Plasma Metabolomics and Olink Proteomics Profiling Predict Outcome of Fufang Duzhong Jiangu Granules in Kashin-beck Disease

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Research Article

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Abstract

Objectives

To investigate predictive biomarkers that could be used to identify patients’ response to treatment, plasma metabolomics and proteomics analyses were performed in Kashin-beck disease (KBD) patients treated with Fufang Duzhong Jiangu Granules (FDJG).

Methods

Plasma were collected from twelve KBD patients before treatment and one month after FDJG treatment. LC-MS was employed for plasma metabolomics profiling, while the olink inflammatory protein panel was used for obtaining inflammatory protein profiles. Patients were classified into responders and non-responders based on drug efficacy after one-month treatment. Enrichment analyses of differential metabolites and proteins of the responders at baseline and after treatment were conducted to study the mechanism of drug action. Differential metabolites and proteins between the two groups were screened as biomarkers to predict drug efficacy. ROC curve was used to evaluate the prediction accuracy of biomarkers.

Results

The changes of metabolites and inflammatory proteins in responders after treatment reflected the mechanism of FDJG treatment for KBD, which may act on glycerophospholipid metabolism, D-glutamine and D-glutamate metabolism, nitrogen metabolism, and NF-kappa B signaling pathway. We identified three metabolites as potential predictors: N-undecanoylglycine, beta-aminopropionitrile, and PC (18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z)). For inflammatory protein, IL-8 was identified as predictive biomarkers to detect responders. Additionally, IL-8 persisted significant in comparison responders to non-responders after one month of therapy. Combined use of these four biomarkers had high predictive ability (AUC = 0.972).

Conclusion

Metabolomics and olink proteomics provide new insights for precision therapy. In our study, IL-8 may be a novel stable potential biomarker for predicting the efficacy of FDJG in KBD treatment.

Introduction

Kashin-beck disease (KBD) is an endemic chronic degenerative osteochondral disease caused by the intricate interplay of genetic and environmental factors, significantly impacting patients’ ability to work and daily life1. KBD involves multiple joints which commonly occurs in children, resulting in short stature and eventual disability later in life. The pathogenesis of KBD is characterized by chondrocyte apoptosis, epiphyseal cartilage necrosis, proliferative inflammation and secondary osteoarthropathy1–3. For a long time, researchers have delved into understanding the causes of KBD from the environmental, cellular, genetic and molecular level4–7. Under comprehensive control measures, the occurrence and development of KBD has been effectively controlled8,9. The drugs used to treat KBD mainly focused on relieving pain and improving joint dysfunction, including non-steroidal anti-inflammatory drugs and chondroitin10. Selenium supplements have been shown to be effective in the treatment of KBD in children11. At present, the majority of patients with KBD are middle-aged and elderly. Researchers have been searching for more effective treatment options in combination with patients’ current physical conditions.

Fufang Duzhong Jiangu Granules (FDJG) is a classic prescription for the treatment of osteoarthritis. It has the effects of nourishing liver and kidney, nourishing blood and tendons, clearing collages and relieving pain, and has achieved good therapeutic effect in clinic12. The anti-osteoarthritis mechanism of FDJG was to act on arachidonic acid metabolic pathway13. In recent years, FDJG has been used in the treatment of KBD, and achieved good therapeutic effect. However, due to inherent individual variabilities, patients exhibit diverse responses to drug therapy. Recognizing the need for precise and individualized treatments, the identification of biomarkers predicting drug efficacy becomes imperative. Our goal is to uncover such biomarkers, allowing for the classification of responders and non-responders before treatment initiation, thereby providing more effective and targeted treatment options.

The rapid advancement of biotechnology offered the possibility of precision treatment. The small molecules identified by metabolomics represent substrates, intermediates and products of all biochemical pathways. Thus, metabolomics, reflecting the closest biological representation of clinical features, holds immense potential for predicting disease progression and therapeutic efficacy. Serum metabolomics has been used to assess treatment response in patients with rheumatoid arthritis and breast cancer14–16. In addition, protein biomarkers could provide additional insights into drug response prediction. Olink is a high throughput, low volume and high sensitivity proteomics method based
on proximity extension assay (PEA) technology\textsuperscript{17, 18}. A previous study showed that olink proteomics has good repeatability and stability in detecting proteins in plasma samples\textsuperscript{19}. It provides multiple immunoassay panels for a variety of disease processes, which was widely used to screen biomarkers for cardiovascular diseases, orthopedic diseases, cancer and so on\textsuperscript{20, 21}. Therefore, we combined metabolomics and olink proteomics to identify biomarkers that could predict drug efficacy of FDJG for KBD treatment.

We aimed to identify predictive drug response biomarkers that could be used to guide drug therapy. In this study, after FDJG treatment one month, we classified KBD patients as responders and non-responders according to drug efficacy, and then analyzed differential plasma metabolites and inflammatory proteins between the different groups. The predictive ability of the biomarkers was further tested by receiver operating characteristic (ROC) curve analysis. In addition to the identification of predictive biomarkers, we also clarified the mechanism of FDJG therapy for KBD.

**Method**

**Sample collection and preparation**

Twelve KBD patients who chose to take FDJG for treatment were randomly selected. They never used this medicine before our study. The patients were diagnosed according to the Chinese national diagnostic criteria for Kashin-Beck Disease (WS/T207-2010). Peripheral blood specimens (5ml) were collected from each participant at two time points – before treatment and 1 month after treatment. Plasma was separated by initial centrifugation of 1200g for 10min and secondary centrifugation of 13000g for 2min. The plasma was divided into two parts. The 500µl plasma was stored at -80°C for olink analysis. And the other 100µl plasma sample was mixed with 400µl methanol for extracting metabolites. After centrifugation at 20000 g for 15 min, the supernatant was transferred into a new tube and dried under vacuum. Then it was dissolved with 100µl 80% methanol and stored at -80°C for non-targeted metabolomics analysis.

This study was approved by the Human Ethics Committee of Xi'an Jiaotong University (approval number: 2022 - 1375). All the participants signed informed consent.

**Questionnaire survey**

At baseline, we surveyed the information of patients' general condition using a well-standardized questionnaire, including age, sex, height, weight, medical history, cigarette smoking (yes/no), hypertension (yes/no), and alcohol drinking (yes/no). The disease was graded according to the clinical criteria (WS/T207-2010). At baseline and one month after FDJG treatment, relevant scales were used to assess drug efficacy and joint pain. Joint dysfunction index (JDI), an evaluation index of therapeutic efficacy for KBD, was calculated according to the standards of Assessment for Therapeutic Efficacy on Kashin-Beck Disease (WS/T 79-2011)\textsuperscript{22}. Joint pain was measured by visual analogue scale (VAS).

**Non-targeted metabolomics analysis**

First, the chromatographic separation was performed using the UltiMate 3000 UPLC system (Thermo Fisher Scientific, Bremen, Germany). The separation was performed on ACQUITY UPLC T3 column (Milford, USA) at 40°C and flow rate of 0.3 ml/min. The mobile phase contains solvent A (5mM ammonium acetate and 5mM acetic acid) and solvent B (acetonitrile). Gradient elution conditions were set as follows: 2% B (0–0.8 min); 2–70% B (0.8–2.8 min); 0–90% B (2.8–5.6 min); 90–100% B (5.6–6.4 min); 100% B (6.4–8.0 min); 100–2% B (8.0–8.1 min); 2% B (8.1–10 min).

Follow-up mass spectrometry (MS) analyses were performed using a high-resolution tandem mass spectrometer, TripleTOF 6600 (SCIEX, Framingham, MA, USA). Q-TOF operated in both positive and negative ion modes. Screen gas was set to 30 PSI, ion source gas 1 was set to 60 PSI, ion source gas 2 was set to 60 PSI, and interface heater temperature was set to 500 ° C. For positive and negative ion modes, the ion spray floating voltage was set to 5000 V and −4500V, respectively. The mass spectrum data were obtained using IDA model. The mass range of TOF was 60–1200 Da. Dynamic exclusion was set to 4s. During the collection process, the quality accuracy was calibrated for every 20 samples.

**Olink proteomics analysis**

Plasma samples from baseline and one month after FDJG treatment were analyzed for 92 selected inflammation-related proteins by using the OLINK Proseek® multiplex inflammation panels. Briefly, PEA technology combined immunoassay of antibodies with PCR and quantitative real-time PCR (qPCR). Antibody pair probes labeled with unique oligonucleotides bound to specific proteins in solution and then formed DNA amplicons, which were highly specific and quantitatively proportional to the initial concentration of the target protein. The amplicons were subsequently quantified by microfluidic qPCR. The final detection result was represented by log\textsubscript{2} normalized protein expression (NPX) values with relative quantification.

**Pathway analysis**

Metabolic pathways of differential metabolites were identified using MetaboAnalyst (https://www.metaboanalyst.ca/). STRING database (https://cn.string-db.org/) was performed to analyze pathway of differential proteins. An online tool bioinformatics...
Graphical visualization.

**Statistical analysis**

The LC-MS raw data files were converted into readable data format and then processed with the XCMS, CAMERA and metaX implemented by R software. The retention time (RT) and m/z data were used to identify each ion. The online KEGG (https://www.genome.jp/kegg/) and HMDB (https://hmdb.ca/) databases were used to annotate metabolites to explain the physical and chemical properties and biological functions of metabolites. MetaX software was applied to evaluate the quality of metabolite data, estimate the missing value, and normalize the data. Then, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were conducted through metaX software. Variable importance in the projection (VIP) value was calculated from the PLS-DA model. The differentially expressed metabolites detected in serum samples were selected according to the criteria of VIP > 1, | log₂ FC | > 0.58 and p < 0.05.

Continuous variables were presented as mean ± standard deviation ( X ± S). Categorical variables were presented as number (percentage). Statistical significance analysis between responders and non-responders was performed using student's t-test with p < 0.05 as the level of significance. ROC curve was used to test predictive ability of multiple biomarkers. All statistical analyzes were carried out using SPSS 26.0.

**Result**

**Patient characteristics**

A total of 12 patients with KBD were included in the plasma metabolite and olink analysis. In our study group, six patients were classified as responders (significant and effective) and six as non-responders (ineffective). Detailed characteristics of patients were shown in Table 1. Age, gender, BMI, disease grade, hypertension, smoking, and alcohol drinking were similar between the two groups. Following one month of treatment, values of VAS and JDI in the two groups of patients showed a trend of decreasing at different levels, which were significantly higher in the non-responder group (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Responder (n = 6)</th>
<th>Non-responder (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60.50 ± 6.80</td>
<td>60.16 ± 5.41</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td>BMI, (kg/m²)</td>
<td>24.42 ± 3.94</td>
<td>24.31 ± 4.02</td>
</tr>
<tr>
<td>Grade , n (%)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3 (50.0)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Alcohol Drinking, n (%)</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as mean ± standard deviation. Categorical variables are presented as number (percentage).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before treatment</th>
<th>After 1 month of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS</td>
<td>5.83 ± 2.78</td>
<td>1.67 ± 1.86</td>
</tr>
<tr>
<td>JDI</td>
<td>8.16 ± 0.98</td>
<td>4.33 ± 0.52</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation. Student's t-test was performed for comparison of values for responders and non-responders at different time points; *p < 0.05. VAS: visual analog scale; JDI: joint dysfunction index.

**Metabolic and Olink profiling in responders before treatment vs responders after one month of treatment**
To determine the mechanism of drug therapy, we compared metabolomics and olink proteomics characteristics of responders before and after treatment. A total of 79 differentially expressed metabolites were identified in responders at baseline and after one month of treatment (VIP > 1, \(|\log_2 FC| > 0.58, p < 0.05\), 63 of which were downregulated after treatment (Fig. 1a). These differential metabolites belonged to lipids and lipid molecules, alkaloids and their derivatives, organic acids and derivatives, organic nitrogen compounds, of which lipids and lipid molecules accounted for the largest proportion (67%). Enrichment analysis showed that the differential metabolites were mainly enriched in glycerophospholipid metabolism, D-glutamine and D-glutamate metabolism, nitrogen metabolism, and aminoacyl-tRNA biosynthesis (Fig. 1b).

Compared with baseline, 4 proteins significantly changed \((p < 0.05)\) with FDJG treatment one month. CD40, CXCL1, and IL-20RA decreased; while FGF-21 increased (Fig. 1c). The biological function enrichment analysis suggested that cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor, and NF-kappa B signaling pathway may play important roles in FDJG treatment for KBD (Fig. 1d).

**Metabolic and olink profiling in responders vs non-responders at baseline**

To identify biomarkers that could predict the response of KBD patients to FDJG treatment, we compared the metabolomic and olink signatures between responders versus non-responders before treatment. Metabolites were analyzed by univariate analysis to determine the significantly different metabolites. Three metabolites were significantly different between two groups: N-undecanoylglycine \((p = 0.03)\), beta-aminopropionitrile \((BAPN) (p = 0.04)\), PC \((18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z)) (p = 0.03)\) (Table 3). When comparing changes in inflammatory proteins before treatment in responders and non-responders, we observed that only the concentration of IL-8 \((P = 0.04)\) had significant difference, which was higher in responders (Table 4). We next combined N-undecanoylglycine, BAPN, PC \((18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z))\), IL-8 as multiple biomarkers to detect responders. A ROC curve was plotted to evaluate the performances of these multiple biomarkers, which showed an AUC of 0.972 (95% CI: 0.889–1, \(p = 0.006\)) (Fig. 2).

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>HMDB ID</th>
<th>M/Z</th>
<th>RT</th>
<th>Fold Change</th>
<th>VIP</th>
<th>P-value</th>
<th>Status</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Undecanoylglycine</td>
<td>HMDB0013286</td>
<td>242.18</td>
<td>7.25</td>
<td>0.59</td>
<td>1.92</td>
<td>0.03</td>
<td>down</td>
<td>Amino acid metabolism</td>
</tr>
<tr>
<td>Beta-Aminopropionitrile</td>
<td>HMDB0004101</td>
<td>71.06</td>
<td>8.91</td>
<td>1.72</td>
<td>2.84</td>
<td>0.04</td>
<td>up</td>
<td>Beta-Alanine metabolism; Cyanoamino acid metabolism</td>
</tr>
<tr>
<td>PC ((18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z)))</td>
<td>HMDB0008181</td>
<td>804.55</td>
<td>6.78</td>
<td>0.52</td>
<td>2.96</td>
<td>0.03</td>
<td>down</td>
<td>Glycerophospholipid metabolism; Arachidonic acid metabolism; Linoleic acid metabolism; Alpha-Linolenic acid metabolism</td>
</tr>
</tbody>
</table>

**Metabolic and olink profiling in responders vs non-responders after one month treatment**

After one month of FDJG treatment, eight differentially expressed metabolites were identified in responders versus non-responders. The expression levels of 7 metabolites in responders were lower than those in non-responders (Fig. 3a). In addition, in the data analysis results after treatment, the three metabolites that showed differential expression at baseline were identified no significant difference.

As for the results of olink inflammatory proteins expression profile, a total of five inflammatory proteins \((\text{Flt3L, TNF, CXCL11, LAP TGF-beta-1, IL-8})\) were expressed at higher levels in responders than in the non-responders (Fig. 3b). Compared with baseline levels, IL-8 showed an overall decrease in both groups after treatment, and it was noteworthy that the expression level of IL-8 was still significantly higher in responders (Fig. 3c).
In this study, we employed a multi-omics approach, integrating metabolic and olink inflammatory protein profiling, to identify potential biomarkers for predicting the treatment response of Fufang Duzhong Jiangu Granules (FDJG) in Kashin-Beck Disease (KBD) patients. We identified three metabolites (N-undecanoylglycine, beta-aminopropionitrile, PC (18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z))), and one protein (IL-8) as biomarkers for predicting KBD patient response to FDJG. Furthermore, analysis of the responder group showed that therapeutic mechanism of FDJG for KBD was through the regulation of glycerophospholipid metabolism, D-glutamine and D-glutamate metabolism, and NF-κB signaling pathway.

After taking FDJG for one-month, joint pain and dysfunction of KBD patients improved, with more significant change in responders. By analyzing the sample data of the responders at baseline and one month after administration, we confirmed the mechanism of drug action. According to the results of metabolic profile, FDJG may treat KBD via acting on glycerophospholipid metabolism, D-glutamine and D-glutamate metabolism, and nitrogen metabolism. These findings align with previous studies linking KBD pathogenesis to metabolite disorders, particularly in lipid metabolic networks and D-glutamine and D-glutamate metabolism. Through metabolomic analysis of serum, urine and fecal samples from patients with KBD, studies have shown that the pathogenesis of KBD was related to metabolite disorders. There were some differentially rich metabolites between the KBD group and the NC group, which were involved in perturbed pathways including lipid metabolic networks, D-glutamine and D-glutamate metabolism, selenium-related bioprocesses, et al. In additional, NO, primarily a pro-inflammatory and chondrocyte-destroying mediator, was found to be significantly higher in the plasma of KBD than that of the control, emphasizing its role in the disease. The results of differential inflammatory protein enrichment suggested that FDJG may act on NF-κB signaling pathway to treat KBD. The activation of NF-κB signaling pathway mainly led to antagonized apoptosis and expression of proinflammatory proteins, which may play key roles in KBD occurrence and development. In addition, the decreased expression of inflammatory proteins (CD40, CXCL1 and IL-20RA) may had a positive effect on chondrocytes. Compared with healthy persons, CD40, CXCL1 and IL-20 showed higher expression levels in osteoarthritis patients. CXCL1 induced chondrocyte hypertrophy and apoptosis. CD40 augmented the expression of inflammatory cytokines and matrix metalloproteinases in chondrocytes, contributing to process of cartilage degradation. In conclusion, the mechanism of FDJG in the treatment of KBD included regulating the metabolite disorders, reducing the level of inflammation, and inhibiting the apoptosis of chondrocytes.

Compared to non-responders, the group of responders showed higher level of beta - aminopropionitrile, a specific LOX inhibitor. BAPN could lead to the reduction of collagen crosslinking and thus damage collagen structure and morphology. In addition, BAPN could also reduce the expression of genes related to bone formation or osteoblast differentiation, hindering the differentiation of osteoblasts. This indicated that responders had more severe cartilage structural damage and osteogenesis disorder before treatment. Additionally, we observed that the responding patients exhibited decreased levels of N-undecanoylglycine and phosphatidylcholine (PC). N-undecanoylglycine was usually a secondary metabolite of fatty acids. Elevated levels of certain acylglycines were associated with fatty acid oxidation disorders. Quercetin, an active ingredient in FDJG, could restore N-undecanoylglycine to the normal level. High-fat diet-induced accelerated osteoarthritis was associated with plasma metabolite signature rich in PC. Responders showed decreased levels of N-undecanoylglycine and phosphatidylcholine (PC), suggesting less severe lipid metabolism disorders compared to non-responders. These insights into pre-treatment metabolic distinctions provide valuable information for understanding patient responses.

Studies have shown that inflammatory cytokines play an important role in the pathogenesis of KBD. Therefore, we chose inflammatory protein panel as the observation indicator. IL-8, a key inflammatory cytokine, played a crucial role in predicting treatment response. Firstly, IL-8 was associated with the pathogenesis of KBD, with high expression in KBD patients compared with normal control. Meanwhile, IL-8 also showed significant differences in KBD patients. We observed that before treatment the expression levels of IL-8 were higher in responders compared to non-responders, indicating higher levels of inflammation in responders. In our study, FDJG treatment led to a reduction in IL-8 expression, demonstrating the therapeutic effect on inflammation in KBD patients. The consistently higher expression of IL-8 in responders, both before and after treatment, suggests its potential as a stable inflammatory biomarker for predicting drug efficacy.

According to the specific situation and characteristics of the patient, the use of precise and individualized treatment could help the patient avoid drug therapy that will be either ineffective or unsafe. This study, the first of its kind in predicting biomarkers of drug efficacy in KBD, presents a promising approach for precision medicine. The integration of multi-omics data provided an opportunity to increase our understanding of the disease and identify valuable biomarkers. We found that using of multiple biomarkers was likely to be better for distinguishing responders and non-responders with high sensitivity and specificity. We used the screened differential metabolites and proteins as multiple biomarkers to predict KBD patients who would be effective in FDJG treatment, which had good predictive accuracy (AUC = 0.972). The research not only enhanced understanding of FDJG’s therapeutic mechanism but also held implication for clinical applications, providing insights into personalized medicine approaches for KBD patients. However, as a preliminary screening in a small patient cohort, further validation in larger populations and additional basic experiments are essential to solidify the application of these identified biomarkers.

Conclusion
In conclusion, our study revealed that the identified differential metabolites and proteins hold significant promise as biomarkers for predicting the efficacy of FDJG in KBD patients. We revealed the metabolic profiles of different responders, and the multiple biomarkers we identified have high predictive ability, offering valuable insights into the pharmacological efficacy and potential therapeutic mechanisms of FDJG as a treatment agent for KBD.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Data and sample collection were performed by Deng XX and Niu H, Zhang Q, Wen JF, Zhao YJ, and Naren GW. Deng XX and Niu H analyzed the data and drafted the manuscript. Wu CY, Liu H and Guo X designed the experiment and revised the manuscript. Zhang F provided financial support. All authors read and approved the final manuscript.

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References


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**Figures**

**Figure 1**

Difference and enrichment analysis of plasma metabolites and inflammatory proteins in responders.

a. Volcano map of differential metabolites in responders at baseline vs responders after one month of treatment. Blue dots represent down regulated, orange dots represent up regulated. b. Pathway enrichment bubble map of differential metabolites. c. Box plots of four differential proteins NPX levels in responders at baseline (red), and responders after one month of treatment (blue). *p* < 0.05. d. Pathway enrichment bubble map of differential proteins.
Figure 2

ROC curves for multiple biomarkers.

ROC curves for four predictive biomarkers (N-undecanoylglycine, beta-aminopropionitrile, PC (18:3(6Z,9Z,12Z)/20:4(8Z,11Z,14Z,17Z)), IL-8) to detect responders to FDJG. ROC: receiver operating characteristic.
Figure 3

Plasma differential metabolites and inflammatory proteins in responders vs non-responders after one month treatment.

a. Heat map of differential metabolites in responders vs non-responders after one month treatment. Redder colors indicate higher levels of expression, bluer colors indicate lower levels of expression.  
b. Volcano map of differential inflammatory proteins. Red dots represent significantly different proteins ($p < 0.05$).  
c. Box plots of IL-8 normalized protein expression (NPX) levels in: responders before treatment, non-responders before treatment, responders after one month treatment, non-responders after one month treatment. * $p < 0.05$. 

- $1H$-Indole-3-propanoic acid
- 5-[(2-Furanyl)-3,4-dihydro-2H-pyrrole
- Hippuric acid
- Indole-3-propionic acid
- Isoquinoline
- LysoPC 22:5
- Oleic acid
- Piperine

\[ \text{Group} \]

\[ \text{Log}_2 \text{fold change} \]

\[ \text{Log}_{10} \text{p-value} \]

\[ \text{IL-8} \]

\[ \text{NPX} \]

\[ \text{Res.} \quad \text{Non-Res.} \quad \text{Res.} \quad \text{Non-Res.} \]

\[ \text{Before} \quad \text{After} \]

\[ * \]