PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	Couderc, Marion; Pereira, Bruno; Schaeverbeke, Thierry; Thomas, Thierry; Chapurlat, Roland; Gaudin, Philippe; Morel, Jacques; Dougados, M; Soubrier, M.

VERSION 1 – REVIEW

REVIEWER	Joanna Rog 1st Department of Psychiatry, Psychotherapy and Early Intervention, Medical University of Lublin, Poland
REVIEW RETURNED	06-Apr-2020

GENERAL COMMENTS	The protocol of the study is really interesting. Taking into account more and more information about the relationship between diet and axial spondyloarthritis it is worth to realize this study. Some questions for Authors: „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. „ Please including the source of information – the reference. ASAS-HI – please including long-form name (not only abbreviation). To my mind, more information/the description of the using tools is necessary: what the specific scales measure, citation of the source of the scales Information about blood collection - the glucose and insulin will be analyzed after blood collection or will be frozen? ASAS – as mentioned above - not only abbreviation Why authors will not include other biomarkers related to inflammation or intestinal permeability? Line 47: "stable treatment (NSAID and/or DMARD) for at least 3 months but no corticosteroid infiltration in the month prior to inclusion": stable treatment is regarding dosage or type of treatment or both of them? Exclusion criteria: a. there is a lack of criteria related to other autoimmune/inflammation diseases f.e. Hashimoto thyroiditis or other rheumatological/allergy symptoms or inflammatory bowel diseases b. there is no information about whether the patients will be ambulatory or during hospitalization?? c. there are any age or BMI-related restriction? Intervention/Protocol

	The description of the methodology is not enough accurate and comprehensive: a. from where the patients will receive gluten-free or gluten bread and other foods? When they will receive it? Every day? b. If the study is blinded, there is no information about differences on taste, smell, other organoleptic features of bread. Authors mentioned limited information about it. To my mind, it is not easy to make gluten-free foods which will be looks and taste as same as gluten-containing foods. Who will prepare it? c.Something about nutrition value should be mentioned in the protocol. Taking into account differences in the levels of vitamins, minerals etc. in gluten-free and gluten products, the results of the study could be misleading. People on a gluten-free diet could more often experience more GI symptoms (less fibre in diet) or some nutritional deficiencies... Methods section There was no information about using SPIRIT checklist the article may not currently address all the items on the checklist, f.e - Specific objectives or hypotheses - Intervention adherence – monitoring of dietitian to my mind could be included here - Explanation of the clinical relevance of chosen efficacy and harm outcomes - This item is important and I strongly recommended to include it

REVIEWER	Mahmoud Slim The Hospital for Sick Children, Canada
REVIEW RETURNED	29-Apr-2020

GENERAL COMMENTS	The current study protocol titled “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” provides a detailed description of the GlutenSpA study activities and procedures. This is a 24-week randomized double-blind placebo controlled study. Patients with axial spondyloarthritis will be randomized to either a 16-week gluten-free arm or a placebo arm. This will be followed by a second phase of an 8-week open label period in which study participants will be given the chance to follow their diet of preference. The primary end point is the change in ASAS-HI scores between baseline and week 16. Secondary endpoints will include several patient reported outcomes including SpA activity, fatigue, depression, functional disability index, anthropometric and gut microbiota in a subset of participants (n=40). Please find below my comments: Title and abstract: - Please add GlutenSpA to the first portion of the title. - Please replace S16 with week 16. - The authors interrupt the flow of listing the primary and secondary outcomes by talking about the 8-week open label period. The reader gets the impression that the secondary outcomes are going to be assessed after 24 weeks. However, after going through the methods section, we become aware that the secondary outcomes will be assessed also after 16 weeks. Thus, it is advised to mention the 8-week period after listing the primary/secondary endpoints. - Some of the abbreviations have not been defined before their use in the manuscript: ASAS-HI, BASDAI, FACIT, HAD, BASFI, BMI, HOMA. Article summary and limitations: The authors missed including some of the potential limitations for their study such as “adherence to the assigned dietary program” as this is one of the major limitations in dietary-based studies.

	Background: - Please cite the information appearing in the first paragraph. - Page 3 line 31- please define BASMI. Line 41- NSAID - There is a recent study published by Isasi et al. (https://www.sciencedirect.com/science/article/pii/S0306987720301493) which the authors can refer to in their background to further strengthen the rational of their study. Although the outcomes is limited to back pain, but it was interesting to see that 23 out of 28 patients with axial SpA reporting significant improvement. Methods: - Page 7, line 21: please define ASAS-HI - Eligibility criteria: o Please provide further details on the diagnostic criteria or at least cite the reference. o Authors state “no corticosteroid infiltration in the month prior to inclusion”. What if the patient received corticosteroid infiltration two months prior to inclusion? Would the patient be excluded? Does this break the “3 months stable treatment” condition? - Recruitment: o Authors state “the participants will submit to an individual rheumatology evaluation”. I believe that they meant that participants will undergo and not submit, right? Please clarify - Intervention/protocol: o Authors lead with the statement “All patients (n=200) will be on a GFD …” and then later in the paragraph we discover that 100 will be randomized to GFD and 100 to control diet. Please correct the leading sentence. o I believe that by S, the authors were referring to “semaine”, however it is recommended to use Week or W. o Please provide further details on the 3-day alimentary questionnaire. o Are the patients in the GFD arm going to receive non-gluten containing capsules? Because if they don’t, there is a high risk for identifying patients’ diet (at least by the healthcare provider if not the patients themselves). o In Table 1: patient-reported outcomes is too broad. Authors added an asterisk but didn’t define it. - Statistical analysis: o What is the reason for comparing patients for their compliance with eligibility criteria? Aren’t they all eligible and comply with the eligibility criteria? Or are the authors referring to select criteria (such as medication use, etc…)? It is advised to be more specific. o What is the reason for using the Student’s t-test for the primary endpoint analysis given that the authors mentioned that they will be using linear mixed models later in the same paragraph? o Another factor that is advisable to control for in the linear mixed models is the pharmacologic profiles, i.e., the type of medications used in each group as this might potentially influence the response/QOL. After reading the study protocol, I am left wondering how will the subset of 40 patients who will undergo the microbiota evaluation be selected? During the study, will the healthcare practitioners be allowed to change the treatment regimen (primarily pharmacologic therapy) of patients? Are the patients (especially the subset of 40) allowed to take antibiotics? (antibiotic use appears in the statistical analysis section as a part of subgroup analyses). As I mentioned earlier, the authors missed highlighting the potential limitations in their study: namely, the risk of noncompliance, which is common in studies including dietary interventions.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

The protocol of the study is really interesting. Taking into account more and more information about the relationship between diet and axial spondyloarthritis it is worth to realize this study.
Some questions for Authors:

„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. „ Please including the source of information – the reference.

Answer : This has been corrected as advised.

ASAS-HI – please including long-form name (not only abbreviation).

Answer : ASAS-HI is the abbreviation for "Assessment of SpondyloArthritis International Society – Health Index" and has been added to the text.

To my mind, more information/the description of the using tools is necessary: what the specific scales measure, citation of the source of the scales

Information about blood collection - the glucose and insulin will be analyzed after blood collection or will be frozen?

Answer : Glucose and fasting insulin will be analyzed directly, allowing the calculation of HOMA.

ASAS – as mentioned above- not only abbreviation

Answer : This has been defined in the text.

Why authors will not include other biomarkers related to inflammation or intestinal permeability?

Answer : Blood and feces collection will be set up for further ancillary analyses, including pro-inflammatory cytokines and fecal calprotectin.

Line 47: "stable treatment (NSAID and/or DMARD) for at least 3 months but no corticosteroid infiltration in the month prior to inclusion": stable treatment is regarding dosage or type of treatment or both of them?

Answer : It means both of them. We have clarified this in the revised text.

Exclusion criteria:
a. there is a lack of criteria related to other autoimmune/inflammation diseases f.e. Hashimoto thyroiditis or other rheumatological/allergy symptoms or inflammatory bowel diseases

Answer : Inflammatory bowel disease belongs to the spondyloarthritis spectrum, so we do not want to exclude these patients.

b. there is no information about whether the patients will be ambulatory or during hospitalization??

Answer : Patients will be ambulatory. We have added this information to the recruitment section.

c. there are any age or BMI-related restriction?

Answer : We have chosen to include only adult patients (as noted in the eligibility criteria). BMI was not chosen to be a restricted parameter but any diet at the time of inclusion or in the prior 3 months led to exclusion.

Intervention/Protocol
The description of the methodology is not enough accurate and comprehensive:
a. from where the patients will receive gluten-free or gluten bread and other foods? When they will receive it? Every day?

Answer : Breads, pasta, and capsules will be furnished to each patient by the local investigating center in two stages (D0 and week 2) for an 8-week period each time.

b. If the study is blinded, there is no information about differences on taste, smell, other organoleptic features of bread. Authors mentioned limited information about it. To my mind, it is not easy to make gluten-free foods which will be looks and taste as same as gluten-containing foods. Who will prepare it?

Answer : Pasta and bread have been chosen for their visual similarity between gluten-free and gluten-containing products and are marketed over-the-counter. Capsules will be made by the central pharmacy of the University Hospital of Clermont-Ferrand from rice flour or gluten flour and sent to each center before delivery.

c.Something about nutrition value should be mentioned in the protocol. Taking into account differences in the levels of vitamins, minerals etc. in gluten-free and gluten products, the results of the study could be misleading. People on a gluten-free diet could more often experience more GI symptoms (less fibre in diet) or some nutritional deficiencies...

Answer : The nutritional differences between gluten-free and gluten-containing products are well known (more glucid and lipid and less protein in the gluten-free products), as highlighted in the following table:

Gluten-free breads Gluten-containing breads Gluten-free pasta Gluten-containing pasta Rice flour Gluten-vital Flour
Energy (kcal/100 g) 431 400 352 365 352 370
Protein (g/100 g) 4.1 11 7.7 13 1 75
Glucid (g/100 g) 74 60 75 72 87 15
Lipid (g/100 g) 12 8.5 2 2 0 1.9
Fiber (g/100 g) 5 5 1.7 3.6 0 0.7

The daily food ration provided by the three products (bread, pasta, and capsules) given to each patient is as follows:

Gluten-free products Gluten-containing products
Energy (kcal/day) 293 316

Protein (g/day) 4 11
Glucid (g/day) 36.2 32
Lipid (g/day) 5.6 8.5
Fiber (g/day) 2.6 3.3

Thus, the patients will be closely monitored on the basis of their nutritional balance and weight.

Methods section
There was no information about using SPIRIT checklist the article may not currently address all the items on the checklist, f.e
- Specific objectives or hypotheses
- Intervention adherence – monitoring of dietitian to my mind could be included here
- Explanation of the clinical relevance of chosen efficacy and harm outcomes - This item is important and I strongly recommended to include it

Answer : We have modified the text to match the SPIRIT checklist.

Reviewer 2

Title and abstract:
- Please add GlutenSpA to the first portion of the title.

Answer : We have changed the title to: "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".

- Please replace S16 with week 16.

Answer : This has been corrected.

- The authors interrupt the flow of listing the primary and secondary outcomes by talking about the 8-week open label period. The reader gets the impression that the secondary outcomes are going to be assessed after 24 weeks. However, after going through the methods section, we become aware that the secondary outcomes will be assessed also after 16 weeks. Thus, it is advised to mention the 8-week period after listing the primary/secondary endpoints.

Answer : We have modified the first sentence of the outcome section to clarify.

- Some of the abbreviations have not been defined before their use in the manuscript: ASAS-HI, BASDAI, FACIT, HAD, BASFI, BMI, HOMA.

Answer The abbreviations are defined at first use in the revised manuscript.

Article summary and limitations:
The authors missed including some of the potential limitations for their study such as “adherence to theassigned dietary program” as this is one of the major limitations in dietary-based studies.

Answer : This is an important issue and we have added a sentence in the Discussion section.

Background:
- Please cite the information appearing in the first paragraph.

Answer : The citations have been added.

- Page 3 line 31- please define BASMI. Line 41- NSAID

Answer : Done

- There is a recent study published by Isasi et al. (https://www.sciencedirect.com/science/article/pii/S0306987720301493) which the authors can refer to in their background to further strengthen the rational of their study. Although the outcomes is limited to back pain, but it was interesting to see that 23 out of 28 patients with axial SpA reporting significant improvement.

Answer : Thank you for this interesting reference. We have added it in the Background section.

Methods:
- Page 7, line 21: please define ASAS-HI
Answer Done

- Eligibility criteria:
o Please provide further details on the diagnostic criteria or at least cite the reference.
Answer : We have added the reference in the Eligibility criteria section

o Authors state “no corticosteroid infiltration in the month prior to inclusion”. What if the patient received corticosteroid infiltration two months prior to inclusion? Would the patient be excluded? Does this break the “3 months stable treatment” condition?

Answer : We considered the time of action of corticoid infiltration, which is usually less than 1 month.

- Recruitment: Authors state “the participants will submit to an individual rheumatology evaluation”. I believe that they meant that participants will undergo and not submit, right? Please clarify
Answer : This has been clarified.

- Intervention/protocol:
o Authors lead with the statement “All patients (n=200) will be on a GFD …” and then later in the paragraph we discover that 100 will be randomized to GFD and 100 to control diet. Please correct the leading sentence.

Answer : All patients will actually be on a GFD and then randomly assigned to have gluten-free or gluten-containing products in addition to the GFD. We have clarified this.

o I believe that by S, the authors were referring to “semaine”, however it is recommended to use Week or W.
Answer : Yes, we have corrected this.

o Please provide further details on the 3-day alimentary questionnaire.
Answer : Done

o Are the patients in the GFD arm going to receive non-gluten containing capsules? Because if they don’t, there is a high risk for identifying patients’ diet (at least by the healthcare provider if not the patients themselves).

Answer : Yes, in the experimental arm they receive rice flour-containing capsules, and in the control arm they receive gluten-containing capsules. We have explained it more precisely in the Intervention section.

In Table 1: patient-reported outcomes is too broad. Authors added an asterisk but didn't define it.
Answer : This has been defined.

- Statistical analysis:
o What is the reason for comparing patients for their compliance with eligibility criteria? Aren't they all eligible and comply with the eligibility criteria? Or are the authors referring to select criteria (such as medication use, etc…)? It is advised to be more specific.
Answer : We thank the reviewer for the helpful comment and apologize for this mistake. The Statistics section has been modified accordingly.
What is the reason for using the Student's t-test for the primary endpoint analysis given that the authors mentioned that they will be using linear mixed models later in the same paragraph?
Answer : We thank the reviewer for the comment. As described in the "Primary endpoint analysis" paragraph, the Student's t-test will be used for the primary analysis and performed without the central effect. In a second step ("Secondary analysis" paragraph), the central effect will be evaluated in multivariable analysis as a random effect (to take into account with and without central variability), in addition to covariates (i.e., fixed effects) determined as mentioned in the submitted manuscript.

o Another factor that is advisable to control for in the linear mixed models is the pharmacologic profiles, i.e., the type of medications used in each group as this might potentially influence the response/QOL.
Answer : We thank the reviewer for the comment. We agree and the Statistics section has been completed accordingly.

After reading the study protocol, I am left wondering how will the subset of 40 patients who will undergo the microbiota evaluation be selected?

Answer : For financial reasons, we cannot analyze the microbiota of the entire population, so we will analyze the first 20 patients from Clermont-Ferrand and the first 20 from Bordeaux.

During the study, will the healthcare practitioners be allowed to change the treatment regimen (primarily pharmacologic therapy) of patients? Are the patients (especially the subset of 40) allowed to take antibiotics? (antibiotic use appears in the statistical analysis section as a part of subgroup analyses).

Answer : All concomitant medications, including SpA treatment (NSAIDs or DMARDs), will be recorded at each visit as noted in Table 1. If necessary, antibiotic use is possible and will be mentioned in the e-crf for specific analysis.

As I mentioned earlier, the authors missed highlighting the potential limitations in their study: namely, the risk of noncompliance, which is common in studies including dietary interventions.
Answer : This has been added.

VERSION 2 – REVIEW

REVIEWER	Joanna Rog Medical University of Lublin, Poland
REVIEW RETURNED	26-Aug-2020

GENERAL COMMENTS	Authors have changed some parts of the manuscript, which significantly improved the quality of the paper.

REVIEWER	Mahmoud Slim Hospital for Sick Children
REVIEW RETURNED	01-Sep-2020

GENERAL COMMENTS	Abstract: Methods, line 3: please revise – "In the experimental arm, patients will receive at least …." As mentioned in my previous review, please list the secondary outcomes before mentioning the second open label period. The authors haven't changed this yet. Strengths and limitations: Except for the first point, the authors only provided a description of their study methods. There is no mention of any limitation. Introduction: There is no need for the full citation at the end of paragraph 2 (The Dignass ref). Sample size calculation: Based on what distribution were the calculations made? Please make sure to include this information.