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Nuclear Magnetic Resonance (NMR)-based Metabonomics Assays to Evaluate Allergic Rhinitis Treatment in Children and Teenagers

Ensaio Metabonômico baseado em Ressonância Magnética Nuclear (RMN) para avaliar o tratamento da rinite alérgica em crianças e adolescentes

Ensayo Metabonómico Basado en Resonancia Magnética Nuclear (RMN) para Evaluar el Tratamiento de la Rinitis Alérgica en Niños y Adolescentes

Denise Maiara da Silva Carneiro[1], Tatiane Priscila Santos Rodrigues da Luz [2], Décio Medeiros Peixoto[1], Ronaldo Dionísio da Silva[3], Ricardo Oliveira da Silva[1].

ABSTRACT

Objective: To identify metabolic profiles potentially associated with treatment adherence and allergic rhinitis severity in children and adolescents. **Methods:** This prospective study was conducted at a university hospital in Brazil, involving 25 volunteers. The ^{1}H Nuclear Magnetic Resonance (NMR) spectra of the volunteers' serum samples were obtained and analyzed using multivariate statistics. Initially, Hierarchical Cluster Analysis (HCA) was employed, resulting in two groups containing 11 and 14 samples, respectively. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to identify the variables responsible for group discrimination. **Results:** Patient-reported adherence to treatment proved unreliable when compared to the HCA results. The primary difference between the groups was their response to treatment: in Group II, 81.8% of volunteers did not respond to treatment, whereas in Group I, this figure was 42.8%. Alanine, serine, arginine, choline, N-acetyl-D-glucosamine, glutamate, lactate, glucose, and VLDL/LDL were the identified metabolites. The metabolic profile of volunteers in Group II suggests a more severe clinical condition compared to those in Group I. **Conclusion:** NMR demonstrated the ability to differentiate patients' responses to allergic rhinitis treatment, effectively identifying those with more severe and chronic forms of the disease.

Keywords: Asthma, Childhood, Metabolomics, Allergic rhinitis.

RESUMO

Objetivo: Identificar perfis metabólicos potencialmente associados à adesão ao tratamento e à gravidade da rinite alérgica em crianças e adolescentes. **Métodos:** Trata-se de um estudo prospectivo desenvolvido em um hospital universitário no Brasil, envolvendo 25 voluntários. Os espectros de Ressonância Magnética Nuclear (RMN) ^{1}H do soro dos voluntários foram obtidos e processados utilizando estatística multivariada. Inicialmente, a Análise de Agrupamentos Hierárquicos (HCA) resultou em dois grupos, contendo onze e quatorze amostras cada. Utilizou-se a Análise Discriminante Ortogonal por Mínimos Quadrados (OPLS-DA)

[1] Programa de Pós-Graduação em Saúde Translacional, Universidade Federal de Pernambuco (UFPE), Recife - PE.
[2] Programa de Pós-Graduação em Química, Universidade Federal de Pernambuco (UFPE), Recife – PE.
[3] Campus Vitória de Santo Antão, Instituto Federal de Pernambuco, Vitória de Santo Antão - PE.

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para identificar as variáveis responsáveis pela discriminação. **Resultados:** O autorrelato de adesão dos pacientes ao tratamento mostrou-se falho quando confrontados com os resultados da HCA. A principal diferença entre os grupos foi a resposta ao tratamento. No grupo II , 81,8% dos voluntários não responderam ao tratamento, enquanto no grupo I, esse número foi igual a 42,8%. Alanina, serina, arginina, colina, N-acetil D-glicosamina, glutamato, lactato, glicose, VLDL/LDL foram os metabólitos identificados. Observou-se que o perfil metabólico dos voluntários do Grupo II pode indicar uma condição clínica mais grave em comparação aos do Grupo I. **Conclusão:** A RMN demonstrou capacidade de identificar a resposta ao tratamento da rinite alérgica, diferenciando pacientes com formas mais graves e crônicas da doença.

Palavras-Chave: Asma, Infância, Metabonômica, Rinite alérgica.

RESUMEN

Objetivo: Identificar perfiles metabólicos asociados a la adherencia al tratamiento y la gravedad de la rinitis alérgica en niños y adolescentes. **Métodos:** Estudio prospectivo realizado en un hospital universitario en Brasil con 25 voluntarios. Los espectros de Resonancia Magnética Nuclear (RMN) ^{1}H de muestras de suero, procesados mediante análisis multivariado. Inicialmente, el Análisis de Agrupamiento Jerárquico (HCA) generó dos grupos, con 11 y 14 muestras. Se utilizó el Análisis Discriminante de Mínimos Cuadrados Ortogonales (OPLS-DA) para identificar las variables responsables de la discriminación. **Resultados:** El autorreporte de adherencia al tratamiento mostró inconsistencias en relación con los resultados del HCA. La principal diferencia entre los grupos fue la respuesta al tratamiento: en el Grupo II, el 81,8% de los voluntarios no respondieron al tratamiento, mientras que en el Grupo I, este número fue del 42,8%. Los metabolitos identificados incluyeron alanina, serina, arginina, colina, N-acetil-D-glucosamina, glutamato, lactato, glucosa y VLDL/LDL. El perfil metabólico del Grupo II sugiere una condición clínica más grave en comparación con el Grupo I. **Conclusión**: La RMN demostró ser eficaz para identificar la respuesta al tratamiento de la rinitis alérgica, diferenciando a los pacientes con formas más graves de la enfermedad.

Palabras clave: Asma, Infancia, Metabolómica, Rinitis alérgica.

INTRODUCTION

Allergic rhinitis is a non-infectious and immunoglobulin E (IgE) mediated chronic inflammatory disease that affects nasal mucosa due to exposure to the allergen in a sensitized individual (CZECH EJ, et al., 2023; WANG Y, et al., 2023). Its main clinical symptoms include mucus hypersecretion, nasal congestion, sneezing, mucosal itching, and post-nasal drip, which can be reversible with specific treatment or, in some cases, may resolve spontaneously without treatment. Allergic rhinitis affects about 40% of the world population and is a chronic disease widespread in childhood (MA GC, et al., 2020; SCHULER CF and MONTEJO JM, 2019).

Children, teenagers, and young adults are most affected by allergic rhinitis, often presenting with comorbidities such as asthma (PASSALI D, et al., 2018; SCHULER CF and MONTEJO JM, 2021). In the pediatric population, the prevalence is about 8% in children aged 6 to 8 years old and increases to 35% among those aged 13 and 14 (AÏT-KHALED N, et al., 2009; NIETO A, et al., 2011). In these groups, allergic rhinitis is associated with interaction between genetic and environmental factors (LIU YL, et al., 2023). The most common aeroallergens are pollen, dust mites, animal dander, pollutants, and fungal allergens (PASSALI D, et al., 2018).

The classification of allergic rhinitis is based on the duration (intermittent or persistent) and the severity (mild or moderate/severe) of symptoms (BOUSQUET J, et al., 2008). This approach provides a comprehensive understanding of allergic conditions and is valuable for appropriately managing and treating the disease. Various treatment options are available, including corticosteroids, antihistamines, decongestants, oral and intranasal anticholinergic agents, and oral antileukotrienes (PAPI A, et al., 2018). However, whatever the therapeutical option adopted, its effectiveness depends on the patient's adhesion to treatment.

According to the World Health Organization (WHO, 2003), treatment adherence is the degree to which a patient's behavior corresponds to the recommendations mutually established with healthcare professionals,

including medication compliance, dietary modifications, and adopting a healthy lifestyle. Non-adherence to recommendations can lead to worsening symptoms, recurrent hospitalizations, and consequently, increased healthcare system costs. Several factors contribute to this low adherence, including the number of prescribed medications, associated costs, frequency of administration, and potential side effects (HUGTENBURG JG, et al., 2013) In children and adolescents, the barriers to medication adherence faced by caregivers may differ from those experienced in adults, particularly due to children resistance to taking medication (SCHULER CF and MONTEJO JM, 2019).

Thus, monitoring treatment adherence is essential for achieving better outcomes in managing health issues. Common techniques to evaluate adherence include patient self-reports (as used in this study), chart reviews, weighing medications, counting doses, monitoring serum drug levels, and using electronic devices (HOLMES J and HEANEY LG, 2021; HUGTENBURG JG, et al., 2013). Self-report questionnaires are widely used to assess adherence due to their low cost and ease of use in large populations. However, they have limitations in terms of accuracy and sensitivity.

While there is no universally accepted threshold for defining good or poor adherence, an 80% implementation rate of the prescribed treatment is often used to distinguish adherent patients from non-adherent ones. This metric aids in assessing treatment effectiveness and identifying the need for additional interventions to enhance patient adherence (OSTERBERG L and BLASCHKE T, 2005; PAPI A, et al., 2018). Description of clinical symptoms and its duration are used to assess adhesion and therapeutical efficacy, but their sensitivity is limited, especially in children. In this paper, we utilized ^{1}H NMR-based metabonomics assays to monitor the endogenous metabolite profile in serum samples from children and adolescents diagnosed with allergic rhinitis, aiming to associate this profile with adherence level to therapy, as well as to evaluate the employ of adherence treatment self-report as monitoring tools.

Metabonomics was defined by Nicholson JK, et al. (1999) as the “quantitative measurement of the multiparametric time-related metabolic responses of one complex system to a pathophysiological intervention or genetic modification”. By enabling the identification of metabolic alterations, metabonomics can contribute to the discovery of differentially expressed metabolites, which play a key role as biomarkers in diagnosis, disease differentiation, and monitoring the progress of medical therapy (HUANG K, et al., 2022). NMR-based metabonomics was employed with success to evaluate individual responses to treatments (VIGNOLI A, et al., 2023) and diagnosis of different diseases, such as male infertility associated with varicocele (NETO FTL, et al., 2020), liver fibrosis induced by hepatitis C virus infection (EMBADE N, et al., 2016).

However, the metabonomic approach has not been widely applied to the study of allergic rhinitis, despite its great potential to provide relevant information about the metabolic processes involved in the disease (HOLMES J and HEANEY LG, 2021). We hypothesize that the adhesion to treatment will change the endogenous metabolites profile which can be observed using NMR spectroscopy and multivariate statistical tools.

METHODS

The study was conducted at the Allergy and Immunology Service of a university hospital in Brazil. A total of twenty-five volunteers, aged 6 to 14 years, were enrolled for monitoring. The diagnosis of allergic rhinitis was confirmed through IgE testing or skin prick tests. Participation in the study was granted following the acquisition of signed informed consent from the parents. The Research Ethics Committee approved this study under number: 5.242.885 and Certificate of Ethical Appreciation Presentation (CAAE) number: 54080521.3.0000.8807 - February 2022.

The participants were classified into two groups: adherent patients (good adherence) and non-adherent patients (poor adherence) to nasal corticosteroid treatment, based on information provided by the patient or caregiver during follow-up visits. Adherence was considered satisfactory if patients reported taking approximately 80% of the prescribed medication (ensuring proper administration and correct technique), as indicated during the follow-up visit. A team of physicians and healthcare professionals saw participants on average every three months.

Information on diagnosis, clinical symptoms, physical examination including rhinoscopy (observation of mucosal pallor and nasal turbinate hypertrophy), and severity of clinical symptoms were obtained from medical records. Rhinoscopy was employed to monitor the progression of treatment and assess the efficacy of the medication. Blood samples were taken from a peripheral vein (2 mL). These samples were centrifuged at 300 rpm and the serum was separated for analysis by NMR spectroscopy. It is noteworthy that the data obtained from the rhinoscopy were recorded both during the consultation preceding the blood sample collection and on the day of the collection itself.

Serum samples were stored at –40ºC until NMR analysis. 200 µL of deuterium oxide (D_2O) was added to 400 µL of the serum sample and transferred to 5 mm id NMR tubes. ^1H NMR spectral data were obtained using the Agilent VNMRS 400 spectrometer at 400 MHz for the ^1H core using the Presat-CPMG (*Carr-Purcell-Meiboom-Gill*) pulse sequence as follows: Spectral window equal to 4.0 kHz, acquisition time equal to 2.55 seconds, tau equal to 400 µs, 88 cycles, presaturation delay equal to 2.0 seconds, and 128 transients. Each ^1H NMR spectrum was acquired in approximately 10 minutes. A spectral data matrix was created between δ 0.5 and 4.5 ppm, divided into 100 bins (0.04 ppm per bin). The data matrix was pre-processed using row normalization (sample spectra) and column autoscaling (chemical shift), which adjusted metabolite intensities to enable more accurate comparisons.

The metabolomic study was conducted using the free online platform MetaboAnalyst 5.0. The dataset was preprocessed using normalization in row and autoscaling in columns. In the exploratory data analysis phase, Hierarchical Cluster Analysis (HCA) was employed to examine the data distribution and identify potential patterns or groupings. Following group definition, Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was conducted to determine the spectral signals contributing to group differentiation (GAO H, et al., 2016). To evaluate the robustness of the OPLS-DA model, Leave-One-Out Cross-Validation (LOOCV) and permutation testing with 2000 permutations were applied (PONTES TA, et al., 2019).

The key metabolites relevant to model construction were identified using Variable Importance in Projection (VIP). VIP scores are calculated based on the projection of the data onto the variance and covariance components discriminated by OPLS-DA. All identified metabolites were compared with KEGG (*Kyoto Encyclopedia of Genes and Genomes*, www.genome.jp) and HMDB (*Human Metabolome Database),* which provide information on compounds' metabolic pathways and chemical properties.

Rhinoscopic data (mucosal pallor and turbinate hypertrophy), disease severity, and demographic characteristics—including gender and age of the participants—were collected and analyzed using the Chi-square test, with a significance level set at 5% ($p < 0.05$).

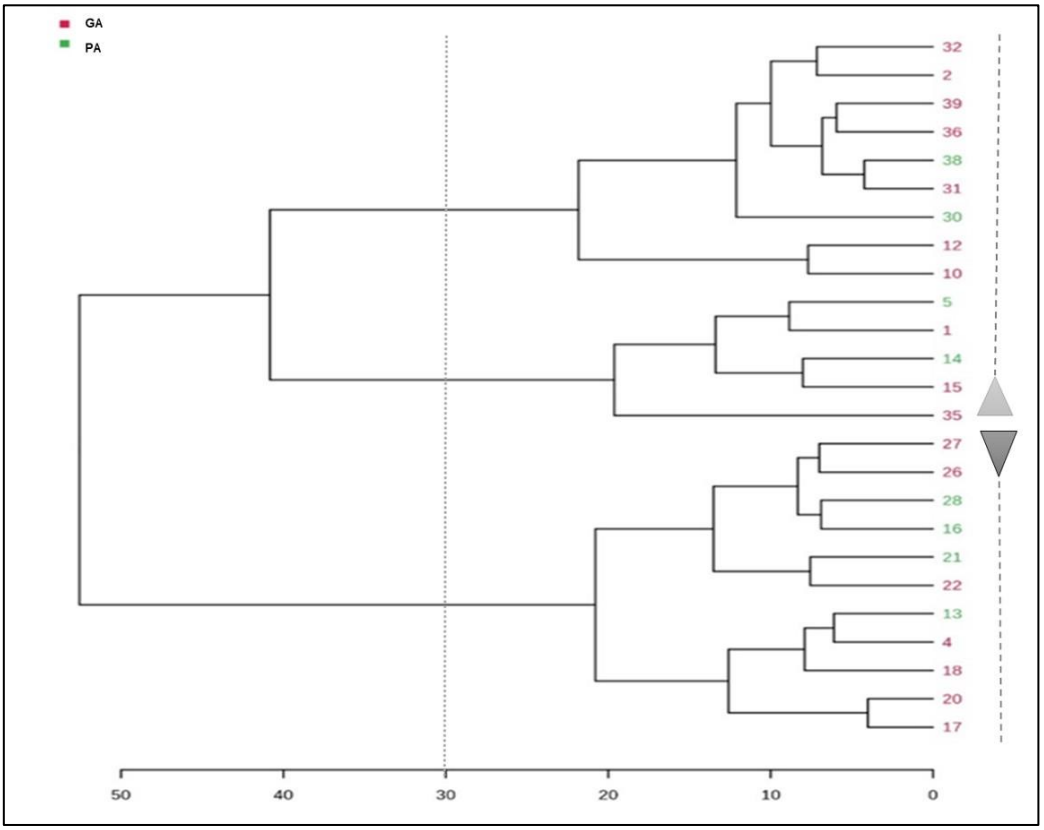
RESULTS

Among the volunteers, 11 were female and 14 were male, with an average age of nine years old (± 2 years old). only six of the participants were diagnosed with allergic rhinitis, while 19 had both allergic rhinitis and asthma. The majority (23 volunteers) had persistent symptoms, while two had intermittent symptoms. In terms of severity, six participants had mild rhinitis while 19 had a moderate to severe form. Regarding treatment adherence (self-report), 17 reported good adherence while eight were classified as inadequate adherence.

The Hierarchical Cluster Analysis (HCA) was used to group and distribute the samples based on the NMR spectra, allowing the identification of patterns and similarities within the analyzed data. We use a threshold value (distance between samples) equal to 30, separating the samples into two groups (**Figure 1**). The upper group consisted of 14 samples, four of which were identified as poor adherent. Conversely, the lower group consisted of 11 samples, four of which were also identified as poor adherents.

However, the volunteers self-reports on treatment adherence were uncertain. This made it difficult to assess this aspect accurately. We decided to perform an exploratory data analysis to define the groups with greater precision and to investigate the clinical history of volunteers. As an analysis criterion, we used the data from the rhinoscopy, which assesses features such as mucosal pallor and hypertrophy of the nasal turbinates. Treatment indifference was arbitrarily defined as the absence of change in results between the rhinoscopy performed at the inclusion visit and that performed during follow-up.

Figure 1 - Hierarchical Cluster Analysis (HCA) using NMR spectral data. GA = Good adherence (adherent patients); PA = Poor adherence (non-adherent patients).



Source: Carneiro DMS, et al., 2025. The image was obtained using MetaboAnalyst.

In the lower group (Group II), nine volunteers (81.8%) did not respond to treatment, and one volunteer exhibited a worsening of their clinical condition. In the upper group (Group I), six volunteers (42.8%) were indifferent to treatment, six exhibited worsening of their clinical condition, and two showed clinical improvement. Demographic and clinical data by group are summarized in **Table 1**.

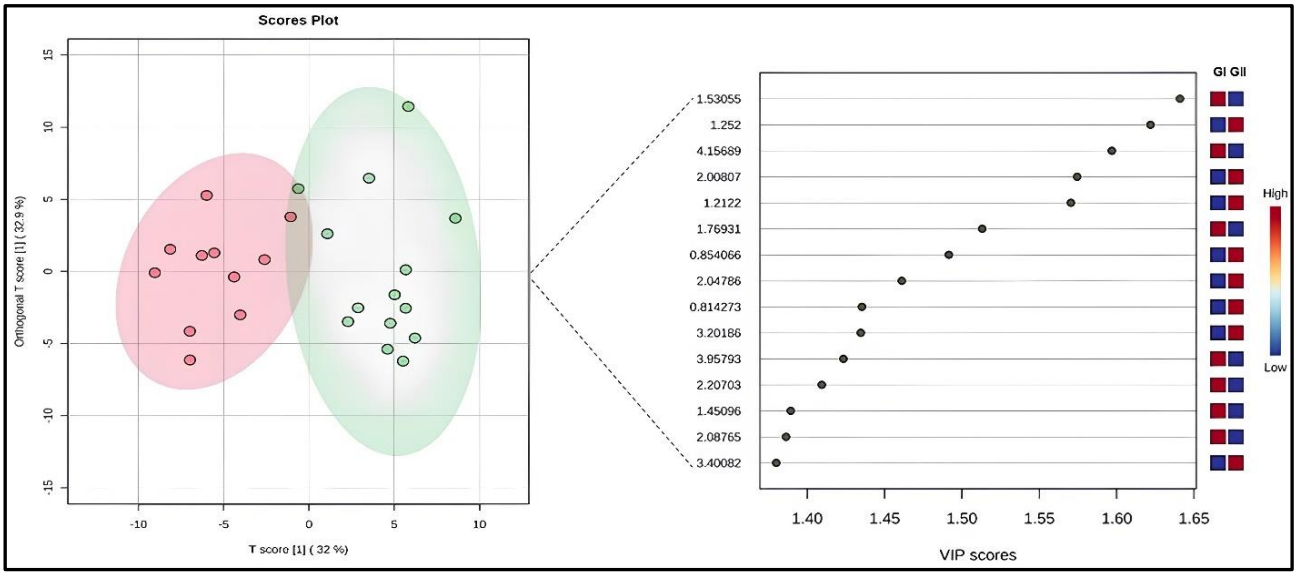
Table 1 - Demographic and clinical data of volunteers by group studied.

	Classification after HCA		*p-value*
	Group I	Group II	
Number of volunteers	14	11	-
Age (years, Average ± standard deviation)	9 ± 2	8 ± 2	0.967
Sex (Female/Male)	8/6	3/8	0.135
Physical exams			
Rhinitis severity (Moderate/Advanced)	10 (71.4%)	10 (90.9%)	0.226
Indifference to treatment (by rhinoscopy)	6 (42.8%)	9 (81.8%)	**0.048**
Good adherence to treatment (by self-report)	10 (71.4%)	7 (63.6%)	0.678

Source: Carneiro DMS, et al., 2025.

The OPLS-DA modeling that uses the classification defined in the HCA as the best-performing response matrix was determined by Leave-One-Out Cross-Validation (LOOCV). A permutation test with 2,000 permutations resulted in R^2 and Q^2 values of 0.823 and 0.738, respectively. The Q^2 value is a cross-validation measure that assesses the model's predictive ability, or its effectiveness in accurately predicting outcomes. **Figure 2** shows the score plot and the VIP scores from the OPLS-DA analysis.

Figure 2 - At left, score plot of OPLS-DA – in green, Group I samples; in red, Group II. At right, the VIP score of OPLS-DA highlights the variable more important for discrimination.



Source: Carneiro DMS, et al., 2025. The image was obtained using MetaboAnalyst.

Variables with VIP scores greater than 1 were selected as potential biomarkers. Using the color scale next to the graph (blue and red), you can see which group has a greater spectral intensity in this region (red). From VIP Score, we observed that variables more intense in Group I had chemical shift (δ) centered at (in ppm): 0.81 – 0.85; 1.21 – 1.25; 2.01 – 2.05; 3.20; and 3.40. Variables more intense in Group II had δ centered at (in ppm): 1.45; 1.53; 1.77; 2.09; 2.21; 3.96; and 4.16.

In general, changes in serum metabolic profiles are related to the activation or disruption of various biological metabolic pathways, primarily energy metabolism, ketone bodies and lipids. **Table 2** details the metabolite assignments for the chemical shifts.

Table 2 - Main variables responsible for discrimination and their assignments by group studied.

Group I (14 volunteers)		Group II (11 volunteers)	
δ (ppm)	**Metabolites**	**δ (ppm)**	**Metabolites**
0.81 – 0.85	VLDL/LDL	1.45	Alanine
1.21 – 1.25		1.53	Arginine
2.01 – 2.05	N-Acetyl D-glucosamine	1.77	Non-Identified
3.20	Choline	2.09	Glutamate
3.40	Glucose	2.21	Acetoacetate
		3.96	Serine
		4.16	Lactate

Source: Carneiro DMS, et al., 2025.

DISCUSSION

In allergic rhinitis therapy, adherence assessment is very important due to the effectiveness of treatment is associated with therapy adhesion. Although patient self-report be used for this end, because of its low cost and easy application, reports are indicating that patients tend to overestimate their adhesion to treatment when asked to report it. This happens due the multiple reasons, such as shame of admitting non-adherence, as well as difficulties in accurately remembering the details of the medication used or understanding the correct way of using it (HOLMES J and HEANEY LG, 2021; HUGTENBURG JG, et al., 2013). Using participants' self-report as a class variable (input) for metabonomic modeling can insert wrong information into analysis. Therefore, exploratory assays were employed to assess the self-report and to define the groups of the study participants.

As expected, PCA (data not shown) and HCA (Figure 1) no relationship was found between the self-reported treatment adherence of the volunteers and the similarity of serum samples analyzed by NMR. However, through HCA, it was possible to identify the formation of two distinct groups were defined: Upper group (Group I) containing fourteen samples, and the Lower group (Group II) containing eleven samples. Among seventeen patients who self-reported good adherence (**Table 1**), ten (58,8%) were included in group I, while seven (41,2%) were included into group II. This distribution suggests that the volunteers' self-reports may not accurately reflect reality, indicating that self-assessment could introduce biases into the analysis models.

When comparing the demographic and clinical data of these groups, only one variable demonstrated a statistically significant difference: indifference to treatment as assessed by rhinoscopy. About 82% of patients in group II did not respond to treatment, while 57,1% of those included in group I showed some type of response to treatment (improvement or worsening of the clinical condition) (**Table 2**). Generally, based on data collected from rhinoscopy examination, the studies tend to explain the response to treatment. It is important to note that most previous studies have focused exclusively on describing the characteristics and evaluating the effectiveness of the treatment concerning participants who responded to the treatment while neglecting to analyze those who did not respond. We chose to investigate those who were indifferent to treatment.

Considering the analysis performed using the OPLS-DA model, it can be stated that the discriminative information between the groups of interest is present in the evaluated data. The characteristics of the samples that differentiate the groups exhibited a predictive capacity of 73%, which can be considered satisfactory. In this study, all volunteers have similar clinical conditions characterized by allergic rhinitis or allergic rhinitis with comorbid asthma. The homogeneity of the clinical conditions between the groups results in subtle differences which pose significant challenges to statistical modeling. This similarity requires a rigorous and sensitive analytical approach capable of identifying variations, albeit discrete, that are critical for differentiating the groups and identifying relevant biomarkers.

VIP scores indicate that alanine, serine, glutamate, and arginine, which are glucogenic amino acids, have higher intensities in the samples of Group II. This suggests that these amino acids are being consumed in gluconeogenesis and, therefore, the homeostatic response is producing these amino acids. However, the synthesis of glucose from amino acids is a strategy used when glycogen stores are compromised, acting as an alternative strategy to meet the increasing energy demands.

Kelly RS, et al. (2017) reported that persistent allergic rhinitis increases metabolic activity of nasal mucosa, increasing glucose caption and its metabolization by glycolytic pathway. Xie S, et al. (2021) reported that the presence of lactate in inflamed nasal mucosa can influence the communication and activation of cells involved in the inflammatory response. This lactate-mediated signaling can increase the inflammatory response in allergic rhinitis, contributing to the persistence of symptoms, as observed in Group II.

Zhou Y, et al. (2019) investigated the metabolic profiling of patients with pollinosis, which is an allergic rhinitis due to pollen exposure. These authors reported that during immunologic stress, these amino acids are redistributed for the synthesis of proteins associated with inflammatory conditions and immune response. Moreover, the increase of acetoacetate serum level of the Group II volunteers indicates that a high metabolic activity resulted in the production of this metabolite, which is the main ketone body produced in the mitochondrial matrix of liver cells in response to carbohydrate deficit (HUANG K, et al, 2022). This indicates that the volunteers in Group II may suffer from a clinically more severe disease, although this severity is not apparent during routine clinical examinations. This finding emphasizes the complexity of allergic rhinitis and suggests that conventional assessment methods may not fully capture the underlying severity of symptoms or metabolic responses of patients.

The metabolites that presented higher intensity in Group I are also associated with modulation of inflammatory response, such as choline which plays an important role in the biosynthesis of phosphatidylcholine (COMHAIR SAA, et al., 2015). During inflammatory processes, phosphatidylcholine can be converted into arachidonic acid, a precursor of inflammatory mediators, such as prostaglandins and

leukotrienes (ROLIM AEH et al., 2015). These mediators are involved in bronchoconstriction and mucus production associated with rhinitis and asthma (ZHANG W, et al., 2021; VILLASEÑOR A, et al., 2017). The expression of VLDL/LDL in Group I volunteers may be associated with oxidative stress, contributing to increased mucus production (SAGDIC O, et al., 2010). The metabolites identified in Group I are related to less severe forms of rhinitis (and asthma). At the same time, those in Group II are associated with greater chronicity and severity of the clinical condition, which highlights the need for individualized treatment.

A major advantage of this study is that it represents a pioneering approach to analyzing metabolic changes in children and adolescents with allergic rhinitis using proton nuclear magnetic resonance (^{1}H NMR). This non-invasive technique requires minimal sample preparation and enables shorter analysis times compared to other analytical methods. Furthermore, by focusing on a specific age group, this study makes a valuable contribution to the understanding of metabolic patterns associated with allergic rhinitis in children and adolescents, an area that remains underexplored in the scientific literature. However, several limitations were noted, including the small sample size, reliance on self-reported adherence measures, and the lack of a control population of healthy individuals. Although only twenty-five samples were used in this preliminary study, the results suggest that the NMR-based method can effectively monitor individual responses to allergic rhinitis treatment.

CONCLUSION

The original aim of this study, to monitor patient adherence to treatment for allergic rhinitis using ^{1}H NMR, was compromised by uncertainty in the definition of good and poor adherence, both by self-report and clinical indication. However, ^{1}H NMR-based metabonomics emerged as a promising tool for predicting treatment responses in patients diagnosed with allergic rhinitis. The analysis of metabolic profiles enabled the early identification of individuals with more severe and chronic forms of the disease. This predictive ability could enable more effective and personalized clinical interventions, improve the treatment of allergic rhinitis and potentially reduce the burden associated with this disease.

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