, BASDAI and BASFI scores at baseline, FIRST score at baseline), and (2) centre as random-

effects (to measure between and within centre variability). The normality of the residuals will be studied. If necessary, a logarithmic transformation of the dependent variable will be proposed. The results will be expressed using effect-sizes and 95% confidence intervals.

The comparisons between the randomized groups will be performed (1) as aforementioned for continuous secondary endpoints and (2) using the chi-squared or Fisher exact tests for categorical variables, such as good response to the regimen, defined by a change of the ASAS-HI score of at least 2 points. For non-Gaussian data, results will be presented using median difference and 95% confidence intervals estimated using quantile regression model. For categorical parameters, the results will be expressed using absolute differences and 95% confidence intervals. The multivariable analysis associated to dichotomous endpoints will be generalized linear mixed model, with logit link function, and center as random-effect. The results will be expressed with of odds ratios and 95% confidence intervals.

Longitudinal data will be analyzed using random-effects regressions, modeling between and within patient effect, as random-effect, in addition to center effect. The following fixed effects will be studied: randomization group, time-points evaluation and their interaction.

According to clinical relevance and to European Medicines and Consolidated Standards of Reporting Trials recommendations, planned subgroup analyses will be proposed after the study of subgroup × randomisation group interaction in regression models. We will study more precisely sex, characteristics of the SpA (HLAB27, inflammatory anomaly with MRI of the sacroiliac, duration of the disease), use of antibiotics (during the study), BMI and variations in weight, type of initial abnormality in the intestinal flora, and immunological profile.

A sensitivity analysis will be performed to study the statistical nature of missing data and to determine the most appropriate approach to the imputation of missing data: maximum bias (e.g., last observation carried forward, baseline observation carried forward) or estimation proposed by Verbeke and Molenberghs for repeated data. A study of patients' abandoning will be proposed considering this parameter as a censored data, and consequently using the Kaplan-Meier to estimate it and log-rank for the comparison between groups.

Patient and public involvement

No patient involved

Discussion

This is the first randomized, double-blind, placebo-controlled clinical trial on the effect of a GFD on quality of life in SpA. Given the popularity of GFDs in patients with inflammatory rheumatisms, data on the efficacy and safety are needed.

The strength of this study lies in its placebo-controlled design, which appears necessary given the importance of the placebo effect of diets. Such an ambitious study could give highlights for understanding the links between symptoms, disease, diet, and microbiome. If this study demonstrates a beneficial effect of the GFD in patients with axial SpA, it could lead to recommendations in current practice or new therapeutics targeting such a manipulation of the microbiome. If it is negative, it will provide an answer to the frequent questions of patients on the benefit of a GFD in this disease.

Regarding the study of the microbiota, it will provide new data on the microbiota of patients with SpA and aid in understanding the interaction between a GFD and the microbiota.

Trial registration number: The study is registered at www.clinicaltrials.gov: NCT04274374.

Trial status

At the time of initial manuscript submission, recruitment had not started and is expected to begin in April 2020. The last patient is expected to be included in october 2022.

Caption Figure 1

Figure 1. GlutenSpa study diagram.

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Author's contributions: All authors contributed to editing of the manuscript. MC and MS with support from BP designed the protocol. The corresponding author attest that all listed authors meet authorsheep criteria.

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Word count: 2815

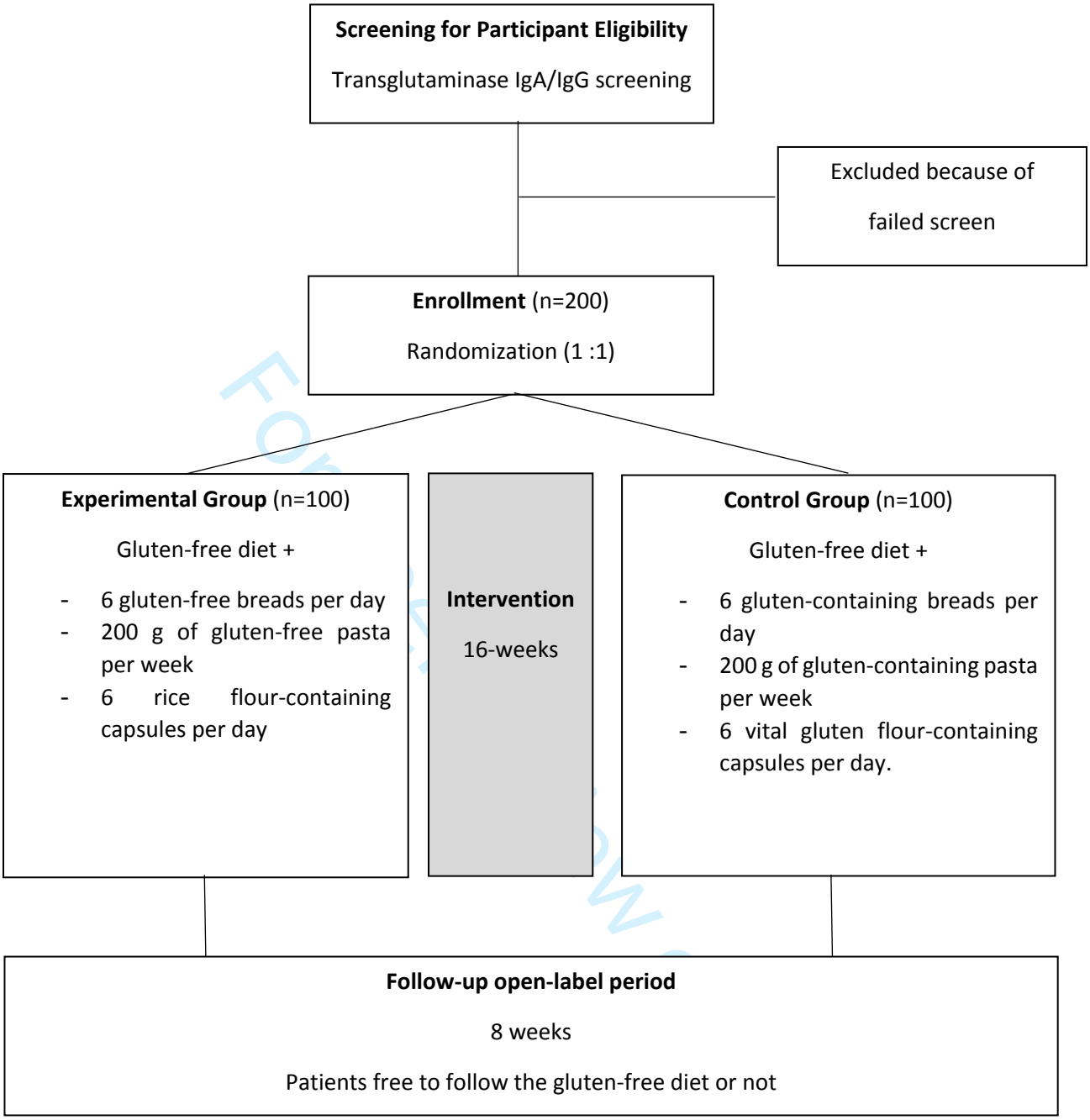

Figure 1. GlutenSpa study diagram.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	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	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	6
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	NA
Roles and responsibilities: sponsor and funder	#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	NA
Roles and responsibilities: committees	#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
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	
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, non-inferiority, exploratory)	6
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)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#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)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#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	8

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)	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	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	
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	7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	7

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#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	

Data collection plan: retention	#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	9
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	
Statistics: outcomes	#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	11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
Statistics: analysis population and missing data	#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)	
Methods: Monitoring			
Data monitoring: formal committee	#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	NA

		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#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	NA
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	NA
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
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)	

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
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	NA
Dissemination policy: trial results	#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	3

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

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

BMJ Open

The GlutenSpA trial: protocol for a randomized double-blind placebo-controlled trial of the impact of a gluten-free diet on quality of life in patients with axial spondyloarthritis.

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The GlutenSpA trial: protocol for a randomized double-blind placebo-controlled trial of the impact of a gluten-free diet on quality of life in patients with axial spondyloarthritis

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Author statement:

- COUDERC MARION: designed and revised critically for important intellectual content of the project; approved the final version of the work; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

- PEREIRA BRUNO: designed and revised critically for important intellectual content of the project; approved the final version of the work; and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Abstract

Introduction

Subclinical intestinal inflammation and gut dysbiosis have been reported in patients with spondyloarthritis (SpA). In common practice, rheumatologists are increasingly confronted with patients with inflammatory rheumatism who are on gluten-free diets (GFDs), despite the lack of reliable data from controlled studies. This study aims to determine the impact of a GFD on the quality of life of patients with axial SpA.

Methods and analysis

The GlutenSpA study is a 24-week, randomized, double-blinded, placebo-controlled, multicenter trial. Patients with axial SpA (n=200) will follow a 16-week GFD and be randomly assigned (1:1) to an experimental or control arm. In the experimental arm with receive at least 6 gluten-free breads per day + 200 g of gluten-free penne pasta per week + 6 rice flavor capsules per day. The control arm will receive at least 6 gluten-containing breads per day + 200 g of gluten-containing penne pasta per week + 6 vital gluten-containing capsules per day. The primary end-point is the variation in Assessment of SpondyloArthritis International Society – Health Index (ASAS-HI) questionnaire between week 16 and baseline. A second open-label period of 8 weeks will follow the intervention period, during which the patient will be free to decide whether they will follow the GFD. The secondary outcomes comprise several patient-reported outcomes (SpA activity [BASDAI], fatigue [FACIT], depression [HAD], functional disability index [BASFI]), variations in BMI and HOMA index, and variations in the abundance and type of bacterial species found in the gut microbiota for a subgroup of patients (n=40). The data will be analyzed using the intention-to-treat principle.

Strengths and limitations of this study:

GlutenSpA is the first randomized, double-blinded, placebo-controlled trial on the effects of a gluten-free diet on quality of life in patients with axial SpA.

Patients with axial spondyloarthritis will be randomized to either a 16-week gluten-free arm or a placebo arm, followed by a an 8-week open label period.

The primary endpoint is the change in ASAS-Health Index between baseline and week 16.

The secondary endpoints will include patient-reported outcomes (SpA activity, fatigue, depression, functional disability index) and gut microbiota.

Key words: spondyloarthritis; ankylosing spondylitis; gluten-free diet; gut microbiota.

Background and rationale

Spondyloarthritis (SpA) is a chronic inflammatory rheumatism affecting the axial skeleton and especially the sacroiliac joints. Ankylosing spondylitis is the prototype disorder. In addition to axial involvement, several other impairments are common, including arthritis, dactylitis, enthesitis, uveitis, and chronic inflammatory bowel disease, defining several subgroups of SpA (1-2). Since the 1990s, subclinical intestinal inflammation has been described in nearly 60% of patients with SpA (3) and is thought to be related to the disease activity (4-5). A more recent histological study of 65 patients with SpA confirmed the presence of gut inflammation in 42% of them, which was closely related to young age, male sex, SpA activity assessed by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and axial mobility assessed by Bath Ankylosing Spondylitis Metrology Index (BASMI) (6). This gut inflammation, which can occur in macroscopically normal regions of the gut, is characterized in the acute phase by infiltrate comprising neutrophil polynuclear cells, which is gradually replaced by mononuclear cells and mediated by different types of immune cells (e.g., macrophages, dendritic cells, Th1, Th17, NK lymphocytes) (7). Fecal calprotectin, one of the markers commonly used to quantify gut inflammation in chronic inflammatory bowel disease (CIDI), could be increased in patients with SpA without gastrointestinal signs (8-9). However, the link with non-steroidal anti-inflammatory drug (NSAID) use remains controversial (10-11). Increased intestinal permeability due to gut inflammation could facilitate the passage of antigens and modulate the immune response (12). It is also enhanced by NSAIDs, the cornerstone of SpA treatment, as well as other treatments and diet (13).

The microbial environment, especially the gut microbiota, has up to 100,000 billion bacteria. High-throughput sequencing has identified tens of millions of bacterial genes attributed to a few thousand bacterial species that would protect the mucosal barrier from invasion by pathogens, metabolizing constituents of food in useful nutrients and contribute to immune system homeostasis. Over the past 10 years, the number of publications on the association between the gut microbiota and chronic pathologies has dramatically increased, first in intestinal diseases (clostridium colitis, celiac disease, CID), but also in other non-intestinal pathologies, such as diabetes, herpetiform dermatitis, nephrotic syndrome, cardiovascular diseases, autism, and schizophrenia (14-15).

In chronic inflammatory rheumatism, whether it is SpA, psoriatic arthritis, or rheumatoid arthritis, there is dysbiosis (gut microbiota imbalance) (16) similar to chronic inflammatory bowel diseases (decreased microbial diversity, scarcity of Firmicutes with anti-inflammatory properties). Several studies in animal models have shown a link between the gut microbiota

and joint inflammation, particularly in the transgenic HLA B27 rat (17). Two very small comparative studies have reported changes in the microbiota of patients with SpA. First, a study of the fecal microbiota in 25 children with a juvenile form of SpA found a decrease in Firmicutes and increase in Bifidobacterium compared to 13 control patients. Another study of gut biopsies in nine patients with SpA compared to nine controls reported changes in Firmicutes and Bacteroidogenes. However, these studies need to be confirmed (18,19).

The main mechanisms of action of a nutrient in inflammatory diseases have been detailed in a recent general review, particularly the direct role of food and nutrients (anti-oxidant effect, anti-inflammatory, immunomodulator, epigenetics, toxic) and the role of food on the gut microbiota (20). Diet clearly alters the microbiota, which has been shown for fasting (21) in mice, and probiotics or long-chain omega-3 fatty acids (22) in patients with CID.

The spectrum of gluten-related disorders has broadened and now includes celiac disease, non-celiac gluten sensitivity, and wheat allergy. Celiac disease is characterized by chronic enteral inflammation that causes malabsorption in genetically predisposed patients (HLA DQ2-DQ8), and alterations in the gut microbiota are thought to be involved in disease pathogenesis. This dysbiosis could be reduced by a gluten-free diet (GFD). In a study on the ileal microbiota of patients with celiac disease, decreased abundance in Bacteroidetes and Firmicutes was reported, as described in the microbiota of patients with SpA (23). Recently, a new clinical entity has emerged called non-celiac hypersensitivity to gluten, which is characterized by a heterogeneous clinical presentation combining intestinal and extra-intestinal signs occurring after gluten ingestion. The pathogenesis could be based on a direct toxic effect of gluten and possibly the gut microbiota (24). This entity could affect 5% of the population, but its diagnosis lacks validated criteria, and the effectiveness of a GFD varies (25).

In common practice, rheumatologists are increasingly confronted with patients with inflammatory rheumatism who are dieting despite the lack of reliable data from controlled studies. A survey presented in 2015 at the Annual Congress of the French Rheumatology Society reported that nearly a quarter of patients with inflammatory rheumatism (216 RA and 166 SpA) followed an eviction diet, 67.1% of which were on a GFD (26). To study the effect of a diet is difficult. The placebo effect or psychological factors may contribute to the response to exclusion diets because patients know that their diet has changed. Patients with a strong belief in alternative treatments report more allergies and food intolerances than other patients and have more psychological facilities to modify their diet. This is important to consider in the design of diet studies, which require the use of randomized, double-blind, placebo trials to really answer the question. To the best of our knowledge, no studies to date have shown the effectiveness of a GFD in SpA.

Objectives

The primary objective is to determine the effects of a 16-week GFD versus placebo diet on quality of life as evaluated by the Assessment of SpondyloArthritis International Society – Health Index (ASAS-HI) questionnaire in a population of patients with axial SpA (27). The secondary objectives are to determine the effects of a 16-week GFD versus placebo diet on the activity of SpA, patient-reported-outcomes (pain, fatigue, depression), the tolerance to and compliance with GFD, and the effect on gut microbiota. We will also determine the factors associated with the response to a GFD.

Trial design

The GlutenSpA study is a 24-week, double-blinded, placebo-controlled, multicenter, randomized trial.

The trial was approved by the French authorities (Comité de protection des Personnes Nord Ouest IV, protocol number: 2018-A00309-46).

Methods: participants, interventions, and outcomes

Study setting

The Rheumatology departments of seven French academic hospitals (Clermont-Ferrand, Saint-Etienne, Lyon, Grenoble, Montpellier, Cochin APHP, Bordeaux) will participate in recruitment.

Patient an public involvement

There is no patient involved in the study.

Eligibility criteria

Eligible participants must meet all of the following inclusion criteria: adult patient with a diagnosis of axial SpA as defined by the Assessment of SpondyloArthritis International Society (ASAS) criteria (28) and for which the rheumatologist does not wish to change the treatment within 4 months of inclusion, stable treatment in dose and type (NSAIDs and/or disease modifying antirheumatic drugs [DMARDs]) for at least 3 months, but no corticosteroid infiltration in the month prior to inclusion, and able to follow a GFD and provide written informed consent and submit to the requirements of the study.

Patients will be excluded if they are on any diet at the time of inclusion or within 3 months prior to inclusion; have a history of celiac disease; received antibiotic treatment within 3 months of inclusion or are taking a probiotic; are pregnant, breastfeeding, or not covered by social security; or are minors or adults under the protection of the law or under the protection of justice. Furthermore, screening for serum anti-transglutaminase IgA or IgG will be performed at the screening visit. Patients with serum anti-transglutaminase antibodies will not be included but referred to a gastroenterologist.

Interventions

All patients (n=200) will be on a GFD from inclusion (day 0 [D0]) to week 16 (W16). At D0, they will be randomly allocated to two intervention groups. The experimental group (gluten-free arm, n=100) will be given at least six gluten-free breads per day (42 g) in addition to the GFD, combined with 200 g (raw weight) of gluten-free pasta per week and six rice flour-containing capsules per day. The control group (gluten-containing arm, n=100) will be given at least six gluten-containing breads per day (50 g) in addition to the GFD, combined with 200 g (raw weight) of gluten-containing pasta per week and six vital gluten-containing capsules.

The bread and penne pasta used for the study are over-the-counter commercial products chosen for their visual resemblance and similar taste to maximize the blindness of the study. They will be repackaged in neutral packaging. In order to reach the daily amount of gluten in a standard diet (estimated in France between 10 and 15 g/d) in the control arm, each day patients will have to ingest six capsules of vital gluten wheat made for the study. Vital gluten flour is an over-the-counter food supplement used to enrich a protein diet or as a base for making products, such as seitan. Capsules will be made using commercially available vital gluten and contain 0.35 g of gluten per capsule. The total amount of gluten in the control arm will be approximately 10.5 g/day. In the experimental group, patients will receive capsules containing rice flour. Capsules containing gluten and capsules containing rice flour are the same color and size. They will be made by the central pharmacy of the University Hospital of Clermont-Ferrand from rice or gluten vital flour and sent to each center before delivery. Breads, pasta, and capsules will be furnished to each patient by the local investigating center in two stages (S0 and S2) for an 8-week period each time.

After the 16-week GFD period, patients will be offered to follow or not follow the GFD according to their own decision for an 8-week open-labelled follow-up period. The study duration for each patient is 24 weeks. The patient recruitment is expected to last 2 years (Figure 1).

Strategies to improve adherence

At baseline, all participants will be told how to properly follow a GFD during a face-to-face interview. They will be given information on gluten-containing food products to avoid. The compliance with the GFD will be evaluated by the dietician at W2, W16, and W24 using a 3-day alimentary questionnaire. The alimentary questionnaire completed by the participants details all food intake during 3 days (2 week-days and 1 week-end day). Conversion in macronutrients (protein, fat, and carbohydrate) will be completed by the dietician using Nutrilog® software. Patients will be closely monitored for their nutritional balance, weight, and body mass index at W2, W16, and W24.

Outcome measures

The primary outcome corresponds to the variation in the quality of life evaluated before and after the intervention (W16) as assessed by the ASAS-HI questionnaire. This self-reported questionnaire measures functioning and health across 17 aspects of health and 9 environmental factors, addressing categories of pain, emotional function, sleep, sexual function, mobility, self-care, community life, support/relationships, attitudes, and health services. ASAS-HI has been validated in patients with radiographic and non-radiographic axial SpA (27,29).

The secondary outcomes will be assessed before and after the intervention (W16) and the open-label 8-week period (W24):

- SpA activity evaluated by the BASDAI (D0, W16, W24).
- Functional status assessed using the Bath Ankylosing Spondylitis Functional Index (BASFI) (D0, W16, W24).
- Parameters of biological inflammation (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]) (D0, W16).
- Fatigue assessed by the Functional Assessment of Chronic Illness Therapy (FACIT) scale (D0, W16, W24). The FACIT is a short, 13-item, easy to administer tool that measures an individual's level of fatigue during their usual daily activities over the past week.
- Depression and anxiety assessed by the Hospital Anxiety and Depression scale (HAD) (D0, W16, W24).
- Fibromyalgia symptoms assessed using the Fibromyalgia Rapid Screening Tool (FiRST) if present at day 0 (D0, W16, W24).

- Compliance with the GFD as evaluated by an interview with the dietician to assess the follow-up of the diet (W2, W16, W24).
- Digestive discomfort assessed by a weekly digestive discomfort questionnaire (D0 to W24).
- Change in weight and body mass index (D0, W16, W24).
- Homeostasis Model Assessment (HOMA) variation: fasting blood glucose (mmol/l) x fasting insulin (µmol/l) /22.5 (D0, W16).
- Variations in the abundance and type of different bacterial species found in the intestinal microbiota for a subgroup of patients (n = 20 at Clermont-Ferrand and n = 20 at Bordeaux) (D0, W16).

Study visit/Participant timeline

Information about the selection, recruitment, and evaluations carried out in each period is given in Table 1.

All concomitant or intercurrent medications, including SpA treatment (NSAIDs or DMARDs), will be recorded at each visit. Antibiotic use will specifically be recorded.

Table 1. Data collected at study visit.

STUDY PERIOD	SCREENING	ENROLLMENT BASELINE	INTERVENTION		FOLLOW-UP
WEEKS	-2	0	2	16	24
Informed consent	X				
Serum anti-transglutaminase	X				
Eligibility criteria		X			
Randomization		X			
ASSESSMENTS					
Socio-demographic characteristics		X			
Medical history		X			
Concomitant medications*		X		X	X
Physical examination		X		X	X
X-ray of the pelvis (if not available)		X			
Primary outcome: Quality of life (ASAS-HI)		X		X	X
Patient-reported outcome **		X		X	X
Dietician consultation		X	X	X	X
Laboratory samples		X		X	
Fecal samples: gut microbiota analysis		X		X	

**Including DMARDs, NSAIDs, painkillers, corticoids.*

***Including BASDAI, BASFI, HAD, FIRST, FACIT, and a digestive discomfort questionnaire.*

Gut microbiota analysis

The microbiota will be analyzed in a subgroup of patients (n = 40, the first 20 from Clermont-Ferrand, and the first 20 from Bordeaux) using stool samples collected at D0 and W16, frozen at -80°C, and then centralized according to the following procedure:

- Extraction of the DNA contained in the feces;

- 16S amplification (specific sequence for bacteria, highly conserved, allowing taxonomic classification);

- sequencing of amplicons;

- bioinformatics analysis for taxonomic assignment of the obtained sequences.

Sample size

The sample size estimation is based on a comparison between randomized groups for the change in ASAS-HI questionnaire score. According to the literature, the smallest detectable and clinically relevant change is 3.0 (22). To take into account a possible Hawthorne effect, a 2-point difference between randomization groups is expected. Considering a two-sided type I error of 5% and statistical power of 90%, an effect size of 0.5 (2-point difference for a standard deviation between 3.5 and 4.5) can be highlighted for the change in ASAS-HI score with 87 patients per group. We propose including 200 patients (100 per group) to consider loss to follow-up.

Recruitment

Patients will be recruited from one of the seven centers participating in the study: Clermont-Ferrand, Saint-Etienne, Lyon, Grenoble, Montpellier, Cochin APHP, and Bordeaux. Eligible patients will be extended an offer to participate during the routine rheumatological consultation. Written informed consent will be obtained for each patient (see supplementary file).

Each of the seven rheumatology departments are well-recognized at the national and international levels for their expertise in managing SpA patients. We estimate that each department is taking care of approximately 50 SpA patients per month, so the inclusion of 1 to 2 patients per month seems to be feasible.

Assignment of interventions

Allocation

The randomization (balanced within random block sizes) will be conducted by an investigator who is not involved in the recruitment, evaluation, and/or treatment of participants.

Blinding

After assignment, study participants and care providers (rheumatologist, nurses, dieticians) will be blinded to the intervention. The analysis will also be performed under blinding. Blinding will be unlocked

Data collection, management, and analysis

Statistical methods

All analyses will be performed by the Biostatistics Unit at the University Hospital of Clermont-Ferrand, which also provides methodological support for the study.

The statistical analysis will be carried out using the software programs Stata (version 13, StataCorp, College Station) and R (http://cran.r-project.org/). All statistical tests will be carried out at the risk of error of the first species α of 5%.

The primary analysis will be assessed as intention-to-treat. In order to prevent attrition bias, imputation of the missing data is planned. The statistical analysis plan also provides for an additional per-protocol analysis. Continuous variables will be presented as mean and standard deviation, subject to the normality of their distribution (Shapiro-Wilk test if necessary). In the case skewed distribution, they will be presented as median, interquartile range, and extreme values. Qualitative variables will be expressed as numbers and percentages. Graphic representations will be associated with these analyses as much as possible. Comparisons between groups will be systematic (1), without adjustment (2) or with adjustment for factors whose distribution could be unbalanced between groups despite randomization.

Patients will be described and compared between groups at baseline in regards to the following variables: demographic characteristics, center, seasonality, clinical characteristics, and medication. The initial comparability of the two arms will be assessed on the main characteristics of the participants and potential factors associated with the primary outcome. A possible difference between the two groups in any of these characteristics will be determined by both clinical and statistical considerations. Deviations from the protocol and causes of abandonment will also be described. The number of patients included and the inclusion curve will be presented by group.

Primary end-point analysis

The primary endpoint will be compared between groups by the Student's t-test, or the non-parametric Mann-Whitney test if Student test conditions are not met (normality verified by Shapiro-Wilk test and equality of variances by Fisher-Snedecor test). The results will be expressed in terms of effect size and 95% confidence interval.

Secondary analysis

In a second step, the analysis described before will be completed by a multivariate analysis of a mixed linear model type (to explain variation in the ASAS-HI score) in order to take into account the covariates retained in the univariate analysis for their clinical relevance (stratification criterion: sex, age, duration of disease, smoking, type of SpA medication [e.g., NSAID or TNF blockers], BASDAI and BASFI scores at baseline, FIRST score at baseline) or the center effect (considered random). The results will be expressed in terms of regression coefficients and 95% confidence intervals. The normality of the residues will be studied; if necessary, a logarithmic transformation of the dependent variable will be proposed.

Comparisons between the groups will be performed (1) in a similar manner as previously presented for quantitative secondary endpoints and (2) the chi-squared or Fisher exact test for categorical variables. Concerning the study of factors associated with a good response to the regimen, defined by a variation of the ASAS-HI score of at least 2 points, comparisons in univariate situations will resume the statistical analyses described previously. We will study more precisely the sex, the characteristics of the SpA (HLAB27, inflammatory anomaly with MRI of the sacroiliac, duration of the disease), the use of antibiotics during the study, BMI, and variations in weight, the type of initial abnormality in the intestinal flora, and immunological profile. The multivariate analysis will consider a logistic regression for which covariates will be determined based on univariate results of clinical relevance. The results will be expressed in terms of odds ratios and

Discussion

This is the first randomized, double-blind, placebo-controlled clinical trial on the effect of a GFD on quality of life in SpA. Given the popularity of GFDs in patients with inflammatory rheumatisms, data on the efficacy and safety are needed.

The strength of this study lies in its placebo-controlled design, which appears necessary given the importance of the placebo effect of diets. Such an ambitious study could give highlights for understanding the links between symptoms, disease, diet, and microbiome. If this study demonstrates a beneficial effect of the GFD in patients with axial SpA, it could lead to recommendations in current practice or new therapeutics targeting such a manipulation of the

microbiome. If it is negative, it will provide an answer to the frequent questions of patients on the benefit of a GFD in this disease.

Regarding the study of the microbiota, it will provide new data on the microbiota of patients with SpA and aid in understanding the interaction between a GFD and the microbiota.

Like other studies on diets, the major limitation of the study will be the possible difficulties in adherence to the GFD. To minimize this potential bias, the dieticians will collect the amount of bread, pasta, and pills ingested during each period, and three face-to-face dietetic consultations (W0, W2, and W16) are planned to educate the patients.

Ethics and dissemination

The regional ethics committee (CPP Nord-ouest IV) has approved the study (IDRCB 2018-A00309-46).

The results of the trial will be submitted for publication in peer-reviewed journals.

The authors have no relationship that may have influenced the submitted work.

Trial status

At the time of initial manuscript submission, recruitment had not started and is expected to begin in September 2020. The last patient is expected to be included in March 2022.

Data statement section: No data is available at this time.

Figure 1 : GlutenSpa study diagram.

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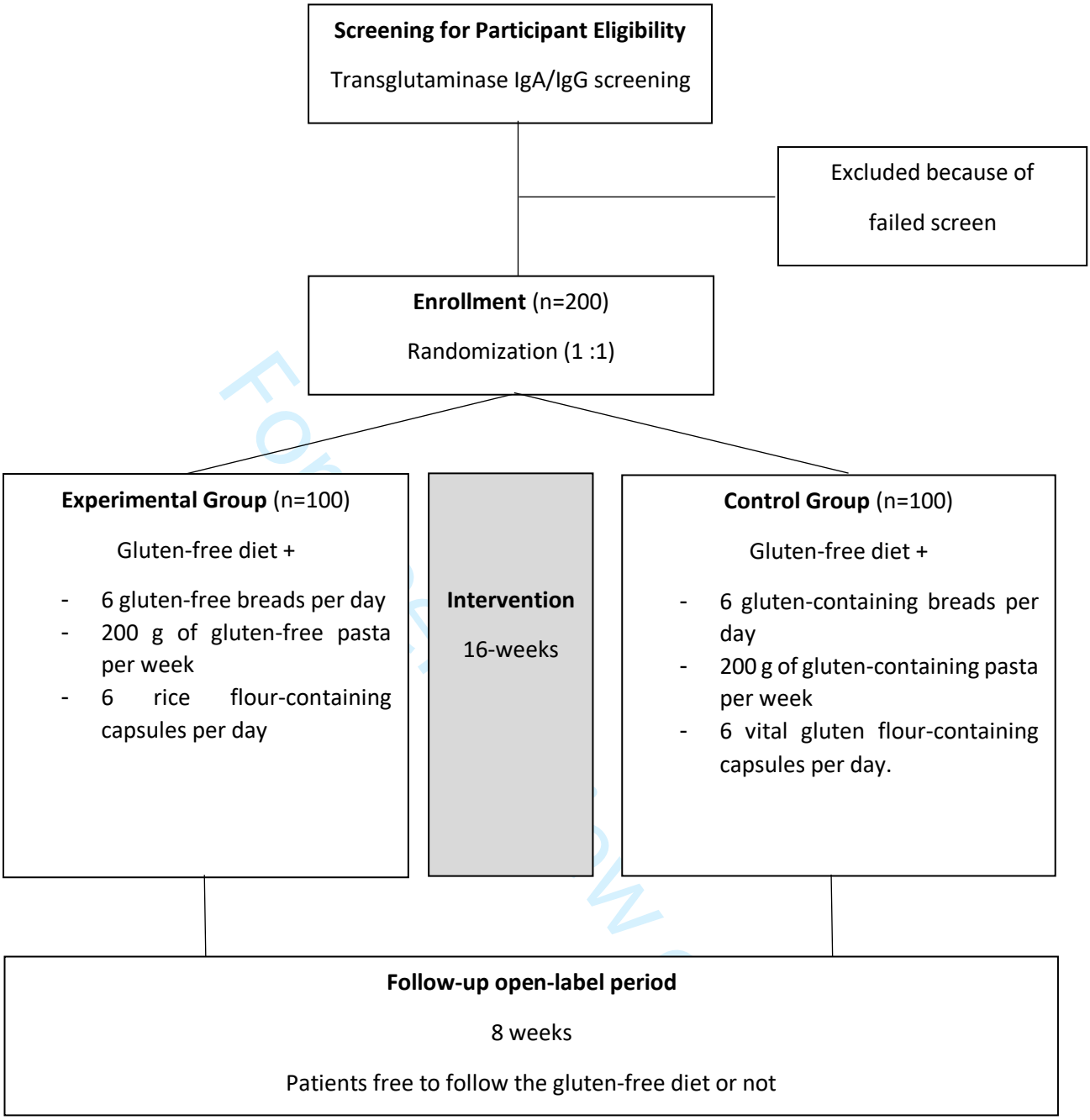

Figure 1. GlutenSpa study diagram.

Formulaire de consentement de participation à une recherche impliquant la personne humaine

Impact d'un régime sans gluten sur la qualité de vie des patients ayant une spondyloarthrite axiale

Je soussigné(e)
M^me^, M^lle^, M. *(rayer les mentions inutiles)* (*nom, prénom*)……
Né(e) le ……..……...
Demeurant……
Déclare :

- que le Docteur (*nom, prénom, téléphone*) ……..……..
  m'a proposé de participer à l'étude sus nommée,
- qu'il m'a expliqué en détail le protocole,
- qu'il m'a notamment fait connaître :
  - l'objectif, la méthode et la durée de l'étude
  - les contraintes et les risques potentiels encourus
  - mon droit de refuser de participer et en cas de désaccord de retirer mon consentement à tout moment
  - mon obligation d'inscription à un régime de sécurité sociale
  - que, si je le souhaite, à son terme, je serais informé(e) par le médecin investigateur de ses résultats globaux
  - que je ne serais pas autorisé(e) à participer à d'autres études cliniques pendant une durée de 24 semaines.
  - que le Comité de Protection des Personnes Nord Ouest IV a émis un avis favorable en date du 24/10/2019
  - que dans le cadre de cette étude le promoteur, le CHU de Clermont-Ferrand, a souscrit à une assurance couvrant cette recherche.
- que j'ai répondu en toute bonne foi aux questions concernant mon état de santé et ma participation à d'autres études,
- que je ne suis pas placé sous sauvegarde de justice.

Les informations relatives à l'étude recueillies par l'investigateur sont traitées confidentiellement. J'accepte que les données enregistrées à l'occasion de cette recherche puissent faire l'objet d'un traitement informatisé sans mention du nom ou du prénom. J'ai bien noté que les droits d'accès, de rectification, d'opposition et de limitation du traitement des données prévus par la loi du 20 juin 2018 relative à la protection des données personnelles s'exercent à tout moment auprès du médecin qui me suit dans le cadre de la recherche et qui connaît mon identité ou du délégué de protection des données du promoteur dont les coordonnées sont mentionnées dans la note d'information qui m'a été remise.

Après avoir discuté librement et obtenu réponse à toutes mes questions, j'accepte librement et volontairement de participer à cette recherche impliquant la personne humaine dans les conditions précisées dans le formulaire d'information et de consentement

☐ Je donne mon accord pour que les prélèvements biologiques effectués sur moi soient conservés et utilisés pour toutes autres études ancillaires portant sur les rhumatismes inflammatoires.

☐ Je m'oppose à ce que les prélèvements biologiques effectués sur moi soient conservés et utilisés pour toutes autres études ancillaires portant sur les rhumatismes inflammatoires.

INVESTIGATEUR Nom et Prénom, date et signature	**PATIENT** Nom et Prénom, date et signature Précédée de la mention « lu et compris»
---------- Le …../…../…… Signature :	---------- Le. …../…../…… Signature :

Ce document est à réaliser en 3 exemplaires originaux, dont le premier doit être gardé 15 ans par l'investigateur, un autre remis à la personne donnant son consentement et le troisième transmis au promoteur.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	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	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	6
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	NA
Roles and responsibilities: sponsor and funder	#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	NA
Roles and responsibilities: committees	#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
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	
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, non-inferiority, exploratory)	6
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)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#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)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#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	8

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)	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	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	
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	7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	7

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#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	

Data collection plan: retention	#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	9
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	
Statistics: outcomes	#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	11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11
Statistics: analysis population and missing data	#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)	
Methods: Monitoring			
Data monitoring: formal committee	#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	NA

		details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#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	NA
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	NA
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
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)	

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
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	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
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	NA
Dissemination policy: trial results	#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	3

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

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