Applications of metabolomics in evaluation of anti-osteoporotic treatments

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Introduction

- Osteoporosis is a hormonal disease characterized by loss of bone mass and deterioration of microarchitecture of bone tissues. This deterioration is caused by an imbalance of bone formation and resorption.
- 10 millions fractures in the people older than 65 years in 2019.
- Multifactorial disease.
- Bone remodeling markers (b-ALP, PINP and CTX) aren't specific to the disease and miss standardization, therefore they can lead to a miss interpretation of results.
- b-ALP (bone Alkaline Phosphatase) and PINP (Procollagen type I N-propeptide) are both used to measure bone formation whereas CTX (collagen type 1 cross-linked C-telopeptide) is used to measure bone resorption.
- Osteoporosis is treated with two modalities; first strategy is to reduce bone resorption (Biphosphonates and Denosumab) and second strategy is to improve bone formation (Teriparatide)
- Metabolomics could be a solution to scale the response of the patient to the treatment and monitoring of the pathology.
- NMR can be used to detect a large panel of metabolites that can be investigated to relate them to the effect of treatment.

Treatment modalities for osteoporosis are complex, which can lead to reduced patient compliance. Consequently, there is a need to develop reliable methods for assessing treatment adherence. The aim of this study is to identify specific metabolic biomarkers that can be used to monitor both efficacy and compliance of osteoporosis therapies.

Material and methods

Cohort
- 49 patients with three times points by patient
- Women (40 to 89 years old)
- Plasma EDTA
- Classification based on osteoporosis treatment. [Figure 1]

Sample preparation [Figure 2]
- Macromolecules ultrafiltration

Analysis [Figure 3]
- Analysis by ^{1}H-NMR (NOESY presat)
- Quantification based on spectral deconvolution with Chenomx® Software

Statistical analysis
- Correlation PLS-R based on treatment group separation.

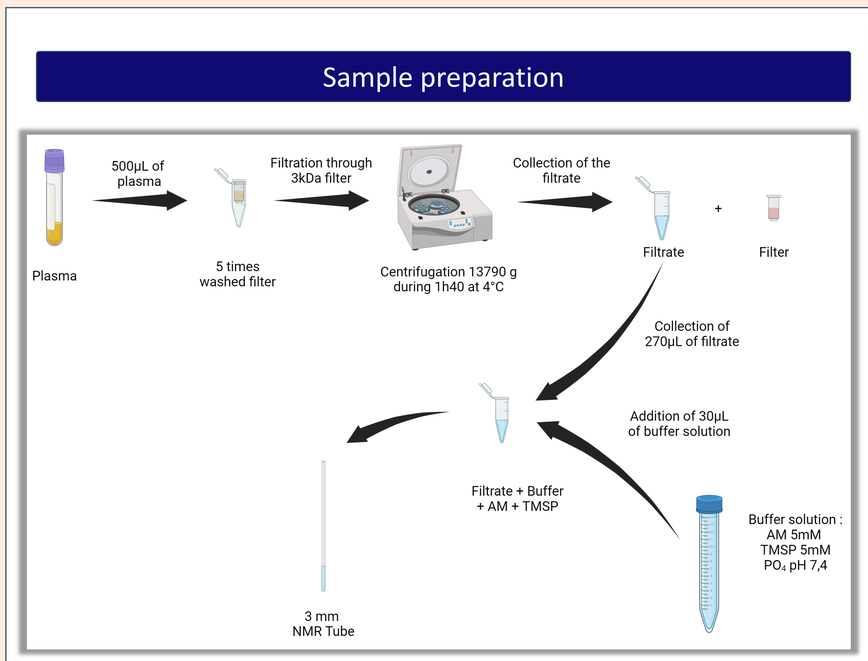

Figure 2. Sample preparation

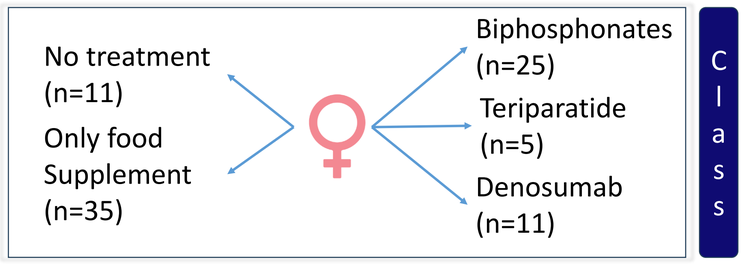

Figure 1. Classification based on treatment

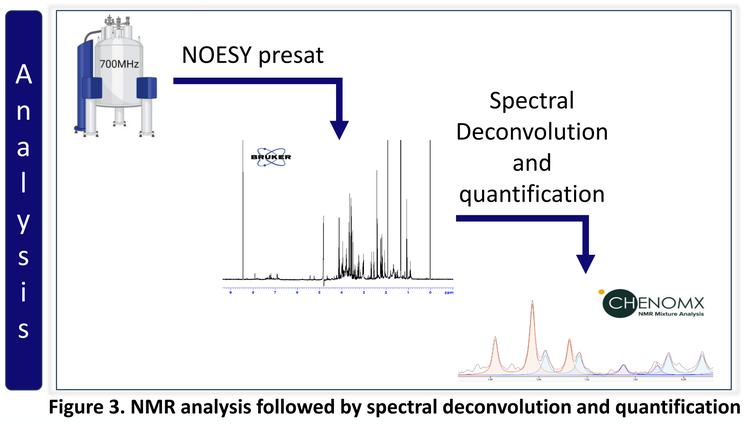

Figure 3. NMR analysis followed by spectral deconvolution and quantification

Results and discussions

PLS-R Correlations were significant in three groups

No treatment : Correlations between metabolome and three biomarkers are significant. For example, figure 4 shows the correlation between PINP and metabolites in the no treatment group. [Figure 4]

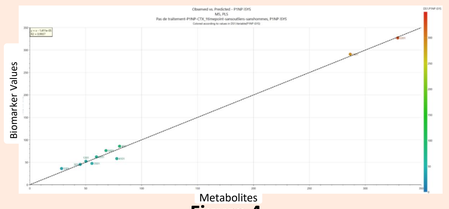

Figure 4.

Teriparatide : Correlations between metabolome and three biomarkers are all significant. These results could be explained by both bone formation and resorption. For example, figure 5 shows the correlation between b-ALP and metabolites in the teriparatide group. [Figure 5]

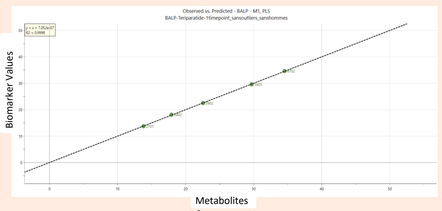

Figure 5.

Denosumab : The only significant correlation observed in this group is the CTX correlation. That result could be explained by the denosumab's main effect which is bone resorption, measured by the CTX level. Figure 6 shows the correlation between CTX and metabolites in the denosumab group. [Figure 6]

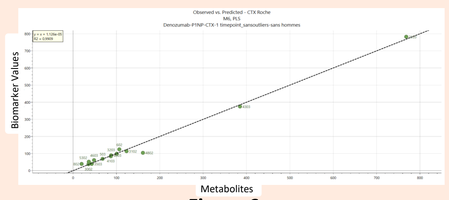

Figure 6.

Metabolites	No treatment	Teriparatide	Denosumab
2-Aminobutyrate	↘^{2}		
2-Hydroxybutyrate	↘^{3}		
2-Oxoisocaproate		↗^{3}	
3-hydroxybutyrate		↗^{3}	↗^{1}
3-methyl-2-oxovalérate	↗^{2}	↗^{3}	
Acetoacetate		↗^{3}	↗^{1}
Acetone		↗^{3}	↗^{1}
Asparagine	↗^{3}	↘^{1}	
Betaïne	↗^{3}		
Creatinine	↗^{3}		↗^{1}
Glucose	↗^{2}	↘^{3}	
Glutamine		↘^{2}	
Glycine	↗^{1}	↘^{2}	

Metabolites	No treatment	Teriparatide	Denosumab
Histidine		↘^{3}	
Mannose	↗^{1}	↘^{3}	↘^{1}
Methionine	↗^{1}		
Thréonine		↗^{2}	
Ornithine	↗^{1}	↘^{3}	
Phenylalanine	↗	↘^{1}	
Uridine	↗^{1}	↘^{1}↗^{2}	
Leucine	↗^{1}		
Pyruvate	↘^{2}	↘^{1}	

$^{1,2 \text{ and } 3}$: number of biomarkers involved in the correlation for each metabolite

↘ Negatively correlated metabolite ↗ Positively correlated metabolite

➡ Out of the 42 metabolites quantified in plasma, 15 are correlated with the no treatment group, 16 are correlated with the teriparatide group and 5 are correlated with the denosumab group. These correlations mean that one metabolite is increased or decreased when the osteoporosis biomarker does.

➡ Some metabolites are specific to the teriparatide group.

➡ Denosumab group is only correlated with the CTX.

Conclusion and perspectives

Before concluding, it is important to highlight the limitations of this study. First, the lack of standardization in the sample collection may lead to greater variability in the dataset. Additionally, we lacked detailed data about patient, which could have helped us to identify and exclude potential outliers. Finally, the groups were not homogenous, and no control group was included, both of which limit robustness of our statistical models.

Despite these limitations, this study is a first step in the use of metabolomics for monitoring of osteoporosis treatment. To summarize, we found that osteoporosis biomarkers could be correlated with some metabolites in context of teriparatide and denosumab treatments. For the teriparatide, we were able to identify and quantify a first panel of potential specific metabolic biomarkers; this observation should be explored in future studies. Regarding denosumab, we observed a correlation with the CTX measurements. Moreover, a small group of metabolites were related to the increase in CTX levels. Unfortunately, the small size of the denosumab group limited us in this study. Finally, no correlation could be established with the biphosphonates group. Future studies, ideally more standardized, are needed to validate and improve our first discovery and to assess the use of metabolomics to monitor the osteoporosis treatment.

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