Nutrition as a risk factor and predictor of the outcome of orthobiological treatments for knee osteoarthritis

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SYNOPSIS

Title	Nutrition as a risk factor and predictor of the outcome of orthobiological treatments for knee osteoarthritis
Protocol number	Nutririsk(L4154)
Type of study	Monocentric, observational with additional procedure (extra routine blood sampling, questionnaires and measurement of Nutritional status), prospective, non-controlled.
Duration	36 months
PI	Paola De Luca
General Aim	The general purpose of this study is to evaluate the relation between the nutritional status of patients, the features of the orthobiologics products used for patients' treatment and the clinical outcomes after one-step conservative regenerative treatment for knee osteoarthritis.
Primary Outcome:	The primary outcome of the study will be the identification of the proportion of responders and non-responders patients to the orthobiologic treatment (according to OMERACT-OARSI [1] criteria) at 6-month follow-up and of their related nutritional status.
Secondary Outcomes	The secondary outcomes of the study will be: - the identification of the proportion of responders and non-responders patients to the orthobiologic treatment (according to OMERACT-OARSI criteria [1]) at 2 and 12-month follow-up and of their related nutritional status; - the characterization of the orthobiologic products used for the patient's treatment.
Patient enrolment	Patients affected by knee osteoarthritis undergoing conservative regenerative medicine procedures with autologous material at IRCCS Istituto Ortopedico Galeazzi and participating to the observational study "REGAIN" will be enrolled for this study: 88 patients will be needed: 1) 22 subjects with body mass index (BMI) considered normal weight (≥18.5 and ≤25) undergo to Platelet-Rich Plasma (PRP) treatment; 2) 22 subjects with BMI values that exceed the normal weight range (BMI>25) undergo to PRP treatment; 3) 22 subjects with BMI within the considered normal weight range (≥18.5 e ≤25) and undergo to microfragmented adipose tissue treatment; 4) 22 subjects with BMI values that exceed the normal weight range undergo to microfragmented adipose tissue treatment. (BMI>25)

Inclusion and exclusion criteria	Inclusion criteria: - males and females ≥18 and ≤ 65 years; - presence of knee osteoarthritis; - indication to regenerative medicine treatments PRP or microfragmented adipose treatment; - participation to the observational study "REGAIN"; - patients with BMI ≥18.5; - signature of Informed Consent for the study. Exclusion criteria: - patients with BMI <18.5; - patients who are not able to comply with the study schedule.
Statistical analysis and Sample Size	Statistical analysis will be performed using R Software. Categorical variables will be compared between cells and groups using Chi-square or Fisher's exact test. Continuous data distribution will be assessed using Shapiro Wilk test. According to the results of the normality test, between groups differences will be assessed by the proper Student's t test (paired or unpaired) or the equivalent non-parametric test (Wilcoxon, Mann-Whitney). Two-way ANOVA or general linear regression models will be used to assess the effect of two or more variable of the study outcomes. P-values <0.05 will be considered statistically significant. The sample size has been calculated considering as the primary outcome the proportion of patients not responsive (according to OMERACT-OARSI [1] criteria) to therapy at the 6-month follow-up in two distinct groups of patients: subjects with body mass index within the reference ranges (Group 1 or 3) and subjects with values outside the range (Group 2 or 4). The proportion of unresponsive patients is expected to be 0.45 in Group 1 or 3 and 0.05 in Group 2 or 4. Considering a power of 80% and a significance level of 0.05 for a one-tailed Fisher exact test, the sample size is 20 subjects per group, and considering 10% of possible dropouts, the number is increased to 22 patients per group. The study will be replicated in two classes of patients, undergoing PRP or microfragmented adipose tissue treatments. Then, 44 patients will be enrolled per class, for a total of 88 patients.

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1.0 Background

Osteoarthritis (OA) is a multifactorial pathology that includes the synovial fluid inflammation and damage to the articular cartilage with consequent loss of it and remodeling of the subchondral bone [2]. Obesity, metabolic syndrome and diabetes can directly influence the development of OA [3]. To date, the main treatments of this disease concern the control of symptoms or, in the most severe cases, the replacement of the joint [4-5]. Although the Osteoarthritis Research Society International guidelines recommend physical exercise and weight loss in overweight and obese subjects, this type of solution is often not implemented [6]. Obesity and therefore the high body mass index (BMI), represent a risk factor for OA not only for the high load that the joint has to bear, but also for the inflammatory state, albeit mild, deriving from it at systemic level, as demonstrated by the presence of OA in obese patient hands [7]. Besides to the fact that the high BMI leads to an increase in the OA incidence, it is interesting to investigate the role of an incorrect and inadequate nutritional status in the arthrosis pathology, with particular attention to the level of the quality of the patient's autologous product (orthobiologic) uses for the treatment. Orthobiologic is a promising nonoperative treatment defined as biological material (including platelet rich plasma (PRP), mesenchymal stem cells, microfragmented adipose tissue) used to improve the healing of a musculoskeletal tissue such as cartilage. They promote healing process through the release of growth factors and molecules involved in healing and inflammation [8].
It is easy to understand how the success of a treatment depends on the quality of the product used and it becomes essential to find strategies capable of improving the quality of starting material especially in terms of anti-inflammatory and immunomodulatory potential. It is known that diet directly influences the inflammatory state both at the systemic level and at the joint tissue level. Just as an incorrect diet causes an increase in the inflammatory state [9], a balanced and controlled diet can modulate the structure of cytokines favoring the anti-inflammatory ones as already demonstrated [10]. In this contest, a clear example is represented by Mediterranean diet, resulted associated with lower prevalence of OA [11]. Between the conservative treatment for OA, PRP [12] and Microfragmented adipose tissue [13] injection represent innovative regenerative approaches more and more widespread. Previous study demonstrated that dietary can induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids [14].
The emerging role of extracellular vesicles (EVs) in the cell communication defining them as biomarkers, has become a source of interest in the study of different metabolic disease [15-17]. For all these reasons a characterization of extracellular vesicles derived from orthobiologics of patients with different nutritional status deserves to be investigated.

2.0 Study aims and flow chart

2.1 General aim

The general purpose of this study is to evaluate the relation between the nutritional status of patients, the features of the orthobiologics products used for patients' treatment and the clinical outcomes after one-step conservative regenerative treatment for knee osteoarthritis.

2.2 Primary Outcome

The primary outcome of the study will be the identification of the proportion of responders and non-responders patients to the orthobiologic treatment (according to OMERACT-OARSI [1] criteria) at 6-month follow-up and of their related nutritional status.

For this purpose the responder or non-responder patients will be identified based on the results of the VAS, Tegner-Lysholm Activity Scale and KOOS scores according to the parameters established by Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT) and Osteoarthritis Research Society International (OARSI).

2.3 Secondary outcomes

The secondary outcomes of the study will be:

- the identification of the proportion of responders and non-responders patients to the orthobiologic treatment (according to OMERACT-OARSI criteria [1]) at 2 and 12-month follow-up and of their related nutritional status;

- the characterization of the orthobiologic products used for the patient's treatment.

2.4 Flow-Chart

	Enrollment	Surgical procedure	After patients discharge 2 month (+/- 2 week)	After patients discharge 6 month (+/- 2 weeks)	After patients discharge 12 month (+/- 1 month)
Sign of Informed Consent	X				
Evaluation of inclusion criteria	X				
Evaluation of nutritional status (anthropometric measurements, plicometry, questionnaires)		X			
Collection of PRP or microfragmented adipose tissue for orthobiologics characterization		X			
Collection of blood sample (concomitant to routine blood sample for PRR; extra routine blood sample for		X			

microfragmented adipose tissue)					
Collection of data from REGAIN questionnaires	X		X	X	X
Collection of food questionnaires		X			

3.0 Study design

Monocentric, observational with additional procedure (extra routine blood sampling, questionnaires and measurement of Nutritional status), prospective, non-controlled.

3.1 Study methodology – subjects

After the ethics committee approval, patients meeting the inclusion criteria undergoing conservative knee regenerative medicine treatments with orthobiologics and participating to the observational study "REGAIN" will be enrolled into the study. The PROMs will be completed by patients based on their clinical condition and on the specific treatment they will receive at the REGAIN Center. The aforementioned PROMs include but are not limited to:
- VAS, Tegner-Lysholm Activity Scale, KOOS.

Patients will be asked to filled the PROMs at the time of the enrollment (before the treatment) and then at 2, 6 and 12 months after the treatment.
Blood and nutritional information will be collected only the day of the treatment to allow for evaluation of the possible association between patients' characteristics and treatment outcomes. Blood sampling will be performed to carry out the following laboratory analysis, in detail: complete blood count, blood glucose, hemoglobin A1C (HbA1c), creatinine, GOT and GPT transaminases, C reactive protein, triglycerides, total cholesterol, HDL.
In particular, for patients undergoing a PRP treatment, a blood sample is already routinely harvested and therefore only an extra test tube will be added to test blood glucose, hemoglobin A1C (HbA1c), creatinine, GOT and GPT transaminases, C reactive protein, triglycerides, total cholesterol, HDL. For patients undergoing a treatment with microfragmented adipose tissue, an ad hoc blood collection will be carried out by filling two test tubes.

Nutritional information include anthropometric measurements, diet history and food consumption questionnaire (24h recall, "Quanto mangio veramente", Italian Mediterranea Index, anamnesi). Anthropometric measurements include weight and height, waist circumference, arm circumference measurements, biceps, triceps, subscapular and suprailiac folds measurement (plicometry) through the use of the skinfolder. It should be noted that the skinfolder is only one of the tools used to evaluate the patient's nutritional status, without diagnostic purposes and without the will to investigate anything about the device.

Concerning the orthobiologics characterization, PRP and microfragmented adipose tissue will undergo to different evaluations as per the observational study protocol "Utilizzo dei Patient-Reported Outcome Measures (PROMs), delle valutazioni cliniche oggettive e biomolecolari per il monitoraggio dei pazienti sottoposti a trattamenti di medicina rigenerativa".

In addition, for the purpose of this study, isolation and characterization of extracellular vesicles derived from PRP and micro fragmented adipose tissue will be performed, in order to deepen the orthobiologic characterization. The following techniques will be applied, where appropriate:

- Count and identification of cells from PRP and microfragmented adipose tissue (hemacytometer, nucleocounter, flow cytometry);
- Extracellular vesicle isolation, count and dimensional analysis (ultracentrifuge, Nanosights);
- Extracellular vesicle marker identification (flow cytometry).

These data will be analyzed for their possible association with nutritional status of the patient and clinical outcomes.

Regardless the specific protocol or investigation, the analysis will not include genomic DNA or diagnostic analysis. All samples (blood and tissues) will be analyzed at Istituto Ortopedico Galeazzi and destroyed at the end of the study.

3.2 Study duration

Total duration of study: 36 months

Starting: after approval of Ethical Committee

Time for sample collection/enrollments: 22 months

Time for data collection at the last follow-up: 12-34 months

Data analysis: 30- 36 months

4.0 Patient enrollment

Will be included patients affected by knee osteoarthritis undergoing conservative regenerative medicine procedures with autologous material at IRCCS Istituto Ortopedico Galeazzi and participating to the observational study [REGAIN]

88 patients will be needed:
1) 22 subjects with body mass index (BMI) considered normal weight (≥18.5 and ≤25) undergo to Platelet-Rich Plasma (PRP) treatment;
2) 22 subjects with BMI values that exceed the normal weight range (BMI>25) undergo to PRP treatment;
3) 22 subjects with BMI within the considered normal weight range (≥18.5 e ≤25) and undergo to microfragmented adipose tissue treatment;

4) 22 subjects with BMI values that exceed the normal weight range undergo to microfragmented adipose tissue treatment. (BMI>25)

4.1 Inclusion criteria

- males and females ≥18 and ≤ 65 years;
- diagnosis of knee osteoarthritis (any Kellgren-Lawrence grade);
- indication to regenerative medicine treatments with PRP or microfragmented adipose treatment;

- participation to the observational study "REGAIN" ;
- patients with BMI ≥18.5;
- signature of Informed Consent for the study.

4.2 Exclusion criteria:

- Patients with BMI <18.5;
- patients who are not able to comply with the study schedule.

4.3 Subject identification

Researchers will prepare an identification list in chronological order of all the subjects recruited for the study to correlate an ID number with the personal information of the subjects preserving the subject anonymity, according to the D.Lgs. 196/2003 and ssm and EU Regulation 679/2016 and Guidelines of the Privacy Authority 24/07/2008.

5.0 Statistical methodology

5.1 Sample size

The sample size was calculated considering as primary outcome the proportion of patients not responder to treatment (according to OMERACT-OARSI [1] criteria) at the 12-month follow-up in two distinct groups of patients: subjects with body mass index within the reference ranges (Group 1 and 3) and subjects with values outside the range (Group 2 and 4). The proportion of unresponsive patients is expected to be 0.45 in Group 1 and 0.05 in Group 2. Considering a power of 80% and a significance level of 0.05 for a one-tailed Fisher exact test, the sample size is 20 subjects per group and considering 10% of possible dropouts, this number is increased to 22 patients. The study will be replicated in two classes of patients, undergoing PRP or microfragmented adipose tissue treatments. Then, 44 patients will be enrolled per class, for a total of 88 patients.

5.2 Analysis methodology

Statistical analysis will be performed using R Software. Categorical variables will be compared between cells and groups using Chi-square or Fisher's exact test. Continuous data distribution will be assessed using Shapiro Wilk test. According to the results of the normality test, between groups differences will be assessed by the proper Student's t test (paired or unpaired) or the equivalent non-parametric test (Wilcoxon, Mann-Whitney). Two-way ANOVA or general linear regression models will be used to assess the effect of two or more variable of the study outcomes. P-values <0.05 will be considered statistically significant.

6.0 Potential risks/benefit

6.1 Benefits

Patients will not have direct benefits by participating in this study. Compared to the enlarged scientific community, the study may indirectly contribute to an extension of knowledge in the direction of potential clinical applications of the results obtained.

The identification of new associations between the evaluation of nutritional status (including anthropometric measurements of body composition, biochemical analysis, evaluation of food consumption and energy expenditure), quality of orthobiologics and clinical outcome of patients will allow to predict which or how nutritional aspects has an impact on clinical result. In this scenario, the results of this study will allow to identify the need of possible modification of patients' food habits to favor better results of the regenerative treatment, improving their life quality and reducing the societal economic burden.

6.2 Potential risks

One risk could be related to blood sampling for biochemical assays: possible complications could be lipotimia, slight pain or irritation at the sampling site. Another risk could be related to the detection of anthropometric measurements such as redness at the landmark with skinfolder.

7.0 Ethical Considerations

The protocol is compliant with current Declaration of Helsinki, with EN ISO 14155: 1 and EN ISO 14155: 2 and the Good Clinical Practice (GCP) and all the staff involved in the study is committed to acting in accordance with the principles contained therein.

7.1 Ethics Committee

All documentation relating to this study must be submitted to the Ethics Committee, for the necessary approvals. The beginning of the study and the inclusion of individuals is subordinate to the favorable opinion expressed by Ethics Committee.

Any amendment to the protocol, information to the patient and the informed consent form must be submitted to the Ethics Committee.

It is the responsibility of the principal investigator of the study to inform the ethics committee of complications or adverse events that may be of interest to the subjects included in the study and any problems that may present a risk to the subject, according to current regulations.

7.2 Subject information and informed consent

Before being included in the study, subjects who meet all the criteria for inclusion and exclusion of the study, should be informed in detail by persons authorized by the principal investigator and be placed in a position to understand objective, meaning, duration of the study. All the risks / benefits, advantages / disadvantages must be discussed with the subject. Before admission to the study, the subject must give their written informed consent by signing and dating the form, of which will receive a copy. The subject is guaranteed that participation in the study is voluntary and that at any moment can withdraw from the study without suffering any consequences.

7.3 Insurance and monitoring

The only additional procedure that implies a minimal risk is blood sample collection. Then, the Sponsor assume that a specific insurance policy is not necessary. For the same reason no monitoring activities will be performed.

7.4 Protection of personal data

The researcher conforms to the principle of the right of the subject to the protection of privacy under Legislative Decree no. 196/2003 and ssm and UE regulation 679/2016 and guidelines of the privacy authority 24/07/2008. All clinical data recorded on the CRF and used for further evaluation are anonymous. Patient's clinical data and are coded by number of subject, date of birth and identification is restricted to authorized personnel involved in the study and under the responsibility of the researcher. Clinical data of the subject are stored and processed electronically for purposes of scientific evaluation, but any discussion or analysis of the data will always be done anonymously.

8.0 Administrative Procedures

8.1 Curriculum vitae

The PI of the project can provide an updated CV demonstrating her experience in research projects in the field of nutrition and regenerative medicine for musculoskeletal disorders. She has a special research interest in the characterization of orthobiologics products such as PRP and microfragmented adipose tissue. In particular, she has been involved in several in vitro and clinical studies including the investigation of the importance of the nutritional

role in post-operative recovery of orthopaedic patients, as well as the role of mesenchymal stem cells and tissue resident progenitor cells for the treatment of musculoskeletal disorders.

8.2 Information about Staff involved

The researcher has the responsibility to inform all personnel involved in the study of all the relevant aspects of the study. A list of personnel designated by the principal investigator to be involved directly or indirectly in the study in question should be set up, updated and stored.

8.3 Publications

All data collected in this study are property of the IRCCS Galeazzi Orthopaedic Institute.

It is foreseeable the production of manuscripts and abstract. The principal investigator is committed to the publication of results and will be responsible for publishing the results of this study. The principal researcher can publish or present the study results in accordance with this section of the protocol.

The principal investigator is entitled to use the results of all the work produced according to this protocol, including, but not limited to, test results and statistical data obtained. The principal institute/researcher agrees that, apart from his or her employees, executives, collaborators, and representatives, the data will not be disclosed except after written consent.

It is the responsibility of the principal investigator to send the study report to the competent authorities.

8.4 Funds

Funds for lab analysis and for preliminary activities of the Ethical Committee will be covered with funds of the Ministry of Health and research funds.

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