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Update of clinical aspects of knee osteoarthritis: data mining from previous work with NMR-based metabolomics of synovial fluid

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ABSTRACT

Introduction: Knee osteoarthritis (OA), which leads to progressive disability, has been associated with metabolic syndrome. Metabolomics emerges as a promising tool for investigation of this connection. The goal of this study was to correlate the metabolic profile of synovial fluid of patients with knee osteoarthritis with clinical factors related to the development of metabolic syndrome (MetS) based on the reanalysis of a patient's database from our previous study.

Methods: Patients were divided in two groups: without osteoarthritis, who underwent knee arthroscopy (n = 8; K-L Grade 0) and with knee OA, who underwent total knee arthroplasty surgery (n = 26, K-L Grades 3-4). From a database of synovial fluid metabolomic analysis by nuclear magnetic resonance, clinical data and collected from medical records, including age, sex, height and weight, and fasting blood glucose levels underwent multivariate analysis.

Results: Based on the metabolic profile, glycerol was increased in the group of patients with osteoarthritis. However, metabolomics was not able to classify patients into subgroups according to blood glucose ranges, body mass index and by age.

Conclusion: In a population with osteoarthritis, metabolic analysis evidences a slightly different profile in the analysis by age and BMI, or age and glycaemia.

Keywords: *Metabolomics; Metabolic Syndrome; Osteoarthritis; Synovial Fluid*

INTRODUCTION

Knee osteoarthritis (OA) is characterized by synovial inflammation, hyaline cartilage degeneration and subchondral bone thickening, affecting the whole joint[1]. Metabolic syndrome (MetS) is defined by the presence of three or more clinical findings of hyperglycemia, obesity, dyslipidemia, and systemic hypertension, hence related to increased rates of cardiovascular disease and mortality[2].

Obesity has long been associated to OA due to mechanical aspects of overweight, but it has also a role in consequence of low-grade inflammation leading to subchondral bone microvasculature alterations and neuromuscular damage[3]. Hypertension seems to have an independent role in OA genesis, compared to other MetS components, since it produces ischemic modifications which can compromise articular cartilage and subchondral bone, hypothetically due to atheroma plates formation[4,5]. Dyslipidemia promotes lipidic deposition in articular cartilage, seeming to damage its structure[5]. Insulin resistance, or lack of this hormone, hinders glucose uptake by cells, leading to an increase of its plasmatic concentration and impairing chondrocyte ability to maintain and repair extracellular matrix[6].

Metabolites constitute a broad range of low molecular weight (less than 1.5KD), which develop important roles in biological systems, allowing the understanding some diseases phenotypes[7]. Body fluids metabolites are influenced not only by genetics, but also by lifestyle, as diet, exercises, drug

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uptake, intestinal microbiome, hormonal homeostasis, and age[8]. Metabolomics is a large-scale analysis of metabolites and is central in flux of information[9]. There are two standard methods for metabolomics, NMR (Nuclear Magnetic Resonance) and MS (Mass Spectrometry). NMR has the advantage to be more reproducible and be indestructible for the sample. In addition, NMR allows in-vivo analysis, and minor liquid biofluid preparation. In the context of OA, NMR metabolomics seems to be a promising tool in finding an OA biomarker useful for diagnosis, stratification, and treatment control[10]. Our group described that OA is related to enhanced levels of glycerol and glucose in the synovial fluid[11].

The goal of this study was to correlate the synovial fluid metabolic profile of patients with and without knee osteoarthritis with clinical data related to metabolic syndrome.

MATERIAL AND METHODS

Study subjects

This study is a secondary analysis performed on clinical data of 34 patients included in a previous study[11] and approved by the Institutional Ethics Board.

The population study was composed of patients who underwent knee arthroscopy (without knee OA, K-L grade 0) or replacement (with knee OA, K-L grades III or IV), classified according to the American College of Rheumatology criteria of OA[12] and Kellgreen-Lawrence OA staging[13]. Patients with gout, pseudogout, rheumatoid arthritis, lupus, psoryasis, hemochromatosis, chronic inflammatory diseases, genetic diseases, autoimmune diseases, and cancer were not included in the study. Patients with infection and previous surgery on the affected knee were also not included, as so as the ones HIV positive, hepatitis B/C positive, in use of immunossuppresants, chemotherapy or corticosteroids. All patients signed the written informed consent.

Radiographic analysis for OA classification was based on the institutional image database and performed with the software mDicom Viewer 3.0.0 (Microdata).

Clinical data evaluation

Clinical data regarding age (in years), height (in meters) and weight (in kilograms) to calculate body mass index (BMI), and serum blood glucose (in milligrams per deciliter) were collected from medical records in the institutional preoperative evaluation form.

Regarding age, patients were stratified in two subgroups: inferior or equal to 70 years and superior to 70 years.

BMI was calculated by the ratio between weight, in kilograms, and height, in square meters, which is the most used method for calculating body adiposity. For BMI analysis we classified the patients according to the Brazilian Consensus on Obesity[14] in normal weight (18.5 to 24.9 kg/m^2), overweight (25 to 29.9 kg/m^2) and obese (≥ 30 kg/m^2).

Blood glucose is measured in serum after 8 to 12 hours fasting, without consumption of food or beverage, except water. In this study we used reference levels adapted from the Brazilian Consensus of Diabetes[15], classified as normal (< 100 mg/dL), prediabetes (100 to 125 mg/dL or diabetes (≥ 126 mg/dL).

Metabolic evaluation of synovial fluid

Synovial fluid harvest, transport and NMR metabolomics analysis from the database samples were described as performed in a previous study[11]. For the new analysis we only used the unsupervised principal component analysis (PCA). All the assignments were performed by COLMARm (https://spin.ccic.osu.edu/index.php/colmarm) with HSQC and TOCSY and 1D spectra by HMDB (https://hmdb.ca/) and BMRB (http://www.bmrb.wisc.edu/metabolomics/).

Metabolic analysis of subgroups first included isolated analysis of Metabolomics by age (below and above 70 years), BMI (normal, overweight, and obese), and blood glucose levels (normal, prediabetes and diabetes). Then, analysis focused only on patients with knee OA and the associations between age and BMI, and age and blood glucose levels, in order to find if there was any metabolite able to differentiate these groups.

Statistical analysis

Data were collected in Microsoft Excel® spreadsheets and statistical analysis was performed by the software GraphPad Prism for Windows 5.0.

Age, BMI, and blood glucose were presented in average and standard deviation and compared by unpaired one-tailed Student´s *t*-test. Chi-square test was used to compare proportion of patients with and without OA and subgroups of BMI and blood glucose.

The matrix with NMR analysis data was performed on AMIX software (Bruker). Multivariate statistical analyses were done on MetaboAnalyst 4.0 (www.metaboanalyst.ca) and normalized by the sum of peak intensities and pareto-scaling, besides multivariate principal component analysis.

Posteriorly, data from metabolomic analysis archives underwent multivariate analysis according to the clinical data obtained from medical records in order to identify metabolites that could discriminate those groups. Analysis involved patients with knee OA which presented age over 70 years and BMI equal or superior to 30 kg/m^2 versus the ones with age

equal or inferior to 70 years and BMI below 30 kg/m^2. In the same manner we investigated patients with knee OA that had age over 70 years and blood glucose levels equal or superior to 100 mg/dL versus the ones with age equal or inferior to 70 years and blood glucose levels below 100 mg/dL. *p* value was considered significant when below 0.05.

RESULTS

Demographics

Clinical profile evidenced that patients with knee OA (n = 26) presented higher age, BMI, and blood glucose levels average than the ones without knee OA (n = 8), as shown in Table 1.

Table 1: Demographic data of patients included in the study.

	W/OA (n=26)	W/O OA (n=8)	*p* value	95% CI
OA Classification	Grade 3: 4 Grade 4: 22	Grade 0: 8	N/A	N/A
Age	67.35 ± 0.97	26.75 ± 2.4	*p*<0.0001*	36.10 – 45.09
Sex	Male:4	Male: 8		
	Female:22	Female:0	N/A	N/A
BMI	32.75 ± 1.1	27.26 ± 1.07	*p*=0.0028**	1.20 – 9.76
Blood glucose	107.6 ± 4.09	85.63 ± 3.38	*p* =0.0401**	6.35 – 37.63

W/OA: with OA; W/O OA: without OA; n: number of patients; CI: confidence interval; BMI: body mass index; N/A: non-applicable; * one-tailed unpaired *t*-test; ** two-tailed unpaired *t*-test.

Clinical data and OA

Patients with and without knee OA were then stratified in subgroups according to the variables BMI (normal, overweight and, obese) and fasting blood glucose level (normal, prediabetes and diabetes).

A significant association between obesity and OA (p = 0.0061) was identified. It´s important to highlight that 7.7% patients with OA did not present abnormal BMI. However, no significant association between fasting blood glucose levels and OA was verified (p = 0.23).

Synovial fluid metabolic profile

To investigate if synovial fluid metabolic profile was related to presence of OA, age, BMI, or fasting blood glucose levels, registries from de Sousa et al study database (10) were re-evaluated by PCA (Principal Component Analysis) guided by clinical data.

Individuals were first classified in two distinct subgroups, without OA and with OA according to metabolic profile (Figure 1A). Original database analysis identified that the metabolite “glucose + glycerol”, chemical shift 3.56 ppm (Figure 1B) was the main responsible for allowing the two groups distinction, which was confirmed in this re-analysis. Based on this separation, we could proceed with further data analysis.

Since all OA patients were over 55 years old, we established a cut point of 70 years for metabolic analysis by age. However, PCA was not able to classify individuals in these two subgroups according to metabolic profile, as there was a clear intersection between the profiles in score plot (Figure 2A), and no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 2B).

PCA analysis evidenced that normal BMI patients presented slightly different synovial fluid metabolic profile than overweight and obese patients (Figure 2C), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 2D).

PCA analysis evidenced that normal glycaemia patients presented slightly different synovial fluid metabolic profile than prediabetic and diabetic patients (Figure 3A), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 3B).

Clinical data from OA patients were combined to verify if the association of the variables age, BMI, and blood glucose levels could help identifying different metabolic profiles.

PCA analysis evidenced, in Score Plot (Figure 4A), that patients from subgroup A (BMI < 30 kg/m^2 and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup B (BMI ≥ 30 kg/m^2 and age > 70 years), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 4B).

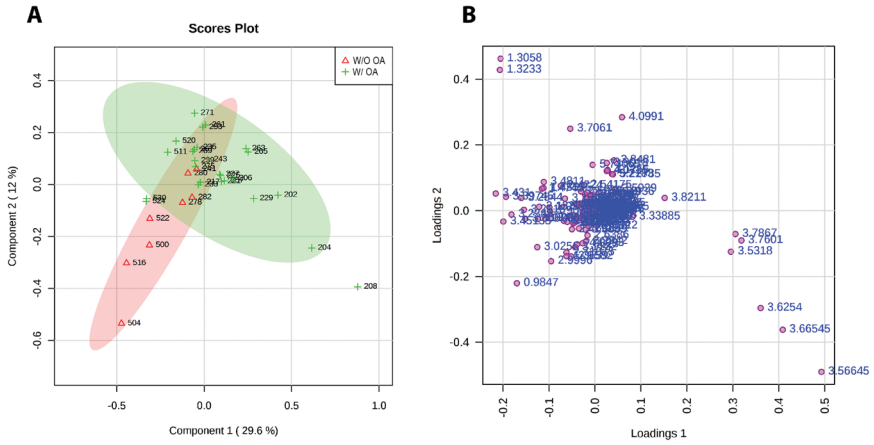

Figure 1: Discrimination of patients without and with OA by metabolic profile. PCA evidenced that it was possible to distinguish patients without OA (red diagram) from those with OA (green diagram) by metabolic profile. A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidence that PC1 can explain 29.6% of variables, and PC2 only 12%; B: Loading-Plot, where each dot represents one bucket, indicated that the metabolite "glucose + glycerol", chemical dislocation 3.56 ppm was the main responsible for this distinction. OA: Osteoarthritis; PCA: Principal component analysis; ppm: parts per million.

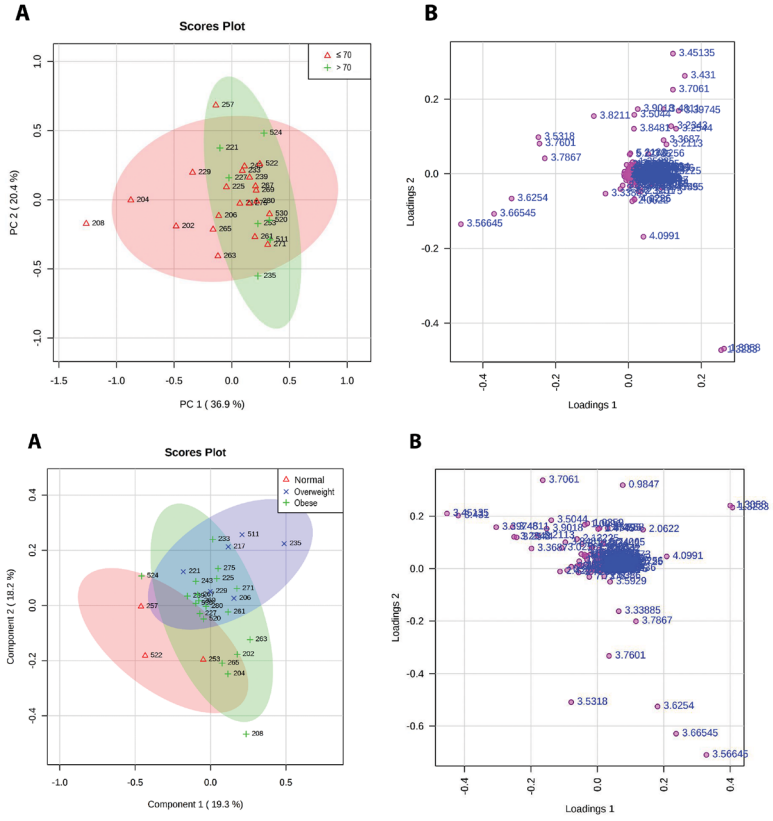

Figure 2: Discrimination of patients according to age and BMI by metabolic profile. A: PCA evidenced that it was not possible to distinguish patients with age equal or below 70 years (red diagram) from those with age above 70 years (green diagram) by metabolic profile B; Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis. C: PCA analysis evidenced that normal BMI patients (red diagram) presented slightly different metabolic profile than overweight (blue diagram) and obese patients (green diagram); D: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis.

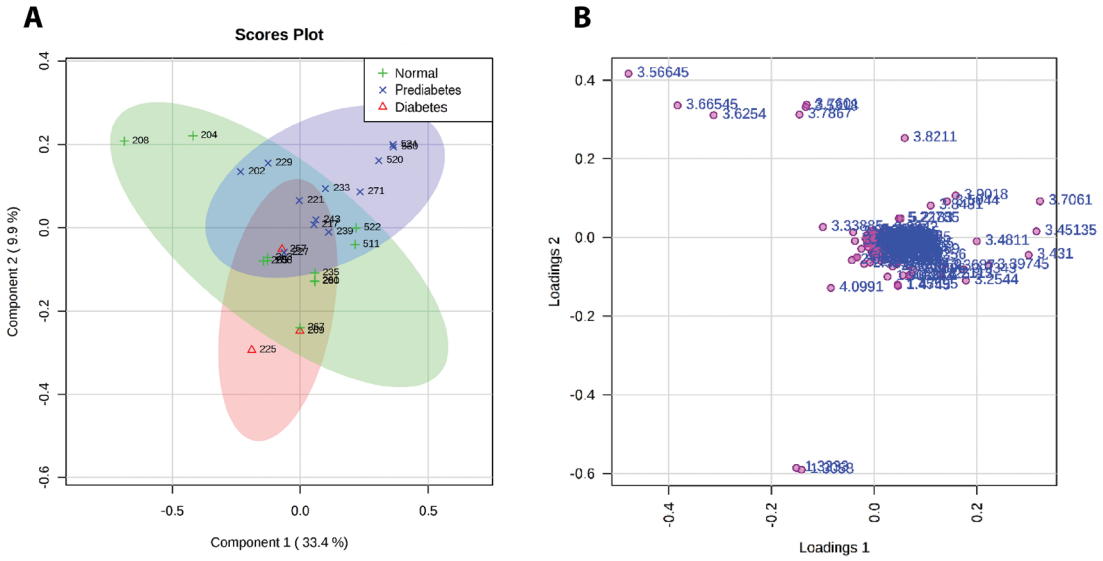

Figure 3: Discrimination of patients according to fasting blood glucose level by metabolic profile. PCA analysis pointed to convergence between normal fasting glycemia (green diagram), prediabetic (blue diagram) and diabetic (red diagram) patients, which impaired their distinction by metabolic profile. A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidence that PC1 can explain 33.4% of variables, and PC2 only 9.9%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; PCA: principal component analysis.

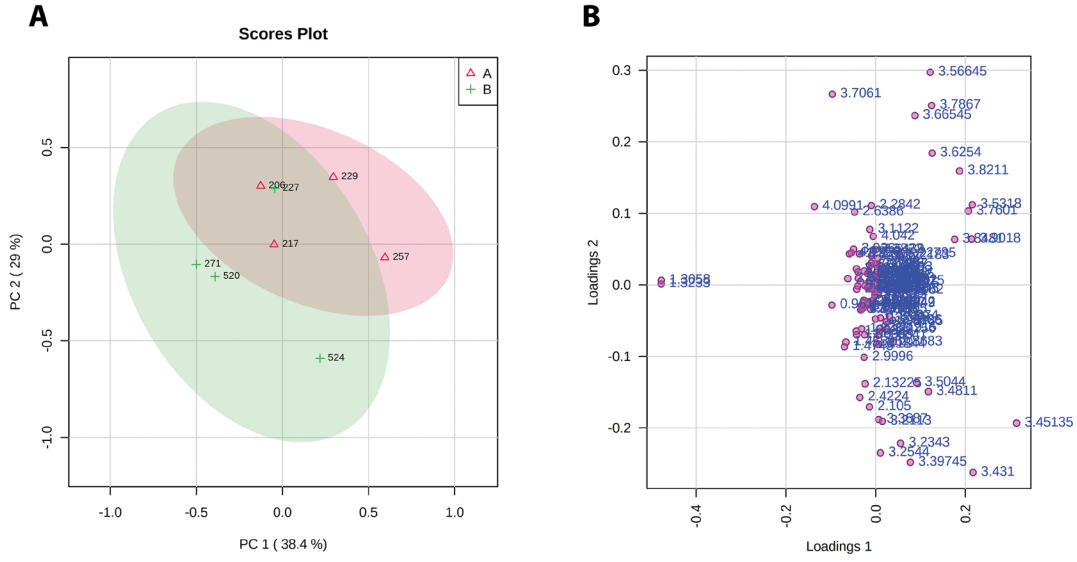

Figure 4: Discrimination of OA patients according to the association between age and BMI by metabolic profile. PCA analysis evidenced that patients in subgroup A (BMI < 30 kg/m2 and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup B (BMI ≥ 30 kg/m2 and age > 70 years). A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidences that PC1 can explain 38.4% of variables, and PC2 only 29%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; BMI: body mass index; PCA: principal component analysis.

Similarly, PCA analysis evidenced, in Score Plot (Figure 5A), that patients from subgroup C (blood glucose < 100 mg/dL and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup D (blood glucose ≥ 100 mg/dL and age > 70 years), but no assigned metabolite was able to distinguish patients group considering the clinical data for each section (Figure 5B).

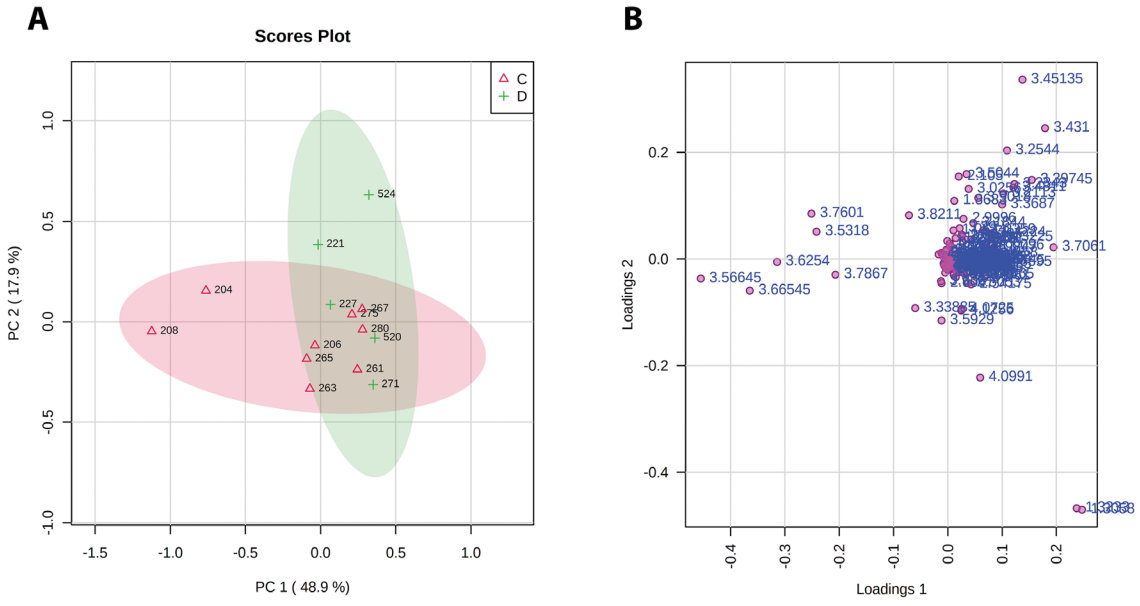

Figure 5: Discrimination of OA patients according to the association between age and fasting blood glucose level by metabolic profile. PCA analysis evidenced that patients in subgroup C (blood glucose < 100 mg/dL and age ≤ 70 years) presented slightly different synovial fluid metabolic profile than patients in subgroup D (blood glucose ≥ 100 mg/dL and age > 70 years). A: Score-Plot, PC 2 versus PC 1, where each dot represents one patient. Percentage in parenthesis evidences that PC1 can explain 48.9% of variables, and PC2 only 17.9%; B: Loading-Plot, where each dot represents one metabolite, indicated that no assigned metabolite was able to distinguish patients group considering the clinical data for each section. OA: osteoarthritis; BMI: body mass index; PCA: principal component analysis.

DISCUSSION

Osteoarthritis (OA) is a chronic complex degenerative disease characterized by cartilage degradation, synovial inflammation, and bone thickening, besides osteophytes formation, affecting the synovial joint as a whole and leading to loss of articular function[16,17].

In this research we performed an analysis of medical records from a database of patients included in a previous study[11] and evaluated their relationship with synovial fluid metabolomic analysis. We confirmed the previous findings that glucose and glycerol were able to classify patients without and with knee OA. However, this classification according to the metabolic profile was not possible neither when considered age, BMI, and fasting blood glucose levels, nor when associating the variables age and BMI, or age and fasting blood glucose levels. In this study, an analysis using principal component analysis (PCA), simpler, was performed, since it was a database re-evaluation, under new parameters, stratified in classes, not performed before. Besides, PCA is less susceptible to data over fitting, permitting visualization without complex statistical analysis.

Synovial fluid is a plasma ultrafiltrate, containing additional molecules produced by cells in joint space, reflecting its biological phenomena. Besides, it is in contact with tissues affected by knee OA, as so as synovial fluid may represent articular tissues alterations more precisely than measurements made in blood or urine[18,19].

In this study, we identified that clinical variables as glycaemia and obesity, as so as age, were not able to distinguish different patient profiles through PCA since this is an unsupervised analysis and, possibly, due to the great variability found in the samples, which can be explained by diet, genetics and ancestry of patients analyzed. Age, sex, BMI, and comorbidities have already been described as possible confusing factors in knee OA patient's synovial fluid metabolic

analysis. However, in a previous study, no metabolic profile related to these factors was found[8].

We chose 70 years old as cut point for age due to the profile of our OA patients include only the ones who underwent total knee replacement, and hence be aged above 55 years old. As knee OA incidence and severity increase with age[17], we thought that the age of 70 years old would be able to identify patients with metabolic profile compatible with later stages of the disease. The age of 50 years old was used as cut point previously in an attempt to create a predictive model of metabolite signatures for knee OA diagnosis, suggesting that phosphatidylcholine and lysophosphatidylcholine would be the predominant indicators of knee OA in older males[20]. Neither alteration in phosphatidylcholine concentrations was found. It had been previously demonstrated that many metabolites concentration is affected by sex, race or age, which is considered preponderant. Hence, those factors may be confounding factors when different groups are compared in clinical studies[21].

Metabolic syndrome, defined by the presence of systemic hypertension, dyslipidemia, hyperglycemia and obesity, seems to increase knee OA severity, notably in relation to the worsening of clinical manifestations and prognosis, due to cumulative influence of metabolic disorders, in a chronic state of low-grade inflammation[2,16,22]. It was suggested that alterations in amino acids metabolism seems to have a central role in MetS[23]. However, although its clinical relevance to public health, a recent systematic review evidenced that few papers were published focusing on MetS biomarkers investigation[24].

In this study, we chose BMI and fasting blood glucose levels, available in institutional medical records of patients, as clinical parameters for stratification analysis of metabolic profile. Analysis stratified by BMI evidenced that normal BMI patients presented slightly different synovial fluid metabolic profile than overweight and obese patients. However, this difference was not statistically significant. It has been reported that other studies tried to stratify patients by BMI, in normal and obese, according to the metabolic profile, using analysis as PCA. However, confounding factors as diet, socioeconomic status, physical activity, tabagism and sex, among others, turn this analysis harder[25].

Knee OA and obesity relationships were investigated by untargeted serum metabolomic analysis and evidenced that obesity related knee OA presents greater oxidative stress and more acid medium. Besides, oxidative stress based on nitrogen reactive species and acidotic problems are increasing in obesity in contrast to non-obesity related knee OA[26]. Metabolic profiles that could distinguish overweight and obese adults presenting progressive and non-progressive signs of radiographic OA were identified, suggesting a role of metabolic factors in disease evolution[27]. In turn, it was also revealed that OA patient's metabolic profile alters robustly over radiographic staging of the disease, with greater concentrations of malate and a tendency to increased concentrations of citrate, fumarate, and succinate in late stages of the disease. These findings suggest that metabolism in OA late stages converges to preserve energy[28]. Our data evidenced a slightly different metabolic profile of normal from abnormal BMI patients, but it did not allow significant distinction of neither of all patients by BMI, nor of OA patients by age and BMI based on synovial fluid metabolic profile.

Regarding diabetes, there are evidences that glucose, fructose, amino acids and lipids are altered both in patients with type I and II diabetes[29]. Similarly, recent study identified 12 plasma metabolites, by mass spectroscopy, in patients without medical records of diabetes (48.1% men, average age 63.3 years), which could be related to different glucose metabolism indexes and insulin sensitivity compared to general population[30]. In the present study, analysis based on fasting glycaemia indicated that normal glycaemia patients presented slightly similar metabolic profile to prediabetic and diabetic patients, although no metabolite could identify this classification. We believe the fact that our analysis been performed in synovial fluid and not on plasma may be one of the factors responsible for this difference in findings between the studies.

Studies pointed out the relationship between type 2 diabetes and knee OA progression in any joint and, specially, with two times chances of association with knee replacement than in normal population[31]. Another study demonstrated that glucose homeostasis can predict individuals at risk for OA development, but are not able to distinguish cases of accelerated knee OA[32]. This is corroborated by our data, which evidenced a slightly different metabolic profile of normal glycaemia from prediabetic and diabetic patients, but it did not allow significant distinction of neither of all patients by blood glucose levels, nor of OA patients by age and blood glucose levels based on synovial fluid metabolic profile.

Finally, we identified that although patients presented different OA grades, age, BMI, and glycaemia, they could only be classified by OA absence/presence, according to metabolic profile.

This study presents some limitations, as the small sample size and incomplete medical records, which turned it difficult to represent all possibilities of profiles. Moreover, lipidogram was not available in medical records, and so it was not used for subgroup stratification for analysis, although dyslipidemia is

one of the components of MetS. However, we could analyze synovial fluid from live patients without and with knee OA, instead of some studies involving cadavers or only diseased samples.

In conclusion, metabolomics was able to classify patients without and with knee OA according to metabolic profile, due to increased glycerol and glucose concentration in the OA subgroup. However, considering only the OA patients, metabolomics evidenced a slightly different metabolic profile according to the associations between age and BMI, or age and glycaemia, which point out to a possible association between MetS with OA.

Conflict of interests

The authors have no conflict of interests to declare.

REFERENCES

1. Goldring MB, Goldring SR. Osteoarthritis. *J Cell Physiol*. 2007;213(3):626-34.
2. Zhuo Q, Yang W, Chen J, Wang Y. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol*. 2012;8(12):729-37.
3. Niu J, Clancy M, Aliabadi P, Vasan R, Felson DT. Metabolic syndrome, its components, and knee osteoarthritis: the framingham osteoarthritis study. *Arthritis Rheumatol*. 2017;69(6):1194-1203.
4. Chan PB, Wen C. Spontaneous hypertensive rat exhibits bone and meniscus phenotypes of osteoarthritis: Is it an appropriate control for MetS-associated OA? *Ann Rheum Dis*. 2018;77(5):e25.
5. Walter SS, Wintermeyer E, Klinger C, Lorbeer R, Rathmann W, Peters A, et al. Association between metabolic syndrome and hip osteoarthritis in middle-aged men and women from the general population. *PLoS One*. 2020;15(3):e0230185.
6. Zhai G. Alteration of metabolic pathways in osteoarthritis. *Metabolites*. 2019;9(1):11.
7. Zhang W, Likhodii S, Zhang Y, Aref-Eshghi E, Harper PE, Randell E, et al. Classification of osteoarthritis phenotypes by metabolomics analysis. *BMJ Open*. 2014;4(11):e006286.
8. Guma M, Tiziani S, Firestein GS. Metabolomics in rheumatic diseases: desperately seeking biomarkers. *Nat Rev Rheumatol*. 2016;12(5):269-81.
9. Santos Jr GCS, Renovato-Martins M, Brito NM. The remodel of the "central dogma": a metabolomics interaction perspective. Metabolomics. 2021;17(48):1-15.
10. Sousa EB, Santos Junior GC, Duarte MEL, Moura Neto V, Aguiar DP. Metabolomics as a promising tool for early osteoarthritis diagnosis. *Braz J Med Biol Res*. 2017;50(11).
11. Sousa EB, Santos Junior GC, Aguiar RP, Sartore RC, Oliveira ACL, Almeida FCL, et al. Osteoarthritic synovial fluid modulates cell phenotype and metabolic behavior *in vitro*. *Stem Cells Int*. 2019;15(2019):8169172.
12. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis: Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum*. 1986;29(8):1039-49.
13. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. *Ann Rheum Dis*. 1957;16(4):494-502.
14. Associação Brasileiro para o Estudo da Obesidade e Síndrome Metabólica. *Diretrizes brasileiras de obesidade*. São Paulo: ABESO; 2016.
15. Sociedade Brasileira de Diabetes. *Consenso Brasileiro de Diabetes, Sociedade Brasileira de Diabetes Diretrizes 2017-2018*. São Paulo: Sociedade Brasileira de Diabetes; 2017.
16. Berenbaum F. Deep phenotyping of osteoarthritis: a step forward. *Ann Rheum Dis*. 2019;78(1):3-5.
17. Munjal A, Bapat S, Hubbard D, Hunter M, Kolhe R, Fulzele S. Advances in molecular biomarker for early diagnosis of osteoarthritis. *Biomol Concepts*. 2019;10(1):111-9.
18. Carlson AK, Rawle RA, Wallace CW, Brooks EG, Adams E, Greenwood MC, et al. Characterization of synovial fluid metabolomic phenotypes of cartilage morphological changes associated with osteoarthritis. *Osteoarthritis Cartilage*. 2019;27(8):1174-84.
19. Watt FE, Hamid B, Garriga C, Judge A, Hrusecka R, Custers RJH, et al. The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis. *Osteoarthritis Cartilage*. 2020;28(3):324-33.
20. Rockel JS, Kapoor M. The metabolome and osteoarthritis: Possible contributions to symptoms and pathology. *Metabolites*. 2018;8(4):92.
21. Lawton KA, Berger A, Mitchell M, Milgram KE, Evans AM, Guo L, et al. Analysis of the adult human plasma metabolome. *Pharmacogenomics*. 2008;9(4):383-97.
22. Dickson BM, Roelofs AJ, Rochford JJ, Wilson HM, De Bari C. The burden of metabolic syndrome on osteoarthritic joints. *Arthritis Res Ther*. 2019;21(1):289.
23. Roberts JA, Varma VR, Huang CW, An Y, Oommen A, Tanaka T, et al. Blood Metabolite Signature of Metabolic Syndrome Implicates Alterations in Amino Acid Metabolism: Findings from the Baltimore Longitudinal Study of Aging (BLSA) and the Tsuruoka Metabolomics Cohort Study (TMCS). *Int J Mol Sci*. 2020;21(4):1249.
24. Monnerie S, Comte B, Ziegler D, Morais JA, Pujos-Guillot E, Gaudreau P. Metabolomic and lipidomic signatures of metabolic syndrome and its physiological components in adults: a systematic review. *Sci Rep*. 2020;10(1):669.
25. Rauschert S, Kirchberg FF, Marchioro L, Koletzko B, Hellmuth C, Uhl O. Early programming of obesity throughout

the life course: a metabolomics perspective. *Ann Nutr Metab*. 2017;70(3):201-9.

26. Senol O, Gundogdu G, Gundogdu K, Miloglu FD. Investigation of the relationships between knee osteoarthritis and obesity via untargeted metabolomics analysis. *Clin Rheumatol*. 2019;38(5):1351-60.

27. Loeser RF, Goldring SR, Scanzello CR, Goldring MB. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64(6):1697-707.

28. Kim S, Hwang J, Kim J, Ahn JK, Cha HS, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. *Joint Bone Spine*. 2017;84(5):605-10.

29. Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. *Int J Mol Sci*. 2019;20(10):2467.

30. Bos MM, Noordam R, Bennett K, Beekman M, Mook-Kanamori DO, van Dijk KW, et al. Metabolomics analyses in non-diabetic middle-aged individuals reveal metabolites impacting early glucose disturbances and insulin sensitivity. *Metabolomics*. 2020;16(3):35.

31. Courties A, Berenbaum F, Sellam J. The phenotypic approach to osteoarthritis: a look at metabolic syndrome-associated osteoarthritis. *Joint Bone Spine*. 2019;86(6):725-30.

32. Driban JB, Eaton CB, Amin M, Stout AC, Price LL, Lu B, et al. Glucose homeostasis influences the risk of incident knee osteoarthritis: data from the osteoarthritis initiative. *J Orthop Res*. 2017;35(10):2282-87.

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