BMJ Open

Validation of the Adult Asthma Epidemiological Score: a secondary analysis of EPI-ASTHMA

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-086493
Article Type:	Original research
Date Submitted by the Author:	15-Mar-2024
Complete List of Authors:	Laranjeira, Catarina; University of Porto Faculty of Medicine Jácome, Cristina; University of Porto Faculty of Medicine, CINTESIS Amaral, Rita; University of Porto Faculty of Medicine, CINTESIS Bernardo, Filipa; AstraZeneca Correia-de-Sousa, Jaime; Life and Health Sciences Research Institute (ICVS)/3B's — PT Government Associate Laboratory, University of Minho; Horizonte Family Health Unit, Fonseca, Joao A.; University of Porto Faculty of Medicine, CINTESIS
Keywords:	Asthma < THORACIC MEDICINE, Epidemiology < TROPICAL MEDICINE, Patient Reported Outcome Measures

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Validation of the Adult Asthma Epidemiological Score: a secondary analysis of EPI-ASTHMA

Authors

Catarina L. Laranjeira[1] M.D. e-mail: catarina_laranjeira2@hotmail.com

Cristina Isabel O. Jácome[2] Ph.D. e-mail: cjacome@med.up.pt

Rita da S. Amaral[2 3 4] Ph.D. e-mail: rita_amaral@med.up.pt

Filipa Bernardo[5], degree e-mail: filipa.bernardo@astrazeneca.com

Jaime Correia-de-Sousa[6] Ph.D. e-mail: jaimecsousa@gmail.com

João A Fonseca[2 7 8] Ph.D. e-mail: fonseca.ja@gmail.com

Affiliations:

[1]Faculty of Medicine of the University of Porto, Porto, Portugal

[2]CINTESIS@RISE, Department of Community Medicine, Information and Health Decision Sciences (MEDCIDS), Faculty of Medicine, University of Porto, Porto, Portugal.

[3]Department of Cardiovascular and Respiratory Sciences, Porto Health School, Polytechnic Institute of Porto, Porto, Portugal.

[4]Department of Women's and Children's Health, Pediatric Research, Uppsala University, Uppsala, Sweden.

[5]AstraZeneca, Queluz, Lisboa, Portugal.

[6]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal.

[7]MEDIDA, Porto, Portugal.

[8]Allergy Unit, Hospital and Institute CUF, Porto, Portugal.

Corresponding author:

Cristina Jácome

Faculty of Medicine, University of Porto, Portugal

Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

cjacome@med.up.pt

+351 916096244

Abstract word count: 244

Text word count: 2851

ABSTRACT

Background: The A2 score is an 8-question patient-reported outcome measure that has been validated for ruling in (score ≥4) and ruling out (score 0-1) asthma. However, this screening tool has been validated in a cohort similar to the derivation cohort used.

Objective: This study aims to validate the predictive accuracy of the A2 score in a primary care population against general practitioner (GP) clinical assessment and to determine whether the proposed cut-offs are the most appropriate.

Methods: This accuracy study is a secondary analysis of the EPI-ASTHMA population-based study. Random adult participants recruited from primary healthcare centers in Portugal were analyzed. Participants answered the A2 score by telephone interview. Those with an A2 score ≥1 (plus 5% with an A2 score of 0) were invited to a diagnostic visit carried out by a GP to confirm or not a diagnosis of asthma. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curves.

Results: A total of 1283 participants (median 54[p25-p75 43-66] years; 60% female) were analyzed. The A2 score showed high discriminatory power in identifying asthma, with an area under the ROC curve of 82.9(95%CI 80.4-85.4)%. The proposed cut-off ≥4 was the most appropriate to rule in asthma (specificity 83.1%, positive predictive value 62.4%, accuracy 78%). Similarly, the proposed cut-off <2 was the most suitable for excluding asthma (sensitivity 92.7%, negative predictive value 93.7%, accuracy 60.5%).

Conclusion: The A2 score is a useful tool to identify patients with asthma in a primary care population.

Key-words: asthma; epidemiology; diagnostic screening; patient-reported outcome measure

Strengths and limitations of this study

- This study uses a large sample size recruited from the three most populated regions of Portugal.
- An interview guide was used to standardize the procedures among the interviewers, during the phone call interview.
- We include only participants from the primary healthcare centers, which may limit the extrapolation of A2 performance in other settings.
- As the A2 score was applied during a telephone screening interview, we cannot guarantee that all the participants fully understood the questions of this patient-reported outcome measure, and this may have influenced the results obtained.

Abbreviations

ECRHS: European Community Respiratory Health Survey

GA2 LEN: Global Allergy and Asthma European Network

WHS: World Health Survey

COPD: chronic obstructive pulmonary disease

CDQ: COPD Diagnostic Questionnaire

SCSQ: COPD-screening questionnaire

PROM: patient-reported outcome measure

A2 score: Asthma Epidemiological Score

PPV: positive predictive value

NPV: negative predictive value

NHS: National Health Service

GP: general practitioner

ROC: receiver operating characteristic

AUC: area under the ROC curve

ASQ: Asthma Screening Questionnaire

CAPTURE: COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk

INTRODUCTION

Asthma is a chronic disease characterized by a wide range of respiratory symptoms, such as wheezing, shortness of breath, coughing, and chest tightness, and by a variable expiratory airflow limitation, both of which vary in time and intensity.[1] This is a growing health problem that affects more than 262 million people worldwide, making it one of the most prevalent chronic diseases, which reflects a severe burden on the healthcare system.[2] The prevalence of asthma varies considerably across continents, ranging from 3.4% to 8.3%, with Europe having approximately 5.86% of the population suffering from this disease.[3]

Differences in asthma prevalence among regions are mainly due to real regional variations but may also result from using different definitions of asthma. Indeed, the definition of asthma has not been standardized for use in epidemiological studies, so each study uses a different questionnaire-based methodology, leading to varying asthma estimates and the inability to make comprehensive comparisons.[4,5] Ideally, asthma diagnosis is based on the identification of typical symptoms and supported by the performance of lung function tests, such as spirometry with reversibility test.[1] However, this makes the diagnosis more expensive and less accessible, especially in resource-limited regions and in population-based studies.

The prevalence of asthma symptoms in epidemiological studies has been mainly measured through written questionnaires.[5] Commonly, literature reports the use of questionnaires in multinational epidemiological studies on asthma prevalence in adults, mainly the European Community Respiratory Health Survey (ECRHS).[6] The Global Allergy and Asthma European Network (GA2 LEN) also conducted a large multicenter European prevalence study using a questionnaire mostly based on the asthma definitions used in the ECRHS [7], and the World Health Survey (WHS) provides the most information on asthma prevalence in low-income countries [8]. In fact, the World Health Organization Global Alliance against Chronic Respiratory Diseases highlights the importance of the development of simple and affordable diagnostic tools for chronic respiratory diseases, which could be adapted for different realities.[9] A systematic review of the diagnostic accuracy of screening tests for chronic obstructive pulmonary disease

(COPD) compares the use of the COPD Diagnostic Questionnaire (CDQ) against handheld flow meters.[10] Moreover, Martinez et al developed the CAPTURE (COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk) questionnaire to identify subjects who would benefit from further diagnostic investigation.[11] In the specific context of asthma, Sá Sousa et al designed and validated a patient-reported outcome measure (PROM) for asthma screening, the Adult Asthma Epidemiological Score (A2 score), a short and easy-to-use questionnaire.[12] This was the first self-reported questionnaire to be validated against a physician's clinical assessment and diagnostic workup for identifying asthma in adults. Furthermore, it showed the ability to rule in and rule out asthma, meaning that it can be applied in prevalence studies as well as used as a screening tool. The cut-offs suggested – score ≥4 to rule in and scores of 0-1 to rule out – were established based on positive and negative predictive values (PPV and NPV, respectively), which are closely related measurements to the prevalence of asthma, so further testing in epidemiological studies is needed. The A2 score showed high accuracy in a validation cohort extracted from the same population of the derivation cohort.[12] However, until now, no validation study applied the A2 score to another population, lacking external validation.

Therefore, this study aimed to validate the predictive accuracy of the A2 score against a general practitioner clinical assessment and to determine whether the proposed cut-offs are the most appropriate in this population.

METHODS

Patient and Public Involvement

Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of our research.

Study design

This accuracy study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study (NCT05169619). Further details regarding this study can be found elsewhere.[13] We used data collected between May 2021 and September 2023 from 34 primary healthcare centers in the North, Center and Lisbon Metropolitan Area of Portugal. The study was approved by the ethics committees of the Regional Health Administration of North (CE/2022/117), Center (27/2021), and of Lisbon and Tagus Valley (2775/CES/2022) and of the Local Health Units of Matosinhos (38/CES/JAS) and Alto Minho (38/2021). All the participants provided oral informed consent during the telephone interview and latter a written informed consent during the clinical assessment visit. This study was reported according to STARD (The Standards for Reporting of Diagnostic Accuracy Studies) guidelines.

Participants

This secondary analysis included part of the patients included in the EPI-ASTHMA study. The EPI-ASTHMA study included a random sample of subjects aged ≥ 18 years who were registered in the primary care National Health Service (NHS) database and provided voluntary consent during an invitation phone call. Those with any specific physical and/or cognitive disabilities that prevented them from cooperating with the study procedures (including lung function tests) and/or understanding/answering the self-reported questionnaires were excluded.

Data collection

Participants who fulfilled the eligibility criteria were invited for a telephone screening interview performed by a centralized team of experienced interviewers. During the interview, they answered the A2 score [12]. This score includes 8 questions: about previous physician diagnosis ("Did a

physician confirm you had asthma?" and "Do you still have asthma (previously diagnosed by a physician)?"; about asthma medication intake and asthma symptoms. The resulting score for each patient is the direct sum of all positive answers, ranging from 0 to 8. The original authors suggested that asthma presence could be ruled in for scores of 4 or more (PPV of 93.3%, with 99.2% specificity and 89.4% accuracy) and ruled out for scores of 0 to 1 (NPV of 98.2%, with 93.1% specificity and 89.4% accuracy).[12] Participants with an A2 score ≥1 were invited to a diagnostic visit undertaken in a mobile outpatient clinic. For quality control, ~5% of those with an A2 score of 0 were also invited. The confirmation of an asthma diagnosis was carried out by a general practitioner (GP) and was based on clinical history, physical examination, lung function tests (spirometry pre- and post-bronchodilator; fractional exhaled nitric oxide measurement), peripheral blood counts (eosinophil), and electronic PROMs (e.g., Control of Allergic Rhinitis and Asthma test).[14,13] Subjects diagnosed with asthma were included and those without asthma were randomly selected, resulting in a final sample of ~30% with asthma and ~70% without, in each region.

Analysis

To describe the characteristics of the participants, mean and standard deviation were used for normally distributed variables, while median and interquartile range (p25-p75) were used for skewed distributions. As for categorical variables, absolute frequencies, proportions, and 95% confidence intervals (95% CI) were performed. To compare continuous variables between patients with and without asthma, t-tests for independent samples or Mann-Whitney tests were used depending on the normality of variables. To assess associations between two categorical variables, a chi-square (X^2) test was performed. Internal consistency of the A2 score was assessed by Cronbach α, which was considered adequate if ≥ 0.70 [15]. To evaluate the discriminative power of the A2 score in comparison to the physician's final diagnosis, receiver operating characteristic (ROC) curve analysis was carried out. Sensitivity, specificity, PPV, NPV, and accuracy were used as diagnostic accuracy measures. The two cut-off points were validated by analyzing the ROC curve performance, which included calculating the Youden index (sensitivity

+ specificity - 1)[16,17]. Additionally, we considered the combination of PPV, NPV, sensitivity, and specificity that best suited the purpose of this score for each case. Statistical analysis was performed using IBM SPSS Statistics, version 29 (IBM Corp, Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Participants

This secondary analysis included 1283 participants (Figure 1), with a median age (p25-p75) of 54 (43-66) years old, of which 60% were females (Table 1). There were no statistically significant differences between participants with and without asthma regarding age, gender, body mass index, smoking status, or geographic region of residence (p>0.005) (Table 1). Sample characteristics are shown in Table 1.

TABLE 1 - Characterization of the population

	Asthma (N=385)	No asthma (N=898)	Total (N=1283)	*p* value
Age (y), median (p25-p75)	52 (41-66)	54 (44-66)	53 (43-66)	0.074*
Female, n (%)	241 (62.6)	527 (58.7)	768 (60.0)	0.190+
BMI (kg/m^2), median (p25-p75)	27.1 (23.9-30.6)[a]	26.5 (23.9-30.1)[b]	26.7 (23.9-30.4)	0.212*
Smoking status, n (%)				0.196+
Never smoker	216 (56.1)[c]	456 (50.8)[d]	672 (52.4)	
Current smoker	72 (18.7)	198 (22.0)	270 (21.0)	
Ex smoker	96 (24.9)	241 (26.8)	337 (26.3)	
Region, n (%)				1+
North	148(38.4)	345 (38.4)	493 (38.4)	
Center	66 (17.1)	154 (17.1)	220 (17.1)	
Lisbon Metropolitan Area	171 (44.4)	399 (44.4)	570 (44.4)	
A2 score, median (p25-p75)	5 (3-6)	2 (1-3)	3 (1-4)	<0.001*

p25-p75, percentile 25 to percentile 75; BMI, body mass index; *Mann-Witney U test; +Chi-square test; [a]8 missing values; [b]17 missing values; [c]1 missing values; [d]3 missing values

Diagnostic accuracy of the A2 score

Participants with asthma had a A2 score median (p25-p75) significantly higher than those without asthma (5(3-6) vs 2(1-3), p<0.001) (Table 1). Internal consistency of the A2 score was adequate (Cronbach's α 0.746). The graphic representation of the ability of the A2 score to discriminate between participants with and without asthma is shown in Figure 2. As the cut-off point increases, the A2 score becomes more sensitive and less specific, the PPV increases and the NPV decreases (Table 2). Thus, the higher the score, the more likely it is to predict the asthma diagnosis, however, the higher the false positive rate.

The discriminatory capacity of the A2 score, summarized by the area under the ROC curve (AUC), was 82.9% (95% CI 80.4-85.4). The predictive power of each cut-off point is shown in Table 2. The Youden index is at its highest value when the cut point is set at 3 (sensitivity 82%, specificity 69,3%, NPV 89.9%, and PPV 53.3%). This corresponds to the optimal trade-off between sensitivity and specificity. To meet the purpose of our study, a cut-off point of less than 2 positive answers (scores of 0 or 1) was chosen to exclude the presence of asthma. This cut-off point showed a high ability to select individuals who should undergo further diagnostic evaluation, and NPV of 93.7% was obtained, with high sensitivity (92.7%) and an accuracy of 60.5% (Table 2). A cut-off of 4 or higher was selected as the most appropriate to rule in asthma. This cut-off had a reasonable accuracy in identifying asthma cases (78%), a PPV of 62.4%, and a specificity of 83.1% (Table 2).

TABLE 2 – Diagnostic accuracy measures and predictive values

A2 score	N (%)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
≥1	1122 (66.0)	97.9 (96.0-99.1)	17.0 (14.6-19.7)	33.6 (32.2-35.3)	95.0 (90.5-97.5)
≥2	836 (49.2)	92.7 (89.7-95.1)	46.7 (43.4-50.0)	42.7 (41.2-44.4)	93.7 (91.2-95.6)
≥3	591 (34.8)	82.0 (77.6-85.5)	69.3 (66.1-72.3)	53.3 (50.8-55.9)	89.9 (87.7-91.7)
≥4	404 (23.8)	65.5 (60.5-70.2)	83.1 (80.5-85.5)	62.4 (58.5-66.1)	84.9 (83.0-86.6)
≥5	282 (16.6)	54.3 (49.2-59.4)	91.9 (89.9-93.6)	74.1 (69.8-78.4)	82.4 (80.8-83.9)
≥6	188 (11.1)	38.7 (33.8-43.8)	95.7 (94.1-96.9)	79.3 (73.8-84.2)	78.5 (77.1-79.8)
≥7	108 (6.4)	22.6 (18.5-27.1)	97.7 (96.5-98.6)	80.6 (72.8-87.8)	74.6 (73.6-75.7)
8	50 (2.9)	10.7 (7.8-14.2)	99.0 (98.1-99.5)	82.0 (69.2-90.3)	72.1 (71.4-72.8)

Definition of abbreviations: A2 score, Adult Asthma Epidemiological Score; CI, confidence interval; PPV, predictive positive value; NPV, predictive negative value.

DISCUSSION

This secondary analysis was the first external validation of the accuracy of the A2 score self-reported questionnaire. The A2 score showed good discriminatory power for asthma diagnosis in a Portuguese primary care population. The proposed cut-offs (scores ≥4 to rule in and scores 0-1 to rule out) were validated in this study population.

There is sparse literature on the performance of predictive scores for adult asthma. In a pilot study, the Asthma Screening Questionnaire (ASQ), an asthma screening tool, showed high sensitivity (96%) and specificity (100%) to discriminate between asthma cases and control subjects.[18] Accuracy of the A2 score could be interpreted as lower than this ASQ. However, it is important to note that the study conducted by Shin et al was based on a small sample size of 50 participants.[18] Additionally, the high accuracy reported may be attributed to the methodology used: the cases were recruited from a clinical setting so they may report more symptoms, while the controls were healthy and asymptomatic subjects, and all confounding comorbid conditions were excluded.[18] In contrast, all participants in our study were randomly recruited from primary care centers, better mimicking the performance of a screening tool in clinical practice.

Pekkanen et al used the ECRHS definitions to develop a continuous asthma score that can identify individuals for further investigation.[19] This method uses the same number of questions as the A2 score questionnaire, mainly based on symptom evaluation. However, the main difference lies in the comparator used: the ECRHS score only compares its results with bronchial hyperreactivity; while the A2 score incorporates a physician's clinical assessment that includes clinical history, physical examination, pulmonary function tests, peripheral blood counts, and PROMs. The ECRHS questionnaire was applied to the original A2 score study's data. The study reported an AUC of 86.8% (95%CI: 82.8-90.8%), a sensitivity of 87.2% (95%CI: 80.3-92.4%), and a specificity of 98.4% (95%CI: 96.7-99.3%).[12] Compared to the ECRHS questionnaire, the A2 score showed, in our sample, overlapping discrimination power (AUC 82.9%, 95%CI: 80.4-85.4%), higher sensitivity to exclude the presence of asthma (92.7%, 95%CI: 89.7%-95.1%) and low specificity to identify asthma (83.1%, 95%CI: 80.5%-85.5%).

The accuracy of A2 score is also high when compared with the accuracy of other known COPD screening tools. A systematic review found a pooled sensitivity of 64.5% (95%CI: 59.9-68.8%) and specificity of 65.2% (95%CI: 52.9-75.8%) for the CDQ.[10] In our sample, the cut-off selected to rule out asthma (scores of 0 or 1) had higher sensitivity than that reported for the CDQ (92.7% vs. 64.5% respectively), and the cut-off to rule in asthma (scores ≥4) had higher specificity (83.1% vs. 65.2% respectively).[10] The CAPTURE questionnaire had lower discrimination power than the A2 score (AUC of 79.5% vs. 82.9% respectively).[11] This case-finding questionnaire showed a sensitivity of 95.7% and a specificity of 67.8% in differentiating cases from the control subjects with no COPD.[11] Compared to the CAPTURE questionnaire's diagnostic accuracy, our validation study had slightly lower sensitivity to exclude the presence of the disease (92.7% vs. 95.7% respectively) and higher specificity to identify the presence of the disease (83.1% vs. 67.8% respectively).[11]

To select the optimal cut-off points a balance between sensitivity and specificity is necessary and should be adapted to meet the specific purposes of the score.[20] When conducting prevalence studies, it is more crucial to have a cut point with high specificity rather than sensitivity, as the focus is to rule in asthma with few false positives. However, high sensitivity is preferable when the focus is on identifying patients who are candidates for further diagnostic investigation. Therefore, we believe that a cut point with few missed cases is better suited for use as a screening tool or, in this case, to rule out asthma. For this reason, even though the cut point of 3 corresponds to the highest Youden index, we considered that a cut point of 4 or higher to rule in asthma and a cut point of less than 2 to rule out asthma as the most appropriate in our sample, validating the cut-offs proposed by the authors of this score. Moreover, they reported a specificity of 96.7% (95%CI: 94.6-98.2%) and PPV of 85% (95%CI: 76.8-90.6%) for the rule in cut-off [12], while in our sample, this cut-off showed lower specificity (83.1%, 95%CI: 80.5-85.5%) and PPV (62.4%, 95%CI: 58.5-66.1%).

According to Price et al, a PPV of at least 50% is reasonable [21], so although the PPV found in our study is lower than that reported by Sá-Sousa et al, it is still very reasonable. For the rule out

cut-off, the authors reported a sensitivity of 85.7% (95%CI: 78.6-91.2%) and a NPV of 95% (95%CI: 92.5-96.6%).[12] In our sample, this cut-off point had overlapping sensitivity (92.7%, 95%CI: 89.7-95.1%) and NPV (93.7%, 95%CI: 91.2-95.6%). The discriminative power (AUC; 95%CI) found is slightly lower than that reported by the authors (82.9%; 80.4-85.4% and 90.4%; 87.0-93.9%, respectively). These differences in the measures of diagnostic accuracy and discriminative power may be attributed to variations in symptom prevalence and asthma severity in the specific settings, as well as differences in sample sizes. In fact, our study has a considerably larger sample size compared to the original A2 score study.

This study has strengths and limitations that should be acknowledged. The large sample size recruited from the three most populated regions of the country is an important strength. However, we did not include any participants from the primary healthcare centers of southern Portugal. This study used a sample taken only from primary care, which may limit the extrapolation of results to other settings. In future studies, researchers should validate this score in other settings.

Another strength is the fact that we excluded patients with any cognitive disability that would prevent them from understanding or answering the A2 score autonomously. However, as this eligibility screening was made during a phone call interview, we cannot guarantee that all the participants fully understood the questions of the score, and this may have influenced the results. In addition, the A2 score was applied by different healthcare professionals which may also have led to small differences in the administration of the A2 score. To overcome this limitation, an interview guide was used to standardize the procedures among the interviewers. Future studies could compare the reliability of the A2 score applied as an electronic PROM and as a telephone interview.

Of note, validation against a GP clinical assessment grounded in objective measures and diagnostic tests is also a major strength. This differs from other asthma screening questionnaires, which were only validated against a physician's diagnosis [22], or based solely on lung functional tests such as spirometry and methacholine challenge test [18].

Moreover, the choice of cut-offs was not based solely on positive and negative predictive values, but also on the ROC curve performance and the Youden index, which is a strength of this study compared to other questionnaire validation studies, including the A2 score original study. The advantage of the ROC curve analysis is that since it is based on sensitivity and specificity, it is independent of disease prevalence.[17]

Conclusions

The A2 score is a simple and easily self-administered 8-question case-finding tool that has demonstrated good discriminatory power in a large primary care population of Portugal. In this validation study, the A2 score showed good diagnostic accuracy to be used in epidemiological studies, assessing the prevalence of asthma, as well as a screening tool in clinical settings, to identify individuals who would benefit from further investigation. Future studies are necessary to validate this score in different settings and countries, and to adapt the questionnaire for use in other languages and cultural contexts.

Funding: This study was sponsored and funded by AstraZeneca, Portugal

Conflict of interest: none

Author's contribution:

JC-d-S, JAF and FB conceptualized the EPI-ASTHMA study and CJ and RA conceptualized this secondary analysis. CL wrote the first draft of the manuscript. CL, CJ, and RA performed the statistical analysis. CJ, RA, FB, JC-d-S, and JAF contributed to and refined the manuscript for scientific content. All authors read and approved the final version of the manuscript.

REFERENCES

1. Global Initiative for Asthma – GINA. Global strategy for asthma management and prevention, 2023 [Internet]. [cited 2023 Oct 18]. Available from: www.ginasthma.org

2. Wang Z, Li Y, Gao Y, Fu Y, Lin J, Lei X, et al. Global, regional, and national burden of asthma and its attributable risk factors from 1990 to 2019: a systematic analysis for the Global Burden of Disease Study 2019. Respir Res. 2023 Dec 1;24(1).

3. Rabe AP, Loke WJ, Gurjar K, Brackley A, Lucero-Prisno III DE. Global Burden of Asthma, and Its Impact on Specific Subgroups: Nasal Polyps, Allergic Rhinitis, Severe Asthma, Eosinophilic Asthma. J Asthma Allergy. 2023 Oct;16:1097–113.

4. Sá-Sousa A, Jacinto T, Azevedo LF, Morais-Almeida M, Robalo-Cordeiro C, Bugalho-Almeida A, et al. Operational definitions of asthma in recent epidemiological studies are inconsistent. Clin Transl Allergy. 2014;4:24.

5. Innes Asher M, García-Marcos L, Pearce NE, Strachan DP. Trends in worldwide asthma prevalence. Eur Respir J. 2020 Dec 1;56(6).

6. Janson C, Anto J, Burney P, Chinn S, De Marco R, Heinrich J, et al. The European Community Respiratory Health Survey: what are the main results so far? Eur Respir J. 2001;18:598–611

7. Jarvis D, Newson R, Lotvall J, Hastan D, Tomassen P, Keil T, et al. Asthma in adults and its association with chronic rhinosinusitis: The GA 2LEN survey in Europe. Allergy: European Journal of Allergy and Clinical Immunology. 2012 Jan;67(1):91–8.

8. Sembajwe G, Cifuentes M, Tak SW, Kriebel D, Gore R, Punnett L. National income, self-reported wheezing and asthma diagnosis from the World Health Survey. Eur Respir J. 2010 Feb;35(2):279–86.

9. Bousquet J, Dahl R, Khaltaev N. Global Alliance against Chronic Respiratory Diseases. Allergy: European Journal of Allergy and Clinical Immunology. 2007 Mar;62(3):216-23.

10. Haroon S, Jordan R, Takwoingi Y, Adab P. Diagnostic accuracy of screening tests for COPD: a systematic review and meta-analysis. BMJ Open. 2015 Oct 8;5(10):e008133.

11. Martinez FJ, Mannino D, Leidy NK, Malley KG, Bacci ED, Barr RG, et al. A new approach for identifying patients with undiagnosed chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2017 Mar 15;195(6):748–56.

12. Sá-Sousa A, Pereira AM, Almeida R, Araújo L, Couto M, Jacinto T, et al. Adult Asthma Scores—Development and Validation of Multivariable Scores to Identify Asthma in Surveys. Journal of Allergy and Clinical Immunology: In Practice. 2019 Jan 1;7(1):183-190.e6.

13. Jácome C, Brito D, João C, Lopes F, Santos J, Amorim L, et al. EPI-ASTHMA study protocol: a population-based multicentre stepwise study on the prevalence and characterisation of patients with asthma according to disease severity in Portugal. BMJ Open. 2022 Sep 19;12(9).

14. Fonseca JA, Nogueira-Silva L, Morais-Almeida M, Azevedo L, Sa-Sousa A, Branco-Ferreira M, et al. Validation of a questionnaire (CARAT10) to assess rhinitis and asthma in patients with asthma. Allergy: European Journal of Allergy and Clinical Immunology. 2010;65(8):1042–8.

15. Prinsen CAC, Mokkink LB, Bouter LM, Alonso J, Patrick DL, De Vet HC, et al. COSMIN guideline for systematic reviews of Patient-Reported Outcome Measures. Qual Life Res. 2018 May;27(5):1147-1157.

16. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32–5.

17. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. Caspian J Intern Med. 2013; 4(2):627–635.

18. Shin B, Cole SL, Park SJ, Ledford DK, Lockey RF. A New Symptom-Based Questionnaire for Predicting the Presence of Asthma. J Investig Allergol Clin Immunol. 2010;20(1):27-34.

19. Pekkanen J, Sunyer J, Anto JM, Burney P, Abramson M, Kutin J, et al. Operational definitions of asthma in studies on its aetiology. European Respiratory Journal. 2005;26(1):28–35.

20. Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: The case of tests with continuous results. Biochem Med (Zagreb). 2016 Oct 1;26(3):297–307.

21. Price DB, Tinkelman DG, Nordyke RJ, Isonaka S, Halbert RJ. Scoring system and clinical application of COPD diagnostic questionnaires. Chest. 2006;129(6):1531–9.

22. Burney PGJ, Laitinen LA, Perdrizet S, Huckauf H, Tattersfield AE, Chinn S, et al. Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: An international comparison. European Respiratory Journal. 1989;2(10):940–5.

Figure legends

FIGURE 1 – Study flow diagram (n=1283)

FIGURE 2 – Receiver operating characteristics (ROC) curve for the A2 score

—— sum of all positive answers in the questionnaire (result score)

------- reference line

The solid line indicates the levels of sensitivity and false positive rate, for each cut-off point.

The area under the ROC curve is 0.829.

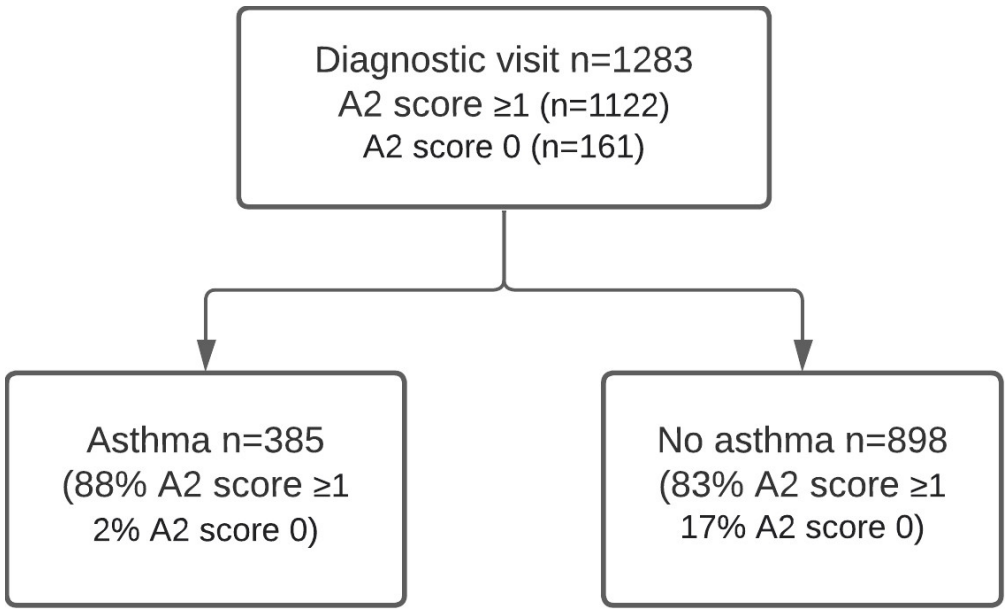
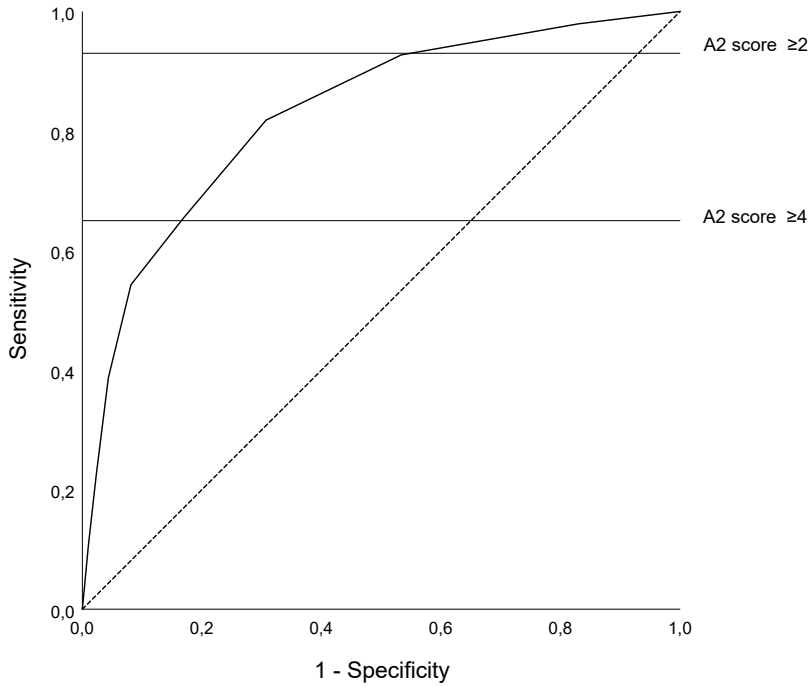


FIGURE 1 – Study flow diagram (n=1283)

95x57mm (300 x 300 DPI)



Reporting guidelines STARD (The Standards for Reporting of Diagnostic Accuracy Studies)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	p.2 "with an area under the ROC curve of 82.9"
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	p.2 "This accuracy study is a secondary analysis of the EPI-ASTHMA"; "methods"; "results"; "conclusions"
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	p.6 "Sá Sousa et al designed and validated a patient-reported outcome measure (PROM) for asthma screening, the Adult Asthma Epidemiological Score (A2 score) […] Furthermore, it showed the ability to rule in and rule out asthma, meaning that it can be applied in prevalence studies as well as used as a screening tool."
	4	Study objectives and hypotheses	p.6 "this study aimed to validate the predictive accuracy of the A2 score against a general practitioner clinical assessment and to determine whether the proposed cut-offs are the most appropriate in this population."
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. p.7 "Further details regarding this study can be found elsewhere.[13]"
Participants	**6**	Eligibility criteria	p.8 "Participants with an A2 score ≥1 were invited to a diagnostic visit undertaken in a mobile outpatient clinic. For quality control, ~5% of those with an A2 score of 0 were also invited."
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	p.7 "a random sample of subjects aged ≥ 18 years who were registered in the primary care National Health Service (NHS) database"
	8	Where and when potentially eligible participants were identified (setting, location and dates)	p.7 "data collected between May 2021 and September 2023 from 34 primary healthcare centers in the North, Center and Lisbon Metropolitan Area of Portugal" p.7 "during an invitation phone call."
	9	Whether participants formed a consecutive, random or convenience series	p.8 "Subjects diagnosed with asthma were included and those without

			asthma were randomly selected, resulting in a final sample of ~30% with asthma and ~70% without, in each region."
Test methods	**10a**	Index test, in sufficient detail to allow replication	Not applicable. Details about the index test can be found in the original A2 score study[12]
	10b	Reference standard, in sufficient detail to allow replication	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. Details about the reference standard can be found in the study protocol.[13]
	11	Rationale for choosing the reference standard (if alternatives exist)	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. Details about the reference standard can be found in the study protocol.[13]
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	p.8 "The two cut-off points were validated by analyzing the ROC curve performance, which included calculating the Youden index (sensitivity + specificity - 1)[16,17]. Additionally, we considered the combination of PPV, NPV, sensitivity, and specificity that best suited the purpose of this score for each case"
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. Details about the reference standard can be found in the study protocol.[13]
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. Details about the reference standard can be found in the study protocol.[13]
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	p.8 "To evaluate the discriminative power of the A2 score in comparison to the physician's final diagnosis, receiver operating characteristic (ROC) curve analysis was carried out. Sensitivity, specificity, PPV, NPV, and accuracy were used as diagnostic accuracy measures."
	15	How indeterminate index test or reference standard results were handled	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	16	How missing data on the index test and reference standard were handled	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Not applicable
	18	Intended sample size and how it was determined	Not applicable. This study is a secondary analysis of the EPI-ASTHMA. Details about the reference standard can be found in the study protocol.[13]
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1

	20	Baseline demographic and clinical characteristics of participants	p.10 "This secondary analysis included 1283 participants (Figure 1), with a median age (p25-p75) of 54 (43-66) years old, of which 60% were females (Table I)." p.11 TABLE I
	21a	Distribution of severity of disease in those with the target condition	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	21b	Distribution of alternative diagnoses in those without the target condition	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	22	Time interval and any clinical interventions between index test and reference standard	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	p.13 Table II
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	p.12 "The discriminatory capacity of the A2 score, summarized by the area under the ROC curve (AUC), was 82.9% (95% CI 80.4-85.4)."
	25	Any adverse events from performing the index test or the reference standard	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	p.16 "This study used a sample taken only from primary care, which may limit the extrapolation of results to other settings."
	27	Implications for practice, including the intended use and clinical role of the index test	p.17 "In this validation study, the A2 score showed good diagnostic accuracy to be used in epidemiological studies, assessing the prevalence of asthma, as well as a screening tool in clinical settings, to identify individuals who would benefit from further investigation"
OTHER INFORMATION			
	28	Registration number and name of registry	p.7 "This accuracy study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study (NCT05169619)"
	29	Where the full study protocol can be accessed	p.7 "Further details regarding this study can be found elsewhere.[13]"
	30	Sources of funding and other support; role of funders	p.1 "This study was sponsored and funded by AstraZeneca, Portugal"

BMJ Open

Validation of the Adult Asthma Epidemiological Score: a secondary analysis of the EPI-ASTHMA population-based study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-086493.R1
Article Type:	Original research
Date Submitted by the Author:	23-Sep-2024
Complete List of Authors:	Laranjeira, Catarina; University of Porto Faculty of Medicine Jácome, Cristina; University of Porto Faculty of Medicine, CINTESIS Amaral, Rita; University of Porto Faculty of Medicine, CINTESIS Bernardo, Filipa; AstraZeneca Correia-de-Sousa, Jaime; Life and Health Sciences Research Institute (ICVS)/3B's — PT Government Associate Laboratory, University of Minho; Horizonte Family Health Unit, Fonseca, Joao A.; University of Porto Faculty of Medicine, CINTESIS
Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Epidemiology, Diagnostics, Respiratory medicine
Keywords:	Asthma < THORACIC MEDICINE, Epidemiology < TROPICAL MEDICINE, Patient Reported Outcome Measures

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Validation of the Adult Asthma Epidemiological Score: a secondary analysis of the EPI-ASTHMA population-based study

Authors

Catarina L. Laranjeira[1] M.D. ORCID 0009-0002-8974-8631

e-mail: catarina_laranjeira2@hotmail.com

Cristina Isabel O. Jácome[2] Ph.D. ORCID 0000-0002-1151-8791

e-mail: cjacome@med.up.pt

Rita da S. Amaral[2 3 4] Ph.D. ORCID 0000-0002-0233-830X

e-mail: rita_amaral@med.up.pt

Filipa Bernardo[5], degree ORCID 0000-0001-8245-0520

e-mail: filipa.bernardo@astrazeneca.com

Jaime Correia-de-Sousa[6] Ph.D. ORCID 0000-0001-6459-7908

e-mail: jaimecsousa@gmail.com

João A Fonseca[2 7 8] Ph.D. ORCID 0000-0002-0887-8796

e-mail: fonseca.ja@gmail.com

Affiliations:

[1]Faculty of Medicine of the University of Porto, Porto, Portugal

[2]CINTESIS@RISE, Department of Community Medicine, Information and Health Decision Sciences (MEDCIDS), Faculty of Medicine, University of Porto, Porto, Portugal.

[3]Department of Cardiovascular and Respiratory Sciences, Porto Health School, Polytechnic Institute of Porto, Porto, Portugal.

[4]Department of Women's and Children's Health, Pediatric Research, Uppsala University, Uppsala, Sweden.

[5]AstraZeneca, Queluz, Lisboa, Portugal.

[6]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal.

[7]MEDIDA, Porto, Portugal.

[8]Allergy Unit, Hospital and Institute CUF, Porto, Portugal.

Corresponding author:
Cristina Jácome
Faculty of Medicine, University of Porto, Portugal
Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal
cjacome@med.up.pt

Abstract word count: 244

Text word count: 2851

ABSTRACT

Objective: The A2 score is an 8-question patient-reported outcome measure that has been validated for ruling in (score ≥4) and ruling out (score 0-1) asthma. However, this screening tool has been validated in a cohort similar to the derivation cohort used. This study aims to validate the predictive accuracy of the A2 score in a primary care population against general practitioner (GP) clinical assessment and to determine whether the proposed cut-offs are the most appropriate.

Design: This accuracy study is a secondary analysis of the EPI-ASTHMA population-based study.

Setting: Primary healthcare centers in Portugal.

Participants: Random adult participants answered the A2 score by telephone interview.

Outcomes: Those with an A2 score ≥1 (plus 5% with an A2 score of 0) were invited to a diagnostic visit carried out by a GP to confirm or not a diagnosis of asthma. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curves.

Results: A total of 1283 participants (median 54[p25-p75 43-66] years; 60% female) were analyzed. The A2 score showed high discriminatory power in identifying asthma, with an area under the ROC curve of 82.9(95%CI 80.4-85.4)%. The proposed cut-off ≥4 was the most appropriate to rule in asthma (specificity 83.1%, positive predictive value 62.4%, accuracy 78%). Similarly, the proposed cut-off <2 was the most suitable for excluding asthma (sensitivity 92.7%, negative predictive value 93.7%, accuracy 60.5%).

Conclusions: The A2 score is a useful tool to identify patients with asthma in a primary care population.

Key-words: asthma; epidemiology; diagnostic screening; patient-reported outcome measure

Strengths and limitations of this study

- This study uses a large sample size recruited from the three most populated regions of Portugal.
- An interview guide was used to standardize the procedures among the interviewers, during the phone call interview.
- We include only participants from the primary healthcare centers, which may limit the extrapolation of A2 performance in other settings.
- As the A2 score was applied during a telephone screening interview, we cannot guarantee that all the participants fully understood the questions of this patient-reported outcome measure, and this may have influenced the results obtained.

Abbreviations

ECRHS: European Community Respiratory Health Survey

GA2 LEN: Global Allergy and Asthma European Network

WHS: World Health Survey

COPD: chronic obstructive pulmonary disease

CDQ: COPD Diagnostic Questionnaire

SCSQ: COPD-screening questionnaire

PROM: patient-reported outcome measure

A2 score: Asthma Epidemiological Score

PPV: positive predictive value

NPV: negative predictive value

NHS: National Health Service

GP: general practitioner

ROC: receiver operating characteristic

AUC: area under the ROC curve

ASQ: Asthma Screening Questionnaire

CAPTURE: COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk

INTRODUCTION

Asthma is a chronic disease characterized by a wide range of respiratory symptoms, such as wheezing, shortness of breath, coughing, and chest tightness, and by a variable expiratory airflow limitation, both of which vary in time and intensity.[1] This is a growing health problem that affects more than 262 million people worldwide, making it one of the most prevalent chronic diseases, which reflects a severe burden on the healthcare system.[2] The prevalence of asthma varies considerably across continents, ranging from 3.4% to 8.3%, with Europe having approximately 5.86% of the population suffering from this disease.[3]

Differences in asthma prevalence among regions are mainly due to real regional variations but may also result from using different definitions of asthma. Indeed, the definition of asthma has not been standardized for use in epidemiological studies, so each study uses a different questionnaire-based methodology, leading to varying asthma estimates and the inability to make comprehensive comparisons.[4,5] Ideally, asthma diagnosis is based on the identification of typical symptoms and supported by the performance of lung function tests, such as spirometry with reversibility test.[1] However, this makes the diagnosis more expensive and less accessible, especially in resource-limited regions and in population-based studies.

The prevalence of asthma symptoms in epidemiological studies has been mainly measured through written questionnaires.[5] Commonly, literature reports the use of questionnaires in multinational epidemiological studies on asthma prevalence in adults, mainly the European Community Respiratory Health Survey (ECRHS).[6] The Global Allergy and Asthma European Network (GA2 LEN) also conducted a large multicenter European prevalence study using a questionnaire mostly based on the asthma definitions used in the ECRHS [7], and the World Health Survey (WHS) provides the most information on asthma prevalence in low-income countries [8]. In fact, the World Health Organization Global Alliance against Chronic Respiratory Diseases highlights the importance of the development of simple and affordable diagnostic tools for chronic respiratory diseases, which could be adapted for different realities.[9] A systematic review of the diagnostic accuracy of screening tests for chronic obstructive pulmonary disease

(COPD) compares the use of the COPD Diagnostic Questionnaire (CDQ) against handheld flow meters.[10] Moreover, Martinez et al developed the CAPTURE (COPD Assessment in Primary Care to Identify Undiagnosed Respiratory Disease and Exacerbation Risk) questionnaire to identify subjects who would benefit from further diagnostic investigation.[11] In the specific context of asthma, Sá Sousa et al designed and validated a patient-reported outcome measure (PROM) for asthma screening, the Adult Asthma Epidemiological Score (A2 score), a short and easy-to-use questionnaire.[12] This was the first self-reported questionnaire to be validated against a physician's clinical assessment and diagnostic workup for identifying asthma in adults. Furthermore, it showed the ability to rule in and rule out asthma, meaning that it can be applied in prevalence studies as well as used as a screening tool. The cut-offs suggested – score ≥4 to rule in and scores of 0-1 to rule out – were established based on positive and negative predictive values (PPV and NPV, respectively), which are closely related measurements to the prevalence of asthma, so further testing in epidemiological studies is needed. The A2 score showed high accuracy in a validation cohort extracted from the same population of the derivation cohort.[12] However, until now, no validation study applied the A2 score to another population, lacking external validation.

Therefore, this study aimed to validate the predictive accuracy of the A2 score against a general practitioner clinical assessment and to determine whether the proposed cut-offs are the most appropriate in this population.

METHODS

Patient and Public Involvement

Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of our research.

Study design

This accuracy study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study (NCT05169619). Further details regarding this study can be found elsewhere.[13] We used data collected between May 2021 and September 2023 from 34 primary healthcare centers in the North, Center and Lisbon Metropolitan Area of Portugal. The study was approved by the ethics committees of the Regional Health Administration of North (CE/2022/117), Center (27/2021), and of Lisbon and Tagus Valley (2775/CES/2022) and of the Local Health Units of Matosinhos (38/CES/JAS) and Alto Minho (38/2021). All the participants provided oral informed consent during the telephone interview and latter a written informed consent during the clinical assessment visit. This study was reported according to STARD (The Standards for Reporting of Diagnostic Accuracy Studies) guidelines.

Participants

The EPI-ASTHMA study included a random sample of subjects aged ≥ 18 years who were registered in the primary care National Health Service (NHS) database and provided voluntary consent during an invitation phone call. Those with any specific physical and/or cognitive disabilities that prevented them from cooperating with the study procedures (including lung function tests) and/or understanding/answering the self-reported questionnaires were excluded. This secondary analysis included part of the patients included in the EPI-ASTHMA study as data collection for EPI-ASTHMA study was still ongoing. All subjects diagnosed with asthma from the 34 participating primary care centers were included and those without asthma were randomly selected, in order to have a final sample of ~30% with asthma and ~70% without. This distribution, similar to the used in the A2 score original study, was chosen as it is known that accuracy measurements such as PPV and NPV are highly dependent on prevalence [12].

Data collection

Participants who fulfilled the eligibility criteria were invited for a telephone screening interview performed by a centralized team of experienced interviewers. During the interview, they answered the A2 score [12]. This score includes 8 questions: about previous physician diagnosis ("Did a physician confirm you had asthma?" and "Do you still have asthma (previously diagnosed by a physician)?"; about asthma medication intake and asthma symptoms. The resulting score for each patient is the direct sum of all positive answers, ranging from 0 to 8. The original authors suggested that asthma presence could be ruled in for scores of 4 or more (PPV of 93.3%, with 99.2% specificity and 89.4% accuracy) and ruled out for scores of 0 to 1 (NPV of 98.2%, with 93.1% sensitivity and 89.4% accuracy).[12] Participants with an A2 score ≥1 were invited to a diagnostic visit undertaken in a mobile outpatient clinic in the following 1-3 weeks. For quality control, ~5% of those with an A2 score of 0 were also invited. The confirmation of an asthma diagnosis was carried out by a general practitioner (GP) and was based on clinical history, physical examination, lung function tests (spirometry pre- and post-bronchodilator; fractional exhaled nitric oxide measurement), peripheral blood counts (eosinophil), and PROMs (e.g., A2 score, Control of Allergic Rhinitis and Asthma test).[14,13] Diagnosis of asthma followed GINA recommendations[1], relying primarily on the presence and pattern of respiratory symptoms (wheeze, shortness of breath, chest tightness, or cough) and supported by objective lung function findings such variable expiratory airflow limitation and high FeNO levels and other objective collected data (eosinophil).

Analysis

To describe the characteristics of the participants, mean and standard deviation were used for normally distributed variables, while median and interquartile range (p25-p75) were used for skewed distributions. As for categorical variables, absolute frequencies, proportions, and 95% confidence intervals (95% CI) were performed. To compare continuous variables between patients with and without asthma, t-tests for independent samples or Mann-Whitney tests were used depending on the normality of variables. To assess associations between two categorical

variables, a chi-square (X^2) test was performed. Internal consistency of the A2 score was assessed by Cronbach α, which was considered adequate if ≥ 0.70 [15]. To evaluate the discriminative power of the A2 score in comparison to the GP asthma diagnosis, receiver operating characteristic (ROC) curve analysis was carried out. Sensitivity, specificity, PPV, NPV, and accuracy were used as diagnostic accuracy measures. The two cut-off points were validated by analyzing the ROC curve performance, which included calculating the Youden index (sensitivity + specificity - 1)[16,17] and the combination of PPV, NPV, sensitivity, and specificity. In making the selection, we also considered the previous cut-offs suggested [12] and the fact that a PPV of at least 50% is reasonable for rule in [21]. Statistical analysis was performed using IBM SPSS Statistics, version 29 (IBM Corp, Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Participants

This secondary analysis included 1283 participants (Figure 1), with a median age (p25-p75) of 54 (43-66) years old, of which 60% were females (Table 1). There were no statistically significant differences between participants with and without asthma regarding age, gender, body mass index, smoking status, or geographic region of residence (p>0.005) (Table 1). Sample characteristics are shown in Table 1.

TABLE 1 - Characterization of the population

	Asthma (N=385)	No asthma (N=898)	Total (N=1283)	*p* value
Age (y), median (p25-p75)	52 (41-66)	54 (44-66)	53 (43-66)	0.074*
Female, n (%)	241 (62.6)	527 (58.7)	768 (60.0)	0.190+
BMI (kg/m^2), median (p25-p75)	27.1 (23.9-30.6)[a]	26.5 (23.9-30.1)[b]	26.6 (23.9-30.4)	0.212*
Smoking status, n (%)				0.196+
Never smoker	216 (56.1)[c]	456 (50.8)[d]	672 (52.4)	
Current smoker	72 (18.7)	198 (22.0)	270 (21.0)	
Ex smoker	96 (24.9)	241 (26.8)	337 (26.3)	
Region, n (%)				1+
North	148(38.4)	345 (38.4)	493 (38.4)	
Center	66 (17.1)	154 (17.1)	220 (17.1)	
Lisbon Metropolitan Area	171 (44.4)	399 (44.4)	570 (44.4)	
A2 score, median (p25-p75)	5 (3-6)	2 (1-3)	3 (1-4)	<0.001*

p25-p75, percentile 25 to percentile 75; BMI, body mass index; *Mann-Witney U test; +Chi-square test; [a]8 missing values; [b]17 missing values; [c]1 missing values; [d]3 missing values

Diagnostic accuracy of the A2 score

Participants with asthma had a A2 score median (p25-p75) significantly higher than those without asthma (5(3-6) vs 2(1-3), p<0.001) (Table 1). Internal consistency of the A2 score was adequate (Cronbach's α 0.746). The graphic representation of the ability of the A2 score to discriminate between participants with and without asthma is shown in Figure 2. As the cut-off point increases, the A2 score becomes more sensitive and less specific, the PPV increases and the NPV decreases (Table 2). Thus, the higher the score, the more likely it is to predict the asthma diagnosis, however, the higher the false positive rate.

The discriminatory capacity of the A2 score, summarized by the area under the ROC curve (AUC), was 82.9% (95% CI 80.4-85.4). The predictive power of each cut-off point is shown in Table 2. The Youden index is at its highest value when the cut point is set at 3 (sensitivity 82%, specificity 69.3%, NPV 89.9%, and PPV 53.3%). This corresponds to the optimal trade-off between sensitivity and specificity. To meet the purpose of our study, a cut-off point of less than 2 positive answers (scores of 0 or 1) was chosen to exclude the presence of asthma. This cut-off point showed a high ability to select individuals who should undergo further diagnostic evaluation, and NPV of 93.7% was obtained, with high sensitivity (92.7%) and an accuracy of 60.5% (Table 2). Both cut-offs ≥4 or ≥5 could be appropriate to rule in asthma based on their accuracy in identifying asthma cases. Nevertheless, a cut-off of 4 or higher, with a PPV of 62.4%, and a specificity of 83.1%, was selected as being reasonable accurate in identifying asthma cases (78%) (Table 2).

TABLE 2 – Diagnostic accuracy measures and predictive values

A2 score	N (%)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
≥1	1122 (66.0)	97.9 (96.0-99.1)	17.0 (14.6-19.7)	33.6 (32.2-35.3)	95.0 (90.5-97.5)
≥2	836 (49.2)	92.7 (89.7-95.1)	46.7 (43.4-50.0)	42.7 (41.2-44.4)	93.7 (91.2-95.6)
≥3	591 (34.8)	82.0 (77.6-85.5)	69.3 (66.1-72.3)	53.3 (50.8-55.9)	89.9 (87.7-91.7)
≥4	404 (23.8)	65.5 (60.5-70.2)	83.1 (80.5-85.5)	62.4 (58.5-66.1)	84.9 (83.0-86.6)
≥5	282 (16.6)	54.3 (49.2-59.4)	91.9 (89.9-93.6)	74.1 (69.8-78.4)	82.4 (80.8-83.9)
≥6	188 (11.1)	38.7 (33.8-43.8)	95.7 (94.1-96.9)	79.3 (73.8-84.2)	78.5 (77.1-79.8)
≥7	108 (6.4)	22.6 (18.5-27.1)	97.7 (96.5-98.6)	80.6 (72.8-87.8)	74.6 (73.6-75.7)
8	50 (2.9)	10.7 (7.8-14.2)	99.0 (98.1-99.5)	82.0 (69.2-90.3)	72.1 (71.4-72.8)

Definition of abbreviations: A2 score, Adult Asthma Epidemiological Score; CI, confidence interval; PPV, predictive positive value; NPV, predictive negative value.

DISCUSSION

This secondary analysis was the first external validation of the accuracy of the A2 score self-reported questionnaire. The A2 score showed good discriminatory power for asthma diagnosis in a Portuguese primary care population. The proposed cut-offs (scores ≥4 to rule in and scores 0-1 to rule out) were validated in this study population.

There is sparse literature on the performance of predictive scores for adult asthma. In a pilot study, the Asthma Screening Questionnaire (ASQ), an asthma screening tool, showed high sensitivity (96%) and specificity (100%) to discriminate between asthma cases and control subjects.[18] Accuracy of the A2 score could be interpreted as lower than this ASQ. However, it is important to note that the study conducted by Shin et al was based on a small sample size of 50 participants.[18] Additionally, the high accuracy reported may be attributed to the methodology used: the cases were recruited from a clinical setting so they may report more symptoms, while the controls were healthy and asymptomatic subjects, and all confounding comorbid conditions were excluded.[18] In contrast, all participants in our study were randomly recruited from primary care centers, better mimicking the performance of a screening tool in clinical practice.

Pekkanen et al used the ECRHS definitions to develop a continuous asthma score that can identify individuals for further investigation.[19] This method uses the same number of questions as the A2 score questionnaire, mainly based on symptom evaluation. However, the main difference lies in the comparator used: the ECRHS score only compares its results with bronchial hyperreactivity; while the A2 score incorporates a physician's clinical assessment that includes clinical history, physical examination, pulmonary function tests, peripheral blood counts, and PROMs. The ECRHS questionnaire was applied to the original A2 score study's data. The study reported an AUC of 86.8% (95%CI: 82.8-90.8%), a sensitivity of 87.2% (95%CI: 80.3-92.4%), and a specificity of 98.4% (95%CI: 96.7-99.3%).[12] Compared to the ECRHS questionnaire, the A2 score showed, in our sample, overlapping discrimination power (AUC 82.9%, 95%CI: 80.4-85.4%), higher sensitivity to exclude the presence of asthma (92.7%, 95%CI: 89.7%-95.1%) and low specificity to identify asthma (83.1%, 95%CI: 80.5%-85.5%).

The accuracy of A2 score is also high when compared with the accuracy of other known COPD screening tools. A systematic review found a pooled sensitivity of 64.5% (95%CI: 59.9-68.8%) and specificity of 65.2% (95%CI: 52.9-75.8%) for the CDQ.[10] In our sample, the cut-off selected to rule out asthma (scores of 0 or 1) had higher sensitivity than that reported for the CDQ (92.7% vs. 64.5% respectively), and the cut-off to rule in asthma (scores ≥4) had higher specificity (83.1% vs. 65.2% respectively).[10] The CAPTURE questionnaire had lower discrimination power than the A2 score (AUC of 79.5% vs. 82.9% respectively).[11] This case-finding questionnaire showed a sensitivity of 95.7% and a specificity of 67.8% in differentiating cases from the control subjects with no COPD.[11] Compared to the CAPTURE questionnaire's diagnostic accuracy, our validation study had slightly lower sensitivity to exclude the presence of the disease (92.7% vs. 95.7% respectively) and higher specificity to identify the presence of the disease (83.1% vs. 67.8% respectively).[11]

To select the optimal cut-off points a balance between sensitivity and specificity is necessary and should be adapted to meet the specific purposes of the score.[20] When conducting prevalence studies, it is more crucial to have a cut point with high specificity rather than sensitivity, as the focus is to rule in asthma with few false positives. However, high sensitivity is preferable when the focus is on identifying patients who are candidates for further diagnostic investigation. Therefore, we believe that a cut point with few missed cases is better suited for use as a screening tool or, in this case, to rule out asthma. For this reason, even though the cut point of 3 corresponds to the highest Youden index, we considered that a cut point of 4 or higher to rule in asthma and a cut point of less than 2 to rule out asthma as the most appropriate in our sample, validating the cut-offs proposed by the authors of this score. Moreover, they reported a specificity of 96.7% (95%CI: 94.6-98.2%) and PPV of 85% (95%CI: 76.8-90.6%) for the rule in cut-off [12], while in our sample, this cut-off showed lower specificity (83.1%, 95%CI: 80.5-85.5%) and PPV (62.4%, 95%CI: 58.5-66.1%).

According to Price et al, a PPV of at least 50% is reasonable [21], so although the PPV found in our study is lower than that reported by Sá-Sousa et al, it is still very reasonable. For the rule out

cut-off, the authors reported a sensitivity of 85.7% (95%CI: 78.6-91.2%) and a NPV of 95% (95%CI: 92.5-96.6%).[12] In our sample, this cut-off point had overlapping sensitivity (92.7%, 95%CI: 89.7-95.1%) and NPV (93.7%, 95%CI: 91.2-95.6%). The discriminative power (AUC; 95%CI) found is slightly lower than that reported by the authors (82.9%; 80.4-85.4% and 90.4%; 87.0-93.9%, respectively). These differences in the measures of diagnostic accuracy and discriminative power may be attributed to variations in symptom prevalence and asthma severity in the specific settings, as well as differences in sample sizes. In fact, our study has a considerably larger sample size compared to the original A2 score study.

This study has strengths and limitations that should be acknowledged. The large sample size recruited from the three most populated regions of the country is an important strength. However, we did not include any participants from the primary healthcare centers of southern Portugal. This study used a sample taken only from primary care, which may limit the extrapolation of results to other settings. In future studies, researchers should validate this score in other settings.

Another strength is the fact that we excluded patients with any cognitive disability that would prevent them from understanding or answering the A2 score autonomously. However, as this eligibility screening was made during a phone call interview, we cannot guarantee that all the participants fully understood the questions of the score, and this may have influenced the results. In addition, the A2 score was applied by different healthcare professionals which may also have led to small differences in the administration of the A2 score. To overcome this limitation, an interview guide was used to standardize the procedures among the interviewers. Future studies could compare the reliability of the A2 score applied as an electronic PROM and as a telephone interview.

Of note, validation against a GP clinical assessment grounded in objective measures and diagnostic tests is also a major strength. This differs from other asthma screening questionnaires, which were only validated against a physician's diagnosis [22], or based solely on lung functional tests such as spirometry and methacholine challenge test [18].

Moreover, the choice of cut-offs was not based solely on positive and negative predictive values, but also on the ROC curve performance and the Youden index, which is a strength of this study compared to other questionnaire validation studies, including the A2 score original study. The advantage of the ROC curve analysis is that since it is based on sensitivity and specificity, it is independent of disease prevalence.[17]

Conclusions

The A2 score is a simple and easily self-administered 8-question case-finding tool that has demonstrated good discriminatory power in a large primary care population of Portugal. In this validation study, the A2 score showed good diagnostic accuracy to be used in epidemiological studies, assessing the prevalence of asthma, as well as a screening tool in clinical settings, to identify individuals who would benefit from further investigation. Future studies are necessary to validate this score in different settings and countries, and to adapt the questionnaire for use in other languages and cultural contexts.

Funding: This study was sponsored and funded by AstraZeneca, Portugal

Conflict of interest: none

Author's contribution:

JC-d-S, JAF and FB conceptualized the EPI-ASTHMA study and CJ and RA conceptualized this secondary analysis. CL wrote the first draft of the manuscript. CL, CJ, and RA performed the statistical analysis. CJ, RA, FB, JC-d-S, and JAF contributed to and refined the manuscript for scientific content. JC-d-S and JAF are the guarantors. All authors read and approved the final version of the manuscript.

REFERENCES

1. Global Initiative for Asthma – GINA. Global strategy for asthma management and prevention, 2023 [Internet]. [cited 2023 Oct 18]. Available from: www.ginasthma.org
2. Wang Z, Li Y, Gao Y, Fu Y, Lin J, Lei X, et al. Global, regional, and national burden of asthma and its attributable risk factors from 1990 to 2019: a systematic analysis for the Global Burden of Disease Study 2019. Respir Res. 2023 Dec 1;24(1).
3. Rabe AP, Loke WJ, Gurjar K, Brackley A, Lucero-Prisno III DE. Global Burden of Asthma, and Its Impact on Specific Subgroups: Nasal Polyps, Allergic Rhinitis, Severe Asthma, Eosinophilic Asthma. J Asthma Allergy. 2023 Oct;16:1097–113.
4. Sá-Sousa A, Jacinto T, Azevedo LF, Morais-Almeida M, Robalo-Cordeiro C, Bugalho-Almeida A, et al. Operational definitions of asthma in recent epidemiological studies are inconsistent. Clin Transl Allergy. 2014;4:24.
5. Innes Asher M, García-Marcos L, Pearce NE, Strachan DP. Trends in worldwide asthma prevalence. Eur Respir J. 2020 Dec 1;56(6).
6. Janson C, Anto J, Burney P, Chinn S, De Marco R, Heinrich J, et al. The European Community Respiratory Health Survey: what are the main results so far? Eur Respir J. 2001;18:598–611
7. Jarvis D, Newson R, Lotvall J, Hastan D, Tomassen P, Keil T, et al. Asthma in adults and its association with chronic rhinosinusitis: The GA 2LEN survey in Europe. Allergy: European Journal of Allergy and Clinical Immunology. 2012 Jan;67(1):91–8.
8. Sembajwe G, Cifuentes M, Tak SW, Kriebel D, Gore R, Punnett L. National income, self-reported wheezing and asthma diagnosis from the World Health Survey. Eur Respir J. 2010 Feb;35(2):279–86.
9. Bousquet J, Dahl R, Khaltaev N. Global Alliance against Chronic Respiratory Diseases. Allergy: European Journal of Allergy and Clinical Immunology. 2007 Mar;62(3):216-23.

10. Haroon S, Jordan R, Takwoingi Y, Adab P. Diagnostic accuracy of screening tests for COPD: a systematic review and meta-analysis. BMJ Open. 2015 Oct 8;5(10):e008133.

11. Martinez FJ, Mannino D, Leidy NK, Malley KG, Bacci ED, Barr RG, et al. A new approach for identifying patients with undiagnosed chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2017 Mar 15;195(6):748–56.

12. Sá-Sousa A, Pereira AM, Almeida R, Araújo L, Couto M, Jacinto T, et al. Adult Asthma Scores—Development and Validation of Multivariable Scores to Identify Asthma in Surveys. Journal of Allergy and Clinical Immunology: In Practice. 2019 Jan 1;7(1):183-190.e6.

13. Jácome C, Brito D, João C, Lopes F, Santos J, Amorim L, et al. EPI-ASTHMA study protocol: a population-based multicentre stepwise study on the prevalence and characterisation of patients with asthma according to disease severity in Portugal. BMJ Open. 2022 Sep 19;12(9).

14. Fonseca JA, Nogueira-Silva L, Morais-Almeida M, Azevedo L, Sa-Sousa A, Branco-Ferreira M, et al. Validation of a questionnaire (CARAT10) to assess rhinitis and asthma in patients with asthma. Allergy: European Journal of Allergy and Clinical Immunology. 2010;65(8):1042–8.

15. Prinsen CAC, Mokkink LB, Bouter LM, Alonso J, Patrick DL, De Vet HC, et al. COSMIN guideline for systematic reviews of Patient-Reported Outcome Measures. Qual Life Res. 2018 May;27(5):1147-1157.

16. Youden WJ. Index for rating diagnostic tests. Cancer. 1950;3(1):32–5.

17. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. Caspian J Intern Med. 2013; 4(2):627–635.

18. Shin B, Cole SL, Park SJ, Ledford DK, Lockey RF. A New Symptom-Based Questionnaire for Predicting the Presence of Asthma. J Investig Allergol Clin Immunol. 2010;20(1):27-34.

19. Pekkanen J, Sunyer J, Anto JM, Burney P, Abramson M, Kutin J, et al. Operational definitions of asthma in studies on its aetiology. European Respiratory Journal. 2005;26(1):28–35.

20. Habibzadeh F, Habibzadeh P, Yadollahie M. On determining the most appropriate test cut-off value: The case of tests with continuous results. Biochem Med (Zagreb). 2016 Oct 1;26(3):297–307.

21. Price DB, Tinkelman DG, Nordyke RJ, Isonaka S, Halbert RJ. Scoring system and clinical application of COPD diagnostic questionnaires. Chest. 2006;129(6):1531–9.

22. Burney PGJ, Laitinen LA, Perdrizet S, Huckauf H, Tattersfield AE, Chinn S, et al. Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: An international comparison. European Respiratory Journal. 1989;2(10):940–5.

Figure legends

FIGURE 1 – Study flow diagram (n=1283)

FIGURE 2 – Receiver operating characteristics (ROC) curve for the A2 score

—— sum of all positive answers in the questionnaire (result score)

------- reference line

The solid line indicates the levels of sensitivity and false positive rate, for each cut-off point.

The area under the ROC curve is 0.829.

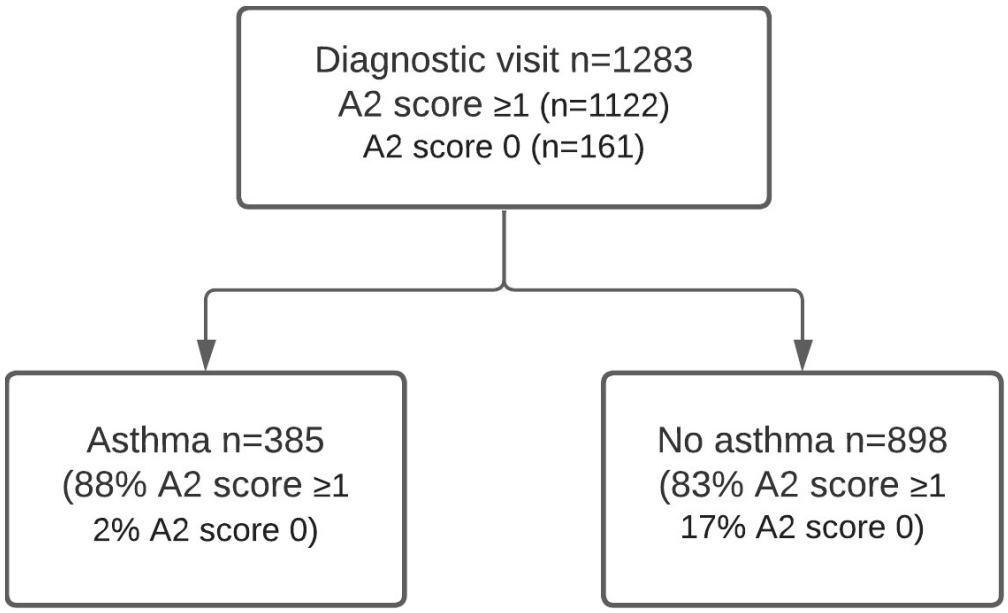
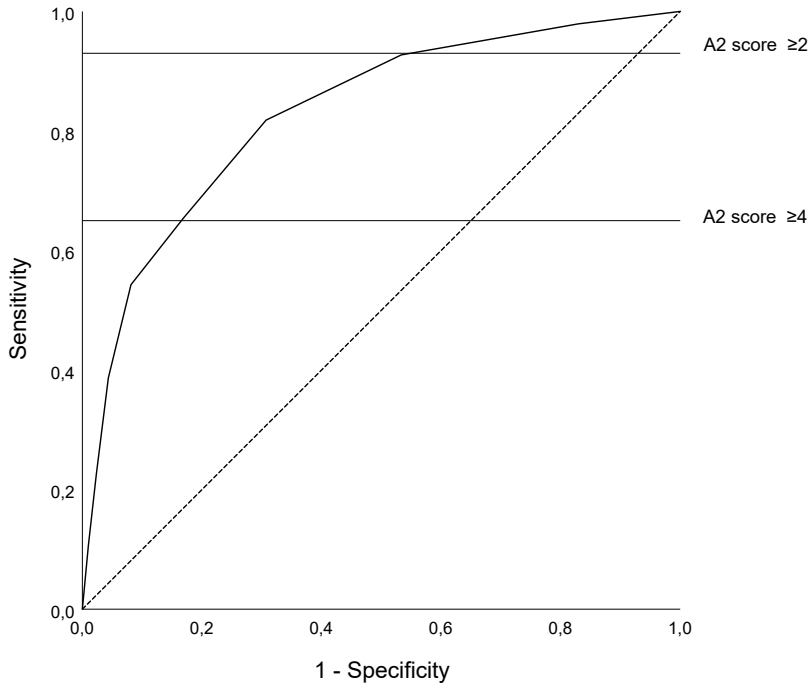


FIGURE 1 – Study flow diagram (n=1283)

95x57mm (300 x 300 DPI)



Reporting guidelines STARD (The Standards for Reporting of Diagnostic Accuracy Studies)

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Page 2, lines 13-14: "Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curves." Page 2, lines 16-17: "The A2 score showed high discriminatory power in identifying asthma, with an area under the ROC curve of 82.9(95%CI 80.4-85.4)%."
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Page 2, lines 8-9: "Design: This accuracy study is a secondary analysis of the EPI-ASTHMA"; Page 2, lines 13-14: Methods "Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curves." Page 2, lines 16-17: Results "The A2 score showed high discriminatory power in identifying asthma, with an area under the ROC curve of 82.9(95%CI 80.4-85.4)%." Page 2, lines 21-22: "Conclusions: The A2 score is a useful tool to identify patients with asthma in a primary care population."
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Page 6 "Sá Sousa et al designed and validated a patient-reported outcome measure (PROM) for asthma screening, the Adult Asthma Epidemiological Score (A2 score) […] Furthermore, it showed the ability to rule in and rule out asthma, meaning that it can be applied in prevalence studies as well as used as a screening tool."
	4	Study objectives and hypotheses	Page 6, lines 17-19: "this study aimed to validate the predictive accuracy of the A2 score against a general practitioner clinical assessment and to determine whether the proposed cut-offs are the most appropriate in this population."
METHODS			

Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	This is a prospective study. Page 7, lines 6-7: "This accuracy study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study"
Participants	**6**	Eligibility criteria	Page 8, lines 10-12 "Participants with an A2 score ≥1 were invited to a diagnostic visit undertaken in a mobile outpatient clinic. For quality control, ~5% of those with an A2 score of 0 were also invited."
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Page 7, lines 17-18: "a random sample of subjects aged ≥ 18 years who were registered in the primary care National Health Service (NHS) database"
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Page 7, lines 8-9: "data collected between May 2021 and September 2023 from 34 primary healthcare centers in the North, Center and Lisbon Metropolitan Area of Portugal"
	9	Whether participants formed a consecutive, random or convenience series	Page 7, lines 22-27: "All subjects diagnosed with asthma from the 34 participating primary care centers were included and those without asthma were randomly selected, in order to have a final sample of ~30% with asthma and ~70% without. This distribution, similar to the used in the A2 score original study, was chosen as it is known that accuracy measurements such as PPV and NPV are highly dependent on prevalence [12]."
Test methods	**10a**	Index test, in sufficient detail to allow replication	Page 8, lines 3-10: "During the interview, they answered the A2 score [12]. This score includes 8 questions: about previous physician diagnosis ("Did a physician confirm you had asthma?" and "Do you still have asthma (previously diagnosed by a physician)?"; about asthma medication intake and asthma symptoms. The resulting score for each patient is the direct sum of all positive answers, ranging from 0 to 8. The original authors suggested that asthma presence could be ruled in for scores of 4 or more (PPV of 93.3%, with 99.2% specificity and 89.4% accuracy) and ruled out for scores of 0 to 1 (NPV of 98.2%, with 93.1% sensitivity and 89.4% accuracy).[12]"
	10b	Reference standard, in sufficient detail to allow replication	Page 8, lines 12-19: "The confirmation of an asthma diagnosis was carried out by a general practitioner (GP) and was based on clinical history, physical examination, lung function tests

			(spirometry pre- and post-bronchodilator; fractional exhaled nitric oxide measurement), peripheral blood counts (eosinophil), and electronic PROMs (e.g., Control of Allergic Rhinitis and Asthma test).[14,13] Diagnosis of asthma followed GINA recommendations[1], relying primarily on the presence and pattern of respiratory symptoms (wheeze, shortness of breath, chest tightness, or cough) and supported by objective lung function findings such variable expiratory airflow limitation and high FeNO levels and other objective collected data (eosinophil)."
	11	Rationale for choosing the reference standard (if alternatives exist)	Page 8, line 16: "Diagnosis of asthma followed GINA recommendations[1]"
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	p.8 "The two cut-off points were validated by analyzing the ROC curve performance, which included calculating the Youden index (sensitivity + specificity - 1)[16,17]. Additionally, we considered the combination of PPV, NPV, sensitivity, and specificity that best suited the purpose of this score for each case"
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Not applicable. This study is a secondary analysis of the EPI-ASTHMA population-based nationwide prevalence study.
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	No
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Yes Page 8, lines 12-16: "The confirmation of an asthma diagnosis was carried out by a general practitioner (GP) and was based on clinical history, physical examination, lung function tests (spirometry pre- and post-bronchodilator; fractional exhaled nitric oxide measurement), peripheral blood counts (eosinophil), and PROMs (e.g., A2 score, Control of Allergic Rhinitis and Asthma test)."
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	p.8 "To evaluate the discriminative power of the A2 score in comparison to the physician's final diagnosis, receiver operating characteristic (ROC) curve analysis was carried out. Sensitivity, specificity, PPV, NPV, and accuracy were used as diagnostic accuracy measures."
	15	How indeterminate index test or reference standard results were handled	Not applicable. A2 score is a numerical score 0-8 and asthma diagnosis (present/absent)

	16	How missing data on the index test and reference standard were handled	No missing data.
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Not applicable. No analyses of variability included.
	18	Intended sample size and how it was determined	Not applicable. There was not an a priori sample size defined.
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	p.10 "This secondary analysis included 1283 participants (Figure 1), with a median age (p25-p75) of 54 (43-66) years old, of which 60% were females (Table I)." p.11 TABLE I
	21a	Distribution of severity of disease in those with the target condition	Not information on severity provided, only presence/absence of asthma. .
	21b	Distribution of alternative diagnoses in those without the target condition	Only information of "absense of asthma was provided by the GP, not if other conditions existed.
	22	Time interval and any clinical interventions between index test and reference standard	Page 8, lines 10-11: "Participants with an A2 score ≥1 were invited to a diagnostic visit undertaken in a mobile outpatient clinic in the following 1-3 weeks."
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	p.13 Table II
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	p.12 "The discriminatory capacity of the A2 score, summarized by the area under the ROC curve (AUC), was 82.9% (95% CI 80.4-85.4)."
	25	Any adverse events from performing the index test or the reference standard	Not applicable. Index test is a PROM answered by phone. Reference standard represents clinical practice.
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	p.16 "This study used a sample taken only from primary care, which may limit the extrapolation of results to other settings."
	27	Implications for practice, including the intended use and clinical role of the index test	p.17 "In this validation study, the A2 score showed good diagnostic accuracy to be used in epidemiological studies, assessing the prevalence of asthma, as well as a screening tool in clinical settings, to identify individuals who would benefit from further investigation"
OTHER INFORMATION			
	28	Registration number and name of registry	p.7 "This accuracy study is a secondary analysis of the EPI-ASTHMA

			population-based nationwide prevalence study (NCT05169619)"
	29	Where the full study protocol can be accessed	p.7 "Further details regarding this study can be found elsewhere.[13]"
	30	Sources of funding and other support; role of funders	p.1 "This study was sponsored and funded by AstraZeneca, Portugal"