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TITLE PAGE

Identification of asthma phenotypes based on extrapulmonary treatable traits

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“Take home” message

This is the first study identifying clusters based on extrapulmonary treatable traits in people with moderate-to-severe asthma. Physical inactivity, higher levels of sedentary time, symptoms of anxiety and depression, and obesity were associated with worse asthma outcomes.

ABSTRACT

Background: Asthma is a heterogeneous and complex disease, and the description of asthma phenotypes based on extrapulmonary treatable traits has not been previously reported. **Objective:** to identify and characterise clusters based on clinical, functional, anthropometrical, and psychological characteristics in participants with moderate-to-severe asthma. **Methods**: This is a cross-sectional multicentre study involving centres from Brazil and Australia. Participants (N=296) with moderate-to-severe asthma were consecutively recruited. Physical activity and sedentary time, clinical asthma control, anthropometric data, pulmonary function, psychological, and health-status were evaluated. Participants were classified by hierarchical cluster analysis and the clusters compared using ANOVA, Kruskal-Wallis, and Chi-square tests. Multiple logistic and linear regression models were performed to evaluate the association between variables. **Results**: We identified four clusters: (1)controlled asthma who were physically active, (2)uncontrolled asthma who were physically inactive and more sedentary, (3)uncontrolled asthma with low physical activity, who were also obese and experienced anxiety and/or depression symptoms (4)very uncontrolled asthma, who were physically inactive, more sedentary, obese and experienced anxiety and/or depression symptoms. Higher levels of sedentary time, female sex, and anxiety symptoms were associated with increased odds of exacerbation risk while being more active showed a protective factor for hospitalisation. Asthma control was associated with sex, the occurrence of exacerbation, physical activity, and health-status. **Conclusion**: Traits such as physical inactivity, obesity, and symptoms of anxiety and/or depression were associated with worse asthma outcomes, and closely and inextricably with asthma control. This cluster analysis supports the importance of assessing extrapulmonary traits to improve personalised management and outcomes for people with moderate and severe asthma.

Keywords: asthma, physical activity, cluster, clinical control, obesity, treatable traits

INTRODUCTION

Asthma is a major health concern, causing a high illness burden for individuals and health systems. Asthma is considered a heterogeneous and complex disease characterised by variability in disease expression and severity(1). People with asthma have different clinical presentations, which, despite being partially explained by disease severity, is insufficient to understand the recognised heterogeneity(2). The current concept of airway diseases may neither recognise the complexity of the disease nor promote individualised management(3). There is increasing appreciation relating to the impact of extrapulmonary features of asthma and associated comorbidities(4), highlighting the need to deconstruct and personalise asthma management by identifying measurable and modifiable traits(5). "Treatable traits" is a taxonomy that has been proposed to recognise the complexity of chronic respiratory diseases. The approach seeks to characterise individuals by the presence of "potentially modifiable elements" that impact symptoms and prognosis(6, 7). Traits are recognised within three domains; pulmonary, extra-pulmonary, and behavioural/risk-factors(7). To be considered a trait, the characteristic must be 1. Identifiable, 2. Clinically relevant, and 3. Modifiable(8).

Physical inactivity, high levels of sedentary time, psychological disturbances, and obesity are extra-pulmonary morbidities/traits frequently reported in people with moderate-to-severe asthma(9-12). People with severe asthma are known to be less active and highly sedentary, and this is associated with worse exercise capacity, poor asthma control, and increased systemic inflammation(13). The lower aerobic capacity in people with asthma is associated with reduced health-status and increased symptoms of depression, regardless of lung function and age(14). Obesity in asthma is also associated with worse prognosis and severity, including increased asthma symptoms and exacerbations, worse lung function, and higher use of oral corticosteroids

(OCS)(1, 15). Therefore, the identification of asthma phenotypes that considers these treatable traits should be included as an integral part of asthma assessment aiming to deliver the most appropriate treatments to patients(6).

Previous cluster analyses have identified asthma phenotypes focusing on characteristics related to disease severity, expiratory airflow limitation, inflammatory biomarkers, and age of asthma onset(1, 16). However, despite these well-conducted cluster analyses, the description of phenotypes based on extrapulmonary traits has not been previously reported. Thus, this study aimed to identify and characterise phenotypes based on clinical, functional, anthropometrical, and psychological characteristics in people with moderate-to-severe asthma. We hypothesised that the severity of these clinical characteristics and modifiable behavioural risk-factors can guide the classification of clinical asthma phenotypes (clusters).

METHODS

A cross-sectional multicentre study involving centres from Brazil and Australia was conducted. Participants were recruited prospectively between July 2012 and March 2019 from tertiary care hospitals during routine medical consultations or using the clinic's research databases.

Participants: Adults (≥18 years old) with a diagnosis of moderate-to-severe asthma according to the GINA criteria(17), who were clinically stable (free from exacerbation in the past 30 days) and receiving optimal treatment according to the international guidelines(17) were eligible for inclusion. Exclusion included COPD or other significant respiratory or cardiovascular diseases, active cancer, uncontrolled hypertension, diabetes, or musculoskeletal condition that could compromise participation in physical activity. The inability to understand the questionnaires, pregnancy and current or past history of smoking (≥10 pack-years) were also listed as exclusions.

Written informed consent was obtained from all participants. Ethical approvals were granted by each centre's respective ethics committees.

Procedures

Data on demographics, anthropometrics(18) (weight, height, Body Mass Index [BMI]), smoking history, comorbidities, lung function, and asthma medications use were extracted from participants' medical records or through interview. Further assessments included:

Asthma control

The Asthma Control Questionnaire (ACQ)(19, 20), consisting of five questions relating to asthma symptoms, bronchodilator use, and lung function (percent predicted of forced expiratory volume in one second [FEV_1] before bronchodilation) was used. Scores range between 0 and 6, where <0.75 and >1.5 are indicative of good and poorly controlled asthma, respectively(21).

Asthma-related health-status

The Asthma Quality of Life Questionnaire (AQLQ)(22) comprising four asthma-related domains: activity limitations, symptoms, emotional function, and environmental stimuli was administered. Scores range between 0 and 7, with higher scores indicating better the health-status.

Asthma exacerbations

Exacerbations were defined as worsening of symptoms that led to: ≥3 days of OCS treatment or a temporary increase in their OCS maintenance dosage; or an asthma-specific hospitalisation; or ED visit requiring systemic corticosteroids(23). Exacerbations were elicited during standardised structured interviewing regarding the last 12 months.

Anxiety and depression symptoms

The Hospital and Anxiety and Depression Scale (HADS)(24) consists of 14 items divided into two domains, 7 for anxiety (HADS-A) and 7 for depression (HADS-D). Each item is scored from 0 to 3, with a maximum score of 21 for each domain. A score of ≥8 in either domain indicates possible anxiety or depression(25).

All questionnaires were validated and applied in the appropriate language (English or Portuguese).

Physical activity and sedentary time

These movement behaviours were objectively measured by the accelerometer Actigraph GT3X (Actigraph, Pensacola, FL, USA)(26). The device was initialised via a computer interface to collect data in 60-second epochs on the three axes by using specific software (ActiLife 6.13.3 Firmware version). Participants wore the device on their waist (using an elastic belt) during wake time for seven consecutive days. Data from valid days (≥4 days and ≥10 hours of recording) were presented as the average number of steps per day (steps/day), the time spent in moderate-vigorous physical activity (MVPA, minutes/day) (≥1,951 counts/min), and time spent in sedentary time (<100 counts/min)(27). Participants performing ≥10,000, ≥7,500 and ≥5,000 steps/day were classified as "physically active", "somewhat active" and "low-level active", respectively(28).

Statistical Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) version 18.0 for Windows (SPSS Inc, Chicago, USA).

A hierarchical cluster analysis was performed to identify the number of clusters and cluster centroids, using the principal component analysis (PCA)(29, 30). The Shapiro-Wilk test evaluated data normality. The between-cluster differences were analysed by the Chi-square test,

ANOVA (plus Holm-Sidak test), or Kruskal-Wallis test (plus Dunn's multiple comparison test). Significance was set at $P<0.05$.

The association analyses were performed using multiple logistic regression between the dependent outcomes (exacerbation, hospitalisation, ED visit, and temporary systemic corticosteroid) and the clinical and behavioural characteristics included in the cluster analysis. The stepwise forward process was used to include variables into the model. Multiple linear regression analysis was performed to identify the independent factors associated with ACQ7(dependent variable), sex, BMI, the occurrence of exacerbation, ICS, daily physical activity, sedentary time, HADS, and AQLQ score. Detailed statistical analyses are presented in the supplementary appendix.

RESULTS

Patient Characteristics

A total of 414 participants were assessed for eligibility, and 118 excluded. The final study population consisted of 296 participants with moderate-to-severe asthma (243 from Brazil and 53 from Australia). Participants were mostly female, who were overweight, had low physical activity and high sedentary time levels, and with mild airway obstruction (Table 1). The majority (68%) of the participants had uncontrolled asthma, and 64% had experienced at least one exacerbation in the last 12 months (10% were hospitalisations, 49% visits to the ED, 60% used systemic corticosteroid). Participants from Australia were older, more physically inactive, and spent more time engaged in sedentary time. However, they had better health-status and fewer symptoms of anxiety and depression (Table 1).

Fifteen comorbidities were identified: osteoporosis, vocal cord dysfunction (VCD), dyslipidemia, bowel disease, hypothyroidism, diabetes, dermatitis, obstructive sleep apnea syndrome (OSAS), sinusitis, musculoskeletal (MSK) impairment, psychological disturbance, hypertension, obesity, gastro-oesophageal reflux disease (GORD) and rhinitis. The frequency of comorbidities ranged from 3% to 78%. Rhinitis, GORD, obesity, hypertension, and psychological disturbance were the most prevalent comorbidities (Figure 1A). Almost all participants (98%) had at least one comorbidity, and more than 50% had more than three comorbidities (Figure 1B).

Cluster Analysis and description

The Kaiser-Meyer-Olkin (0.6) and the Bartlett´s Test of Sphericity ($P<0.001$) confirmed that the cluster analysis was appropriate. The PCA identified two components: component 1 encompassed the variables BMI, ACQ-7, AQLQ, and HADS and component 2 encompassed physical activity levels and sedentary time (Table S1, Supplement).

Ward's cluster analysis was based on the significant components identified by the PCA. Using the hierarchical cluster analysis described in the Methods, a dendrogram was generated, and four clusters were identified. The four clusters differed significantly by sex, BMI, asthma medication, asthma control, physical activity levels, sedentary time, health-status, and symptoms of anxiety and depression (Table 2). Although all clusters had a high frequency of rhinitis (>60%), they differed in the frequency for most other comorbidities (Figure 2).

Cluster 1, "High movers": this cluster comprised 76 participants (25%), of whom 88% were from the Brazilian population. The cluster had the largest percentage of physically active participants (41% classified as "physically active," 79% as "somewhat physically active"), and engaged in less sedentary time than other cluster participants. Additionally, most of the participants (62%) had controlled asthma symptoms, were female, overweight, and used lower doses of ICS compared with the other clusters.

Cluster 2, "Poorly active": this cluster comprised 80 participants (27.3 %, [60% from Brazilian cohort]), and 99% were classified as low-level active and more sedentary. This cluster had fewer female patients, were overweight, but had a smaller number of obese patients than Clusters 3 and 4 (respectively, 37% vs. 69%, and 64%). Most participants (65%) presented with uncontrolled asthma symptoms.

Cluster 3, "Moderately active, obese and distressed": this cluster comprised 69 patients (23%), were mostly female, and 75% presented as uncontrolled asthma. Obesity was present in 71% of participants; they were more physically active, and engaged in less sedentary time than clusters 2 and 4, and had higher anxiety and depression scores than Cluster 1 and 2.

Cluster 4, "Physically inactive, obese and distressed": this cluster comprised 71 participants (24% of the population, [83% from Brazilian cohort]). Obesity was present in 64% of participants, and all were classified as physically inactive and accrued a high volume of sedentary time. Participants in Cluster 4 presented with increased anxiety and depression

symptom scores compared to Cluster 1 and 2. They used higher doses of ICS and long-acting b_2-agonists. Most participants (91%) presented with uncontrolled asthma. Interestingly, our results suggest that only those patients with controlled asthma were physically active, suggesting that both outcomes are firmly linked. There were no between-cluster differences in age, smoking history, asthma onset, lung function, and the frequency of participants treated with long-acting b_2-agonists in combination with ICS (Table 2).

Comorbidities, asthma control, health-status, psychological symptoms and exacerbations within clusters

The most prevalent comorbidities in all clusters were GORD, obesity, and rhinitis (Figure 2). Diabetes, obesity, and psychological disturbances were most prevalent in Clusters 3 and 4. Cluster 3 presented a lower prevalence of musculoskeletal disorders compared with all the other Clusters.

Clusters 3 and 4 reported worse asthma control than Clusters 1 and 2 (Figure 3). The use of short-acting bronchodilators was higher in Cluster 4. No between-cluster differences were observed for lung function. The odds of uncontrolled asthma were higher in Clusters 2 and 3 compared to Cluster 1 (respectively, OR [95% confidence interval (CI)], 3.3 [1.7-6.4]) and 4.6 [2.4-10.1]). Cluster 4 presented even higher odds of uncontrolled asthma (17.6 [6.7-45.6]) (Figure 4A).

Cluster 4 reported the highest exacerbation rate. No difference in the odds of exacerbations and hospitalisations was observed between Clusters 1, 2, and 3. (Figure 4B and 4C). Cluster 4 presented 2.3 (1.1-4.6) higher odds of exacerbation compared with Cluster 1 and 5.5 (1.5-20.0) higher odds of hospitalisation compared with Clusters 1 and 3. Additionally, Clusters 3 and 4 presented higher odds of ED visits (respectively, 1.8 [1.0-3.6] and 1.9 [1.0-3.7]) compared with Cluster 2. No between-cluster differences were observed for the odds of requiring systemic corticosteroid use for asthma exacerbation.

Clusters 3 and 4 reported lower health-status, more symptoms, activity limitation, and impaired mental health than Clusters 1 and 2 (Tables 2).

Clusters 3 and 4 presented with increased anxiety and depression symptoms levels compared with Clusters 1 and 2. However, patients in Cluster 3 presented even higher anxiety and depression symptoms than Cluster 4 (Table 2).

Tree Diagram

The Tree Diagram was performed using discriminatory variables for cluster assignment (physical activity, obesity, and anxiety symptoms) using subsets of these variables to assess the classification of participants (Figure 5). The results of this analysis demonstrate that a greater number of comorbidities/risk-factors (e.g., inactivity, obesity, and anxiety) identifies clusters with worse asthma control. The proportion of participants in each cluster is presented in Figure 6. These figures suggest that a simple method for phenotyping of asthma subclasses can be based on these clinical variables.

Clinical associations

The associations between the dependent outcomes (exacerbation, hospitalisation, ED visit, and bursts of systemic corticosteroids) with the characteristics: sex, BMI, ACQ5, sedentary time, daily physical activity, HADS-A, HADS-D, AQLQ, and ICS dose are described in Table S2. Higher levels of sedentary time were significantly associated with increased odds of exacerbation (OR=1.83, [95%CI 1.02 – 3.30] P=0.04), hospitalization (OR=1.23, [95%CI 1.01 – 1.50] P=0.04) and with greater systemic corticosteroids bursts (OR=1.16 [95%CI 1.02 – 1.32], P=0.02) (Table S2); while being more active was a protective factor for hospitalisation (OR=0.81, [95%CI 0.67 – 0.97] P=0.03) (Table S2). Additionally, female sex was also a risk factor for exacerbation (OR=1.14, [95%CI 1.01 – 1.30] P=0.04), ED visits (OR=1.90, [95%CI 1.02 – 3.53] P=0.04) and for greater systemic corticosteroid bursts (OR=1.95, [95%CI 1.06 – 3.60] P=0.03). (Table S2). Higher levels of anxiety symptoms were

also significantly associated with increased visits to ED visits (OR=1.06, [95%CI 1.02 – 1.14] P=0.01).

Multiple linear regression

The ACQ-7 total score was significantly associated with sex, the occurrence of exacerbation, daily physical activity, and AQLQ total score. *Equation 1*:

$$\text{ACQ-7} = 4.13 + (0.33*\text{Exacerbation}) - (0.35*\text{sex}) - (0.08*\text{number of steps/day}) - (0.34*\text{AQLQ})$$

(Table S3).

DISCUSSION

In this study, we performed a hierarchical cluster analysis to identify clinical asthma phenotypes based on extrapulmonary traits and behavioural/risk-factors in patients with moderate-to-severe asthma and describe the clinical characteristics associated with these phenotypes. We included two populations; from Brazil and Australia. Our analysis identified four distinct phenotypes with relatively even representation of patients within each cluster. The clusters included patients with: 1. controlled asthma who were physically active 2. uncontrolled asthma who had higher levels of inactivity and sedentary time, 3. uncontrolled asthma with low physical activity, who were also obese and experienced symptoms of anxiety and depression and 4. very uncontrolled asthma, who had higher levels of inactivity and sedentary time, were obese and also experienced symptoms of anxiety and depression. We examined the clinical associations of each of these clusters and determined cluster 4 to be associated with worse outcomes in terms of exacerbation, and they had the poorest asthma control.

Previous cluster analyses have been performed in asthma, aiming to identify clinical phenotypes in patients with severe disease. Despite the importance of these phenotypes, new approaches have been recommended for the classification of asthma. Moore and coworkers identified five clusters of patients with different clinical, physiologic, and inflammatory characteristics. Of the 11 most important variables that determined assignment to individual clusters, six were based on pulmonary function tests, two were age-related, two variables reflected medication use, and one was sex(16). In another analysis, Haldar and coworkers defined four clusters in patients from secondary care; the variables included airway inflammation, lung function, symptoms, atopy, and obesity. Patients were classified as “early-onset symptom” and “late-onset inflammation,” and there was observed discordance between asthma symptoms and eosinophilic airway inflammation(1). To the best of our knowledge, no

prior asthma cluster analyses have attempted to phenotype patients based on extrapulmonary treatable traits and risk-factors. This analysis is necessary for several reasons. Traits such as physical inactivity and high sedentary time are common in patients with asthma, especially in severe disease(13), and they are significantly associated with poor clinical outcomes and poor health-status(4). Evidence from the general population and in other chronic diseases has confirmed that these traits are importantly modifiable(31, 32). Therefore understanding the impacts of these traits and how they cluster, is important for the development of treatment interventions beyond the current asthma management paradigm(33, 34).

There have been advances in asthma management for patients with severe asthma over the last decade, including monoclonal antibody therapies(35) and macrolide antibiotics for exacerbation reduction(36). Nevertheless, patients with severe disease continue to experience poor health-status(37), and have a higher comorbidity and risk-factor burden than those with controlled disease(6). Therefore, a current priority is to develop interventions that target clinically important extra-pulmonary traits(6). Our cluster analysis has identified four clusters based on these traits. We have also determined the clinical relevance of these clusters by assessing their associations with important clinical outcomes such as exacerbations, health-status, and asthma control. Targeting these traits may improve these outcomes. The treatable traits paradigm proposes the application of multidimensional assessment to identify traits that are clinically important, measurable, and modifiable, followed by targeted interventions for each trait identified. An RCT of this approach in severe asthma demonstrates its efficacy in terms of improving health-status, airway, and systemic inflammation and reducing primary care visits (8). In this study, all of the traits identified were treated. The presence of the traits of anxiety, depression, and obesity are common and tend to be associated with poorer asthma outcomes independent of the clinical features of the disease. In clusters 2 and 4, the age of asthma onset and lung function were relatively similar; however, the outcomes in cluster 4

were worse, the difference being is the presence of these additional traits. This finding highlights the importance of these traits on disease outcomes.

The impact of obesity and symptoms of anxiety and depression warrants consideration. Cluster 4 (very uncontrolled asthma, highly physically inactive and sedentary with obesity) and Cluster 3 (movers with better asthma control but still obese with anxiety and depression) were the phenotypes that were associated with the poorest clinical outcomes. This further highlights the importance of these extrapulmonary traits in terms of their additive deleterious effects on people with moderate and severe asthma. Obesity is common in more severe asthma and is a recognised risk-factor for increased asthma exacerbations and worse asthma control(1, 10). The synergistic relationship between obesity and reduced physical activity is also well characterised(38). Previous studies in obese(39, 40) and non-obese(41) people with asthma have shown that dietary restriction plus exercise programs have promising effects on asthma control and health status, highlighting their potential as treatable traits.

This is the first study to identify asthma phenotypes considering clinical, functional, anthropometrical, and psychological characteristics collectively, in people with moderate-to-severe asthma. Our findings reinforce the need for an individualised multidimensional assessment of asthma to facilitate the implementation of personalised management(8). For instance, a behaviour change intervention aimed at increasing physical activity could lead to improved asthma control for patients of cluster 2 (poorly active)(33), while a weight-loss and psychological interventions may be proposed for patients within cluster 3 (moderately active, obese and distressed). Regarding cluster 4 (physically inactive, obese, and distressed), a more comprehensive approach, including physical training and programs that address behaviour change techniques, may be more effective in improving asthma control(8, 42, 43). In addition, we have proposed cut-points that allow the identification of these clusters (Figure 5), which

may be applied in clinical practice. However, further studies are required to validate these cut-points to endorse or refute their applicability.

Asthma phenotypes have been applied in clinical practice to allow more precise endpoints according to the main underlying pathology aiming to reduce exacerbations and corticosteroid treatment and to improve clinical control, pulmonary function, and quality of life (1, 16). Despite this, people with moderate to severe asthma continue to experience frequent symptoms and attacks, suggesting that current pharma and non-pharmacotherapies are insufficient faced with the complexity of more severe disease. If the phenotypes identified in this current study are considered in the overall management of people with moderate to severe asthma, there may be greater gains in asthma control and outcomes for this population.

Our study has strengths and some limitations. In terms of strengths, we quantified physical activity and sedentary outcomes using objective measures, which are scarce in these populations. Second, the inclusion of participants from two continents increases the generalisability of our findings, even though these populations were not matched in terms of age, lung function, and the impact of the disease. Another limitation may be the higher proportion of women; however, this is also reflective of a more severe asthma population. Despite the multicentre nature of this study, the sample size is relatively small, indicating the need for further validation of these clusters. We also acknowledge the imbalance in the number of patients between Brazilian and Australian cohorts; however, this imbalance takes into account the populations of each country (210 and 25 million inhabitants, respectively). Finally, due to the cross-sectional nature of the study design, we cannot establish causality. Further studies are needed to understand the bidirectional nature of these traits in this population.

In conclusion, we have identified four asthma phenotypes based on extrapulmonary characteristics through hierarchical cluster analysis. These distinct clusters based on physical

activity levels, obesity, and depressive and anxiety symptoms were associated with important clinical asthma outcomes. Our data reinforce the importance of evaluating extrapulmonary traits in clinical practice to individualise treatments with the goal of improving clinical outcomes in people with moderate-to-severe asthma.

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Author's contributions:

Study concept and design: P.D.F., R.M.C.P., A.C., R.S., C.R.F.C.; data acquisition: P.D.F., V.M.M., L.C.R., K.C.F., J.M.O; data analysis and/or interpretation: P.D.F., R.F.X., V.M.M., P.G.G., L.C.R., K.C.F., J.M.O ; R.M.C.P., A.C., R.S., C.R.F.C.; Manuscript writing and/or critical revisions for important intellectual content: P.D.F., R.F.X., V.M.M., P.G.G., L.C.R., K.C.F., J.M.O ; R.M.C.P., A.C., R.S., C.R.F.C. All the authors have read and approved the final version of the manuscript.

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Conflict of interest:

The authors declare that they have no conflicts of interest to disclose.

TABLES

Table 1. Participant Characteristics

	Brazilian Cohort (n=243)	Australian Cohort (n= 53)	All Participants (n= 296)
Anthropometric data			
Female, n (%)	203 (84)	28 (53) *	231 (78)
Age, yrs	46.0 (39.0 – 53.0)	54.0 (41.0 – 64.2) *	47.0 (39.0 – 45.0)
BMI, kg/m^2	30.4 (26.7 – 34.7)	28.8 (25.2 – 33.8)	29.9 (26.6 – 34.6)
Physical activity			
Number of steps, steps/day	6,480 (5,024 – 8,425)	5,402 (3,951 – 7,744) *	6,246 (4,808 – 8,103)
MVPA, min/day	20.0 (13.1 – 35.1)	22.7 (12.8 – 36.9)	20.4 (12.9 – 35.5)
Sedentary time, hours/day	8.5 ± 1.8	11.2 ± 1.4*	8.9 ± 1.9
Pulmonary function			
FEV_1, % predicted	70.4 ± 18.6	66.8 ± 23.3	69.8 ± 19.5
FVC, % predicted	84.0 ± 16.3	79.8 ± 18.9	83.3 ± 16.8
FEV_1/FVC	0.69 (0.62 – 0.75)	0.62 (0.56 – 0.71)*	0.68 (0.60 – 0.75)
Asthma medication			
ICS dose, µg/day	1,600 (1,600 – 2,400)	2,000 (2,000 – 2,000)	1,600 (1,600 – 2,400)
LABA use, n (%)	231 (95)	47 (87)	287 (94)
Asthma control			

ACQ-7, score	1.8 (1.2 – 2.6)	2.1 (1.4 – 2.6)	2.0 (1.3 – 2.6)
Subjects uncontrolled asthma, n (%)	162 (67)	37 (71)	199 (67)
Exacerbation			
Hospitalization, n (%)	15 (7)	15 (28) *	30 (10)
Emergency department visit, n (%)	117 (52)	17 (32) *	134 (49)
Systemic corticosteroid burst, n (%)	119 (53)	46 (87) *	165 (60)
Exacerbation, n (%)[#]	141 (58)	47 (88) *	188 (64)
Health Status			
AQLQ, total score	4.0 (3.1 – 5.0)	5.4 (4.3 – 6.2) *	4.2 (3.2 – 5.2)
HAD-A, total score	9.0 (5.7 – 12.0)	6.0 (4.0 – 9.0) *	8.5 (5.0 – 11.0)
HAD-D, total score	7.0 (4.0 – 10.2)	4.0 (2.0 – 6.0) *	6.0 (4.0 – 10.0)

Legend: The data are presented as the means ± standard deviation (SD), medians (25^{th}-75^{th}), or n (%). BMI, body mass index; FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity; ICS, inhaled corticosteroids (Total daily dose beclomethasone equivalent); LABA, long-acting B_2 agonists; ACQ-5, asthma control questionnaire with five questions; ACQ-7, asthma control questionnaire with seven questions; MVPA, moderate and vigorous physical activity; AQLQ, asthma quality of life questionnaire; HAD-A, hospital anxiety and depression scale-anxiety; HAD-D, hospital anxiety and depression scale-depression. Exacerbation[#]: hospitalization, emergency department visit, and/or temporary systemic corticosteroid. *P<0.05 between countries.

Table 2. Cluster characteristics of participants with moderate-to-severe asthma

	(1) High movers (n=76; 88% Br, 12% Au)	(2) Poorly active (n=80; 60% Br, 40% Au)	(3) Mod active, obese and distressed (n=69; 100% Br, 0% Au)	(4) Inactive, obese and distressed (n=71; 83% Br, 17% Au)
Anthropometric /asthma data				
Female, n (%)	60 (79)	48 (60) *	62 (90) ⁂	61 (86) * ⁂
Age, yrs	45.0 (38.5 - 53.5)	47.5 (36.5 – 58.0)	49.0 (40.7 – 54.0)	46.0 (41.0 - 55.0)
BMI, Kg/m^2	27.6 ± 4.9	29.3 ± 5.7	33.1 ± 5.8* ⁂	33.2 ± 6.2 * ⁂
Ex-smoker, n (%)	18 (24)	18 (23)	15 (22)	17 (24)
Onset of childhood asthma, n (%)	56 (74)	46 (58)	44 (64)	45 (63)
Pulmonary function				
FEV_1, %	72.8 ± 19.1	69.3 ± 20.8	71.4 ± 18.1	65.6 ± 19.6
FVC, % predicted	86.2 ± 14.7	82.3 ± 17.6	85.1 ± 16.5	79.5 ± 17.9
FEV_1/FVC	0.68 ± 0.11	0.67 ± 0.12	0.69 ± 0.09	0.66 ± 0.10
Asthma medication				
ICS dose, µg/day[#]	1,600 (1,100 – 1,600)	2,000 (1,600 – 2,000) *	1,600 (1,600 – 2,400) *	2,000 (1,600 – 2,400) *
LABA use, n (%)	69 (90.7)	75 (93.7)	67 (97.1)	67 (94.3)
Asthma control				
ACQ-7, score	1.3 (0.7 – 1.8)	1.7 (1.3 – 2.4) *	2.0 (1.5 – 2.7) *	2.7 (2.2 – 3.4) * ⁂⁑
Uncontrolled asthma, n (%)	29 (38)	53 (67) *	52 (75) *	64 (92) * ⁂⁑
Physical activity				
Number of steps, steps/day	9,249 (7,814 – 10,998)	5,193 (4,309 – 6,206) *	7,380 (6,113 – 9,281) * ⁂	4,606 (3,669 – 5,569) *⁑

MVPA, min/day	33.7 (20.3 – 54.8)	19.6 (12.8 – 28.2) *	27.2 (14.7 – 42.2) *	13.6 (8.2 – 19.0) * ⁂
Sedentary time, hours/day	7.9 ± 1.6	10.1 ± 1.8 *	7.8 ±1.4 ⁂	9.8 ± 1.8 *⁂
Health Status				
AQLQ, total score	4.9 (4.1 – 5.8)	5.3 (4.5 – 5.8)	3.2 (2.7 – 3.9) * ⁂	3.5 (2.9 – 4.1) * ⁂
AQLQ, symptoms score	5.2 (4.3 – 6.0)	5.1 (4.4 - 5.8)	3.7 (2.9 - 4.4) * ⁂	3.6 (2.8 - 4.6) * ⁂
AQLQ, activity limitation score	4.5 (3.8 - 5.5)	5.3 (4.5 - 6.0)	3.2 (2.5 - 3.7) * ⁂	3.4 (2.8 - 4.1) * ⁂
AQLQ, emotional function score	5.1 (3.4 - 6.4)	5.6 (4.6 - 6.4)	3.0 (2.0 - 3.8) * ⁂	3.8 (2.6 - 4.6) * ⁂
AQLQ, environmental stimuli score	4.9 (3.2 - 5.7)	5.5 (4.2 - 6.2)	2.5 (1.7 – 3.5)	3.2 (2.5 - 4.8)
HAD-A, score	6.0 (4.0 – 9.0)	5.0 (3.0 – 7.0)	13.0 (10.0 – 15.0) * ⁂	10.0 (8.0 – 12.0) * ⁂
HAD-D, score	4.0 (3.0 – 6.0)	4.0 (2.0 – 6.0)	13.0 (10.0 – 15.0) * ⁂	9.0 (6.0 – 11.0) * ⁂

Legend: The data are presented as the means ± standard deviation (SD), medians (25^{th}-75^{th}), or n (%). Br, Brazilian cohort; Au, Australian cohort; BMI, body mass index; FEV_1, forced expiratory volume in 1 sec; FVC, forced vital capacity; ICS, inhaled corticosteroids ($^{\#}$TDD beclomethasone equivalent); LABA, long-acting B_2 agonists; ACQ-5, asthma control questionnaire with five questions; ACQ-7, asthma control questionnaire with seven questions; MVPA, moderate and vigorous physical activity; AQLQ, asthma quality, and life questionnaire; HAD-A, hospital anxiety and depression scale-anxiety; HAD-D, hospital anxiety and depression scale-depression. *P<0.05 vs. cluster 1; ⁂ P<0.05 vs cluster 2; ⁂ P<0.05 vs. cluster 3

FIGURES LEGENDS

Figure 1. Comorbidities in 296 participants with moderate-to-severe asthma. A) Distribution of 15 comorbidities identified; B) Distribution of the number of comorbidities per patient. Data are presented in percentage (%). VCD, vocal cord dysfunction; OSAS, obstructive sleep apnea syndrome; GORD, gastro-oesophageal reflux disease.

Figure 2. Frequency of comorbidities per cluster. Data are presented as the percentage (%) of each comorbidity in the four Clusters. VCD, vocal cord dysfunction; OSAS, obstructive sleep apnea syndrome; GORD, gastro-oesophageal reflux disease. *P<0.05 vs. cluster 1; ⁑ P<0.05 vs cluster 2; ⁂ P<0.05 vs. cluster 3.

Figure 3. Comparison between clusters of each ACQ-7 question. Data are presented as mean ± standard deviation (SD). ACQ, asthma control questionnaire; FEV_1, forced expiratory volume in the 1 sec. *P<0.05 vs. cluster 1; ⁑ P<0.05 vs cluster 2; ⁂ P<0.05 vs. cluster 3.

Figure 4. Adjusted odds ratios (OR) between clusters. A) Uncontrolled asthma; B) Exacerbation; C) Hospitalization; D) Temporary use of oral corticosteroid. Data are presented as the median and 95% confidence interval. *P<0.05 vs. cluster 1; ⁑ P<0.05 vs cluster 2; ⁂ P<0.05 vs. cluster 3.

Figure 5. Tree analysis. The Figure reports the distribution of each comorbidity in the clusters considering clinical cut-points (darker color) for physical activity (in step counting) (28), body mass index (BMI) (44), and anxiety symptoms (HADS subscale) (24). Participants were assigned to one of the four clusters that range from controlled asthma (Cluster 1) to very uncontrolled asthma disease (Clusters 4).

Figure 6. Tree performance. Using the algorithm generated by the tree analysis, 80% of participants were assigned to the correct cluster according to the number of comorbidities. Colors are in line with the tree diagram (blue, high movers; green, poorly active; yellow, mod active, obese and distressed; and red, inactive, obese, and distressed). The number of

comorbidities (inactive, obese, and distressed/ higher anxiety scores) is represented by numbers from 0 (no comorbidities) to 3 (three comorbidities). The percentage of participants from that cluster that are correctly assigned is indicated numerically within the shape.

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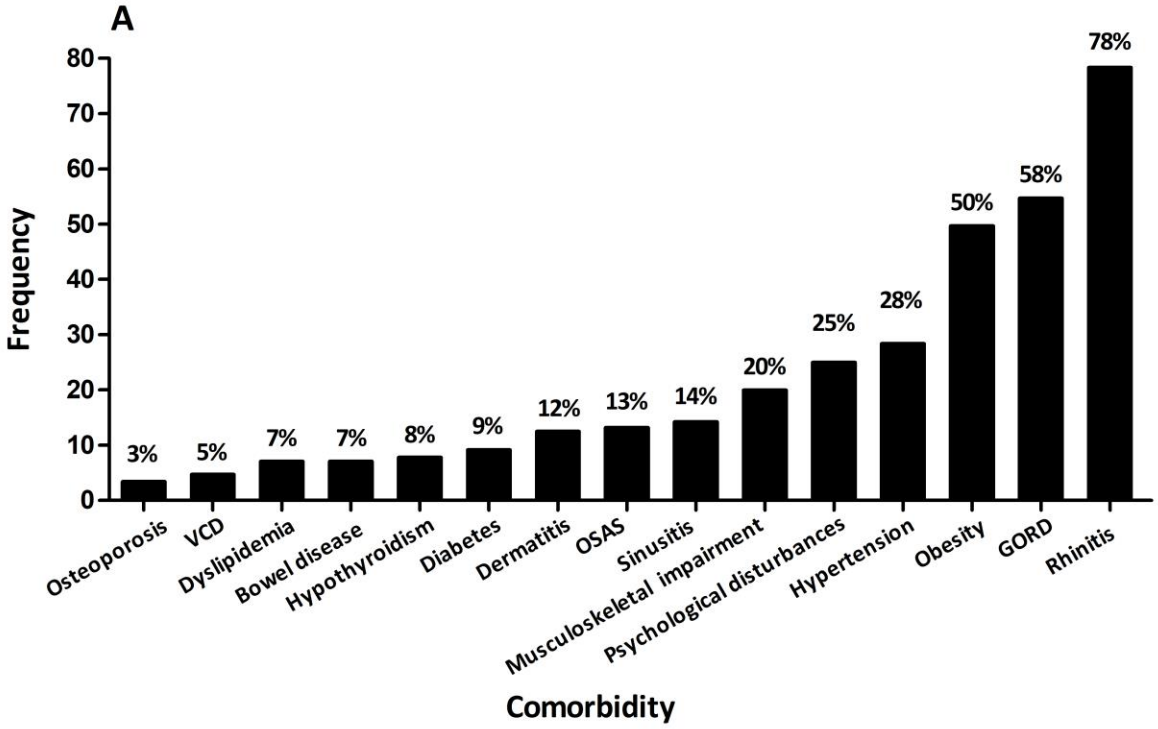
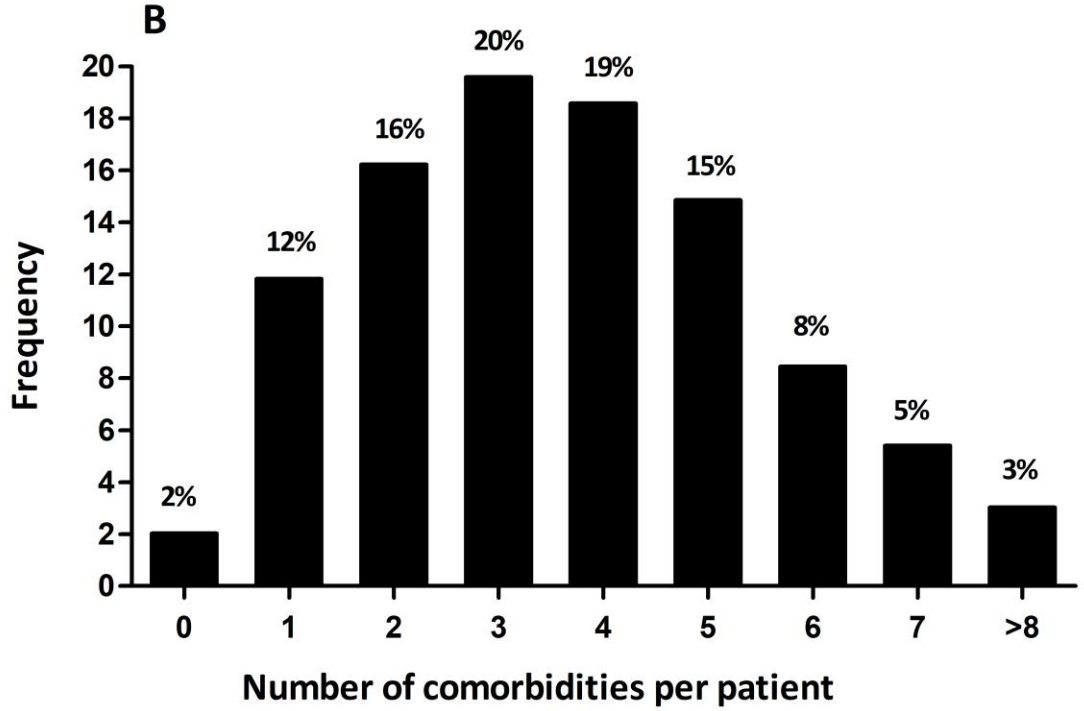
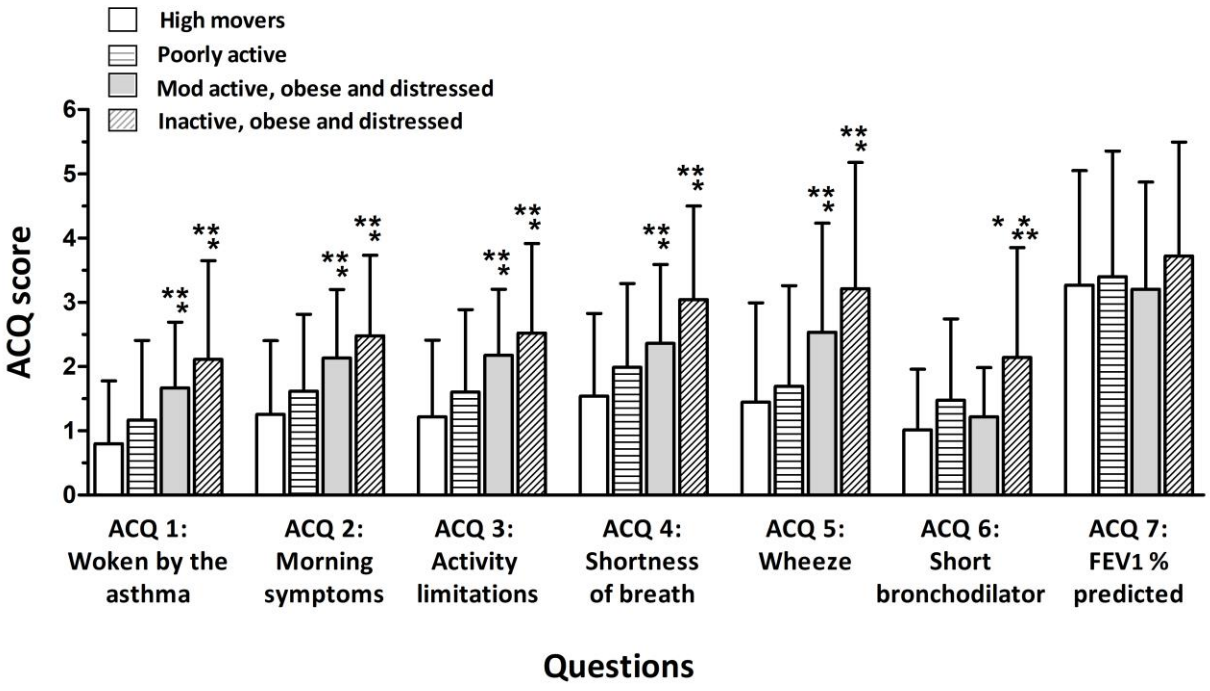
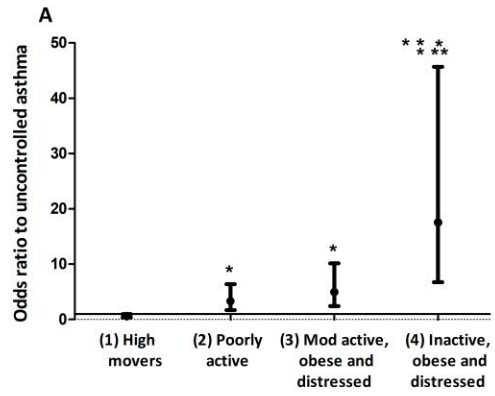
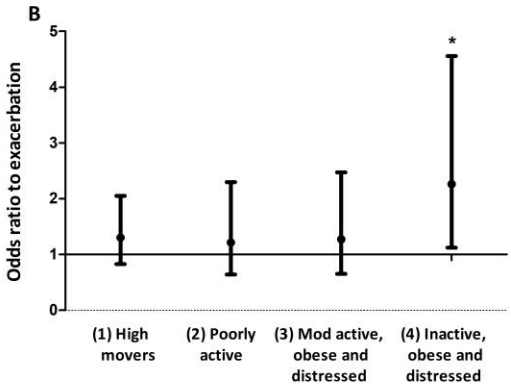

A
Frequency
80
70
60
50
40
30
20
10
0
3%
5%
7%
7%
8%
9%
12%
13%
14%
20%
25%
28%
50%
58%
78%
Osteoporosis
VCD
Dyslipidemia
Bowel disease
Hypothyroidism
Diabetes
Dermatitis
OSAS
Sinusitis
Musculoskeletal impairment
Psychological disturbances
Hypertension
Obesity
GORD
Rhinitis
Comorbidity

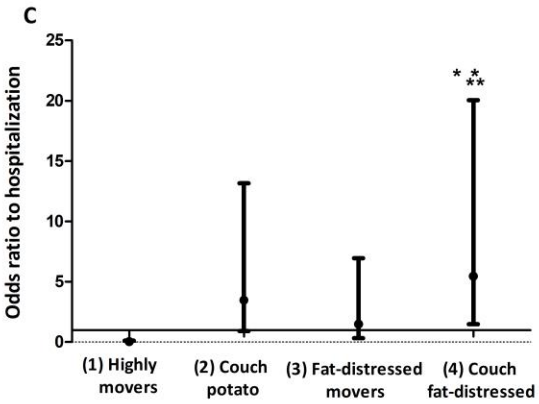

B
Frequency
20
18
16
14
12
10
8
6
4
2
0
2%
12%
16%
20%
19%
15%
8%
5%
3%
0
1
2
3
4
5
6
7
>8
Number of comorbidities per patient

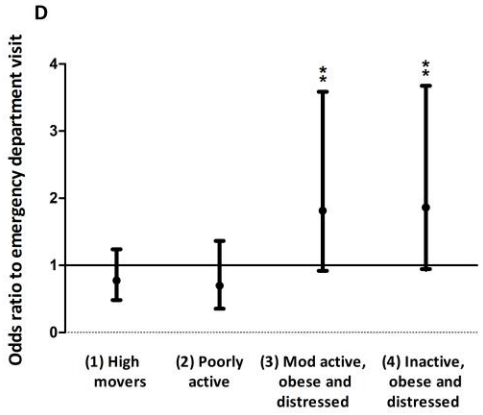
0 - 20% > 20-40% > 40-60% > 60%	% Osteoporosis	% VCD	% Dyslipidemia	% Bowel disease	% Hypothyroidism	% Diabetes	% Dermatitis	% SAOS	% Sinusitis	% MSK impairment	% Psychological disturbance	% Hypertension	% Obesity	% GORD	% Rhinitis
(1) High movers (n=76)	4	4	4	5	8	3	9	9	15	18	13	16	33	42	78
(2) Poorly active (n=80)	1	3	3	8	8	6	21*	10	14	31	21	31*	30	42	61
(3) Mod active, obese and distressed (n=69)	3	3	17*‡	6	12	15*	6	15	17	7‡	32*	38*	70*‡	54	80
(4) Inactive, obese and distressed (n=71)	6	10	6	10	4	14*	13	20	11	21*‡	35*	30	62*‡	72*‡‡	80

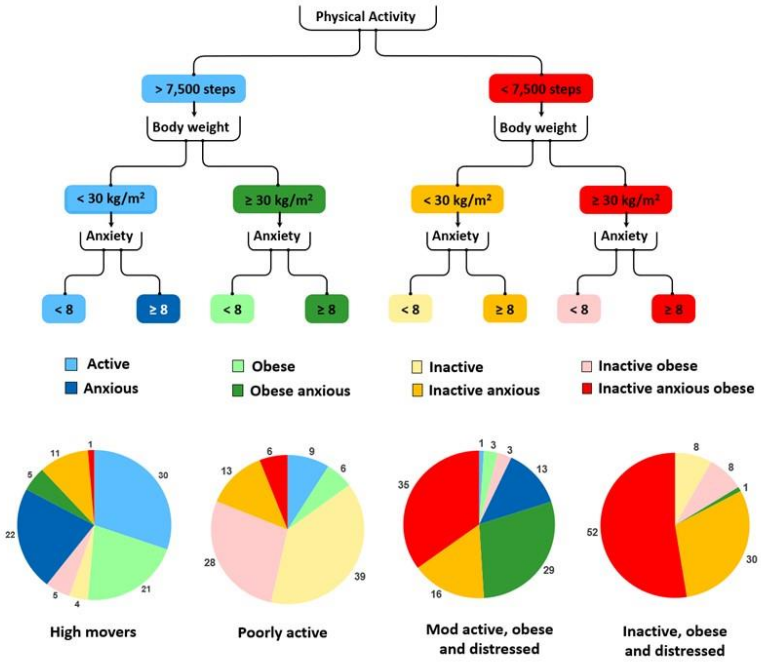

High movers
Poorly active
Mod active, obese and distressed
Inactive, obese and distressed
ACQ score
0
1
2
3
4
5
6
ACQ 1: Woken by the asthma
ACQ 2: Morning symptoms
ACQ 3: Activity limitations
ACQ 4: Shortness of breath
ACQ 5: Wheeze
ACQ 6: Short bronchodilator
ACQ 7: FEV1 % predicted
Questions

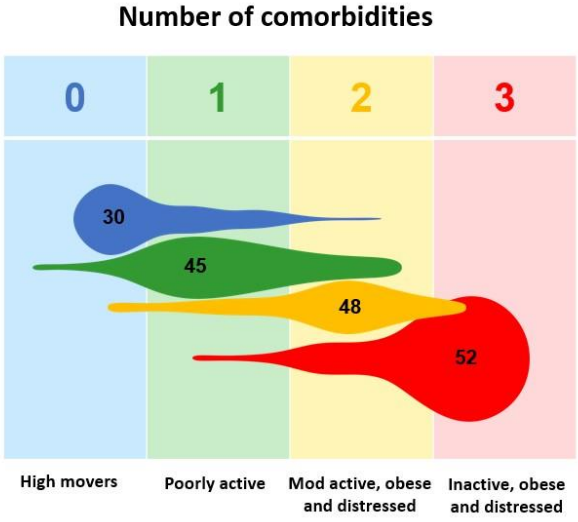

A
Odds ratio to uncontrolled asthma
50
40
30
20
10
0
(1) High movers
(2) Poorly active
(3) Mod active, obese and distressed
(4) Inactive, obese and distressed


B
Odds ratio to exacerbation
5
4
3
2
1
0
(1) High movers
(2) Poorly active
(3) Mod active, obese and distressed
(4) Inactive, obese and distressed


C
Odds ratio to hospitalization
25
20
15
10
5
0
(1) Highly movers
(2) Couch potato
(3) Fat-distressed movers
(4) Couch fat-distressed


D
Odds ratio to emergency department visit
4
3
2
1
0
(1) High movers
(2) Poorly active
(3) Mod active, obese and distressed
(4) Inactive, obese and distressed


Physical Activity
> 7,500 steps
< 7,500 steps
Body weight
Body weight
< 30 kg/m²
≥ 30 kg/m²
< 30 kg/m²
≥ 30 kg/m²
Anxiety
Anxiety
Anxiety
Anxiety
< 8
≥ 8
< 8
≥ 8
< 8
≥ 8
< 8
≥ 8
Active
Anxious
Obese
Obese anxious
Inactive
Inactive anxious
Inactive obese
Inactive anxious obese
30
21
4
5
22
5
11
1
High movers
39
28
13
6
9
6
Poorly active
29
16
35
13
3
1
Mod active, obese and distressed
30
52
1
8
8
Inactive, obese and distressed


Number of comorbidities
0
1
2
3
30
45
48
52
High movers
Poorly active
Mod active, obese and distressed
Inactive, obese and distressed

Online Data Supplement

This appendix has been included by the authors to provide readers with additional information about the manuscript.

Supplement to: Freitas PD, Xavier RF, McDonald VM, Gibson PG, Cordova-Rivera L, Furlanetto KC, de Oliveira JM, Carvalho-Pinto RM, Cukier A, Stelmach A, Carvalho CRF.

Identification of asthma phenotypes based on extrapulmonary treatable traits

Online Data Supplement

This Supplementary Materials section has been included by the authors to provide readers with additional information about the methods and results.

The components of the Supplementary Materials are as follows:

1. METHODS

1.1 Statistical analysis

Principal component analysis (PCA) was used to adjust the correlations between variables. The Kaiser-Meyer-Olkin (KMO) measures of sampling adequacy test and Bartlett's Test of Sphericity were used to confirm whether the PCA was appropriate for these variables.

Cluster analysis: was performed using measures that were randomly chosen to identify the two components identified in the PCA. A hierarchical cluster analysis was used to identify the number of clusters and cluster centroids. Possible cluster divisions were determined by inconsistent jumps between stages in an agglomeration schedule. Once cluster numbers and centroids were decided, a K-means cluster analysis was used to cluster cases to centroids. The stability of the clusters was tested using a repeated K-means clustering with a random sample containing 50% of the cases (1, 2).

Association analyses: were performed using multiple logistic regression between the dependent outcomes (exacerbation, hospitalisation, ED visit and temporary systemic corticosteroid) and the clinical and behavioural characteristics included in the cluster analysis: sex (female=1), BMI ($\leq$30kg/m^2=1), ACQ5 (uncontrolled=1), sedentary behavior (hours/day), daily physical activity (1,000 steps/day), HADS-A and HADS-D (scores subscales), AQLQ (total score), and inhaled corticosteroids (ICS; dose/1,000 µg/day). The stepwise forward process was used to include variables (with $P<0.20$ in the univariate analysis) into the model. Multiple linear regression analysis was performed to identify the independent factors associated with ACQ7(dependent variable): sex (female=1), BMI ($\leq$30kg/m^2=1), the occurrence of exacerbation (exacerbation=1), ICS (dose/1,000 µg/day), daily physical activity (1,000 steps/day), HADS-A and HADS-D

(scores subscales) and AQLQ (total score). Both the regression analyses and predictive equations were conducted to reinforce the clinical relevance of these clusters.

2. RESULTS

2.1. **Table S1**. Rotated component matrix

	Component 1	**Component 2**
BMI, Kg/m^2	0.43	0.13
ACQ-7, score	0.50	0.40
Daily physical level, steps/day	-0.24	-0.85
Sedentary behavior, hours/day	-0.05	0.84
AQLQ, total score	-0.77	0.08
HAD-A, score	0.77	-0.26
HAD-D, score	0.79	-0.20

Legend: Extraction method: Principal component analysis. BMI, body mass index; ACQ7, Asthma Control Questionnaire with 7 questions; AQLQ, Asthma Quality of Life Questionnaire; HAD-A, Hospital Anxiety and Depression scale-anxiety; HAD-D, Hospital Anxiety and Depression scale-depression.

2.2 Table S2. Risk factors associated with exacerbation, hospitalization, emergency department visit, and temporary systemic corticosteroid.

Variables	Univariate analysis		Multiple logistic final model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Exacerbation				
Sex, female	1.55 (0.88 – 2.71)	0.12#	1.14 (1.01 – 1.30)	0.04*
BMI, Kg/m^2	1.10 (0.68 – 1.76)	0.69		
ACQ-5, score	1.92 (1.18 – 3.12)	0.009#		
Sedentary behavior, hours/day	1.11 (0.98 – 1.25)	0.10#	1.83 (1.02 – 3.30)	0.04*
Daily physical level, steps/day	0.95 (0.88 – 1.03)	0.24		
HAD-A, score	1.01 (0.95 – 1.07)	0.76		
HAD-D, score	0.99 (0.94 – 1.06)	0.98		
AQLQ, total score	0.84 (0.69 – 1.02)	0.07#		
ICS dose, µg/day	1.30 (0.98 – 1.75)	0.08#		
Hospitalization				
Sex, female	0.62 (0.27 – 1.43)	0.26		
BMI, Kg/m^2	1.17 (0.55 – 2.51)	0.67		
ACQ-5, score	2.22 (0.91 – 5.29)	0.08#		
Sedentary behavior, hours/day	1.35 (1.12 – 1.63)	0.002#	1.23 (1.01 – 1.50)	0.04*
Daily physical level, steps/day	0.76 (0.63 – 0.90)	0.002#	0.81 (0.67 – 0.97)	0.03*
HAD-A, score	0.99 (0.91 – 1.08)	0.88		
HAD-D, score	0.94 (0.85 – 1.03)	0.19#		

AQLQ, total score	1.04 (0.77 – 1.41)	0.77		
ICS dose, µg/day	1.17 (0.75 – 1.81)	0.48		
Emergency department visit				
Sex, female	2.16 (1.18 – 3.94)	0.01#	1.90 (1.02 – 3.53)	0.04*
BMI, Kg/m^2	1.09 (0.68 – 1.75)	0.71		
ACQ-5, score	1.62 (0.99 – 2.66)	0.05#		
Sedentary behavior, hours/day	0.90 (0.80 – 1.01)	0.08#		
Daily physical level, steps/day	1.01 (0.93 – 1.09)	0.82		
HAD-A, score	1.07 (1.01 – 1.14)	0.01#	1.06 (1.02 – 1.14)	0.01*
HAD-D, score	1.06 (0.99 – 1.12)	0.06#		
AQLQ, total score	0.71 (0.58 – 0.86)	0.001#		
ICS dose, µg/day	1.21 (0.90 – 1.61)	0.20		
Temporary systemic corticosteroid				
Sex, female	1.59 (0.89 – 2.54)	0.17#	1.95 (1.06 – 3.60)	0.03*
BMI, Kg/m^2	0.93 (0.57 – 1.51)	0.78		
ACQ-5, score	1.65 (1.01 – 2.72)	0.05#		
Sedentary behavior, hours/day	1.19 (0.99 – 1.26)	0.07#	1.16 (1.02 – 1.32)	0.02*
Daily physical level, steps/day	0.91 (0.84 – 0.99)	0.03#		
HAD-A, score	1.04 (0.98 – 1.10)	0.21		
HAD-D, score	1.01 (0.95 – 1.07)	0.63		
AQLQ, total score	0.94 (0.78 – 1.14)	0.57		
ICS dose, µg/day	1.07 (0.80 – 1.44)	0.63		

Legend: The data are presented as the odds ratio and 95% confidence interval. OR = odds ratio, 95% CI = 95% confidence interval; BMI, body mass index; ACQ-5, Asthma Control Questionnaire with 5 questions; HAD-A, Hospital Anxiety and Depression scale-anxiety; HAD-D, Hospital Anxiety and Depression scale-depression; AQLQ, Asthma Quality of Life Questionnaire; ICS, inhaled corticosteroids. $^{\#}P<0.20$; $^{*}P<0.05$.

2.3 Table S3. Multiple linear regression analyzing variables associated with ACQ-7.

	Univariate analysis		**Final model**	
			R^2 adjusted = 0.29	*P*<0.001
Variables	**(95% CI)**	**P value**	**(95% CI)**	**P value**
Sex, female	(-0.36 – 0.18)	0.54	(-0.58 – -0.11)	0.004*
BMI, kg/m^2	(-0.17 – 0.28)	0.63		
Exacerbation, yes=1	(0.19 – 0.65)	<0.001#	(0.13 – 0.53)	0.001*
ICS dose, µg/day	(0.13 – 0.49)	0.001#		
Daily physical level, 1,000 steps/day	(-0.13 – -0.06)	<0.001#	(-0.12 – -0.05)	<0.001*
Sedentary behavior, hours/day	(-0.12 – 0.10)	0.12#		
HAD-A, score	(0.007 – 0.06)	0.01#		
HAD-D, score	(0.007 – 0.06)	0.02#		
AQLQ, total score	(-0.42 – -0.26)	<0.001#	(-0.42 – -0.27)	<0.001*

Legend: The data are presented as the 95% confidence interval. OR, odds ratio; 95% CI, 95% confidence interval; R^2, R square; BMI, body mass index; ICS, inhaled corticosteroids; HAD-A, Hospital Anxiety and Depression scale-anxiety; HAD-D, Hospital Anxiety and Depression scale-depression; AQLQ, Asthma Quality of Life Questionnaire. #*P*<0.20; **P*<0.05.

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