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Application of ^{1}H NMR-based serum metabolomic studies for monitoring female patients with rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis is a chronic autoimmune-based inflammatory disease that leads to progressive joint degeneration, disability, and an increased risk of cardiovascular complications, which is the main cause of mortality in this population of patients. Although several biomarkers are routinely used in the management of rheumatoid arthritis, there is a high demand for novel biomarkers to further improve the early diagnosis of rheumatoid arthritis, stratification of patients, and the prediction of a better response to a specific therapy.

In this study, the metabolomics approach was used to provide relevant biomarkers to improve diagnostic accuracy, define prognosis and predict and monitor treatment efficacy. The results indicated that twelve metabolites were important for the discrimination of healthy control and rheumatoid arthritis. Notably, valine, isoleucine, lactate, alanine, creatinine, GPC APC and histidine relative levels were lower in rheumatoid arthritis, whereas 3-hydroxyisobutyrate, acetate, NAC, acetoacetate and acetone relative levels were higher. Simultaneously, the analysis of the concentration of metabolites in rheumatoid arthritis and 3 months after induction treatment revealed that L1, 3-hydroxyisobutyrate, lysine, L5, acetoacetate, creatine, GPC + APC, histidine and phenylalanine were elevated in RA, whereas leucine, acetate, betaine and formate were lower. Additionally, metabolomics tools were employed to discriminate between patients with different *IL-17A* genotypes.

Metabolomics may provide relevant biomarkers to improve diagnostic accuracy, define prognosis and predict and monitor treatment efficacy in rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune-based inflammatory disease that leads to progressive joint degeneration, disability, and an increased risk of cardiovascular complications, which is the main cause of mortality in this population [1]. The etiopathogenesis of RA is multifactorial and not fully known, which is characteristic of most autoimmune diseases. An improved understanding of RA etiopathogenesis and immunological disorders has led to modern therapeutic options, including TNF-α inhibitors. Although therapy with TNF-α inhibitors constitutes a breakthrough in RA management, no improvement is achieved in approximately 30% of cases, and another 20% of patients discontinue therapy because of side effects. There is also an ongoing search for biochemical and clinical markers that would allow the prediction of a good response to therapy with biologicals, including TNF-α inhibitors. Moreover, the results of our recent studies

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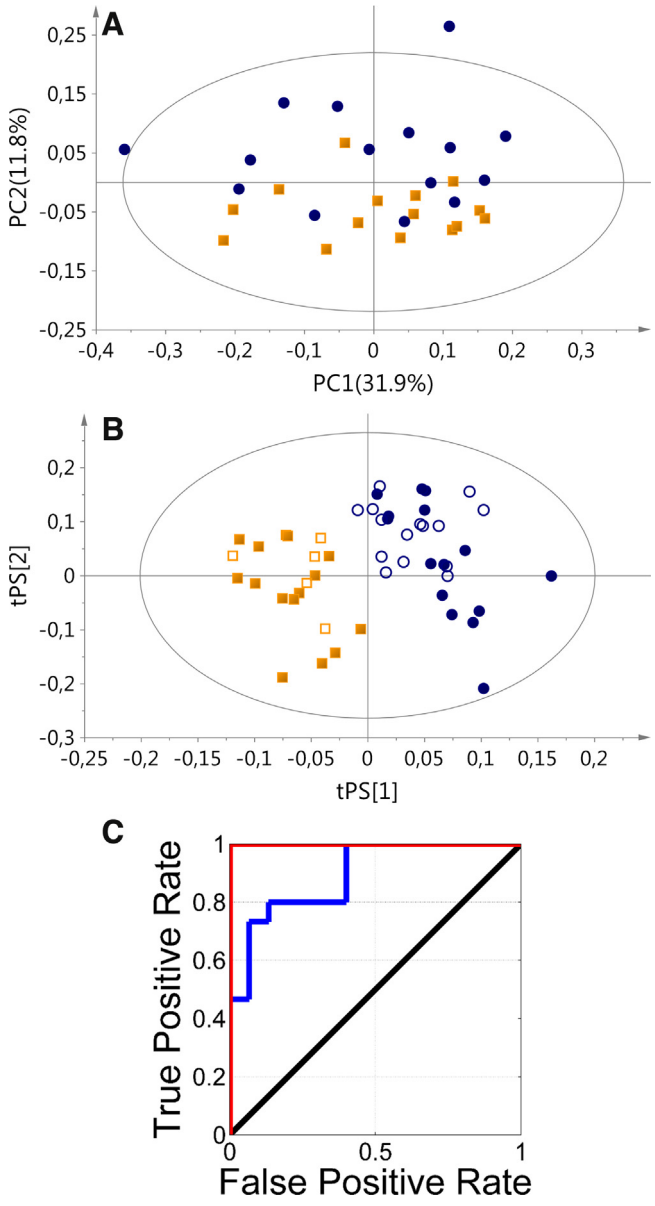

Fig. 1. The PCA model ((A); $R^2X_{cum} = 0.437$, $Q^2_{cum} = 0.272$) and PLS-DA model ((B); $R^2X_{cum} = 0.551$, $Q^2_{cum} = 0.761$) with the ROC curve (C) for comparison of the HC and RA groups. Blue circles—healthy controls; yellow boxes—patients before anti-TNF-α treatment. Empty symbols—the prediction set; solid symbols—the model set. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

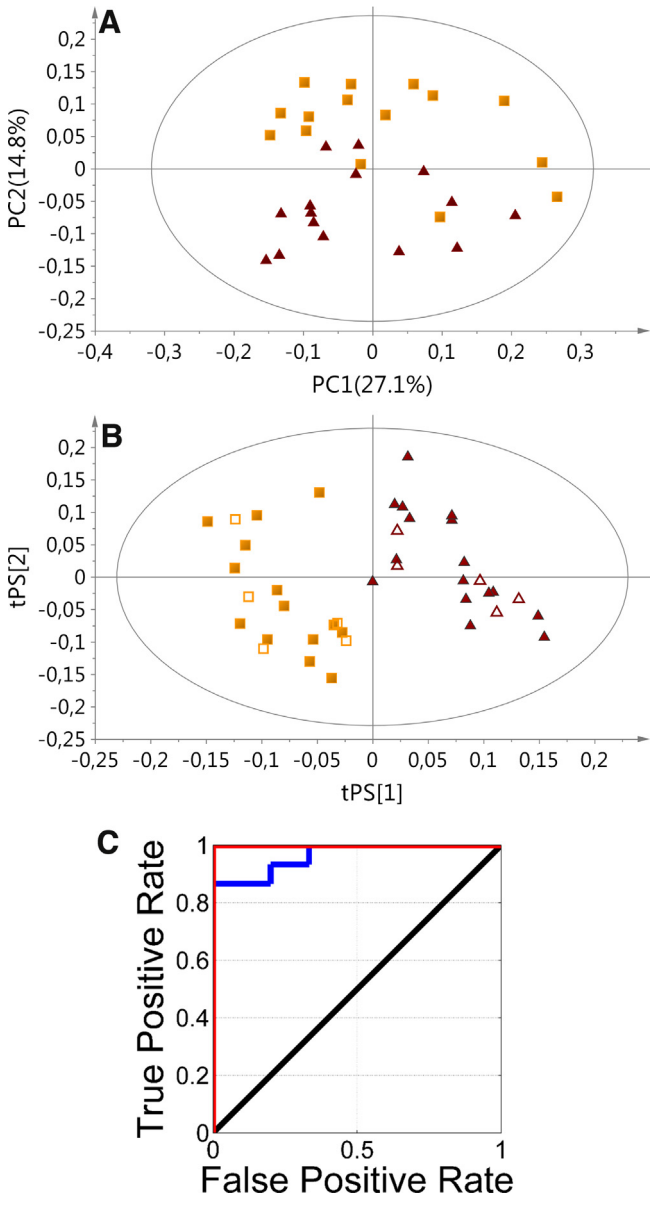

Fig. 2. The PCA model ((A); $R^2X_{cum} = 0.513$, $Q^2_{cum} = 0.351$) and PLS-DA model ((B); $R^2X_{cum} = 0.475$, $Q^2_{cum} = 0.767$) with the ROC curves (C) for comparison of the RA and RAT groups. Yellow boxes—patients before treatment; red triangles—patients after 3 months of anti-TNF-α treatment. Empty symbols—the prediction set; solid symbols—model set. (For interpretation references to color in this figure legend, the reader is referred to the web version of this article.)

have suggested that genetic variations within genes encoding for factors involved in inflammatory processes associated with RA development may play a significant role in disease susceptibility, progression and response to anti-TNF-α treatment [2,3]. In addition to clinical factors, genetic predisposition in combination with metabolomics may be helpful in clinical predictions. Although several biomarkers are routinely used in the management of RA, there is a high demand for novel biomarkers to further improve the early diagnosis of RA, the stratification of patients, and the prediction of a better response to specific therapies. In addition to genomics and proteomics, the implementation of metabolomic techniques may also improve our knowledge of the etiopathology of RA [4], as shown in our previous work in patients with inflammatory bowel disease [5] and pulmonary disease [6].

The present study assessed the potential impact of the application of metabolomic studies in patients with RA to determine whether metabolomic biomarkers of disease diagnostics and disease activity can be identified.

2. Materials and methods

2.1. Study population/characteristics of RA patients

The study included 20 Caucasian women patients meeting the EULAR/ACR (European League Against Rheumatism/American College of Rheumatology) 2010 criteria for (RA) and 30 healthy female volunteers (HC) as the control group. The patient characteristics are presented in Table 1. There was no statistically significant difference in sex and age between the populations.

Seventy serum samples were collected from adult individuals (women), including 40 samples obtained from 20 patients before