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ORIGINAL ARTICLE

Adaptation and Validation of the Autism Mental Status Examination (AMSE) in Chile: seeking to reduce the diagnostic gap

Adaptación y Validación del Examen de Estado Mental del Autismo (AMSE) en Chile: buscando reducir la brecha diagnóstica

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What do we know about the subject matter of this study?

Timely detection of Autism Spectrum Disorder (ASD) is a priority public health challenge, especially in low- and middle-income countries. Both qualified clinical assessment and diagnostic tools with solid evidence require a high level of training, are costly and time-consuming to administer.

What does this study contribute to what is already known?

The adaptation and validation of Autism Mental Status Exam (AMSE), a brief, structured, observational and free of charge instrument for clinical use, compared with ADOS-2 is presented. The psychometric properties, capacity and accuracy demonstrated by AMSE to support the diagnostic suspicion of ASD in children and adolescents, make it a useful clinical tool for timely recognition and referral, particularly in Primary Health Care.

Abstract

Early detection of Autism is a challenge in Chile and Latin America. Gold-standard evaluations are expensive and difficult to access. The Autism Mental Status Exam (AMSE) is a brief, structured, observational instrument for clinical use with promising results. **Objective:** To adapt and validate the AMSE in a sample of Spanish-speaking children and adolescents at risk of Autism Spectrum Disorder (ASD) using the ADOS-2 as a comparison. **Subjects and Method:** Children and adolescents aged 15m-17yo consulting due to language and communication problems and/or suspected ASD. The AMSE was administered during clinical evaluation. The ADOS-2 was administered by inde-

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pendent evaluators, blinded to the study, which determined diagnostic categories as: Non-Autism Spectrum Disorder (Non-ASD), Autism Spectrum Disorder (ASD), or Autism (AUT). Psychometric characteristics of the AMSE were assessed and, using the ROC curve, a cut-off point was calculated, identifying sensitivity, specificity, positive, and negative predictive value. **Results:** Sixty-four children participated, 56 were males (87.5%), with a mean age of 6.6y (SD: 3.6). They were distributed as Non-ASD: 12 (18.8%), ASD: 19 (29.7%), and AUT: 33 (51.6%) according to the ADOS-2. The AMSE scores differed significantly among the three categories ($p<0.0001$). Internal consistency was moderate and Cronbach's alpha was 0.61. The cut-off point was ≥6 (Sensitivity 0.79, Specificity 0.92, AUC: 0.91). A second analysis according to language level suggests a greater diagnostic accuracy of the AMSE for the group with lower language development, maintaining the same optimal cut-off point (≥6). **Conclusions**: The AMSE shows adequate psychometric properties, good capacity, and accuracy to support the diagnosis of ASD. Its brevity, low cost, and easy integration into clinical practice make it an alternative with great potential for timely recognition and referral of children with ASD.

Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder, with a reported prevalence of 18.5 per 1,000 persons (one in 54) at age 8 years[1,2]. Both nuclear deficits and comorbidities associated with ASD represent important risk factors for future disabilities, affecting ASD individuals and their families, and representing a high social and economic cost[3,4].

Clinical signs, such as low eye contact or inattention to the immediate environment, which are evident in the first years of life[5] offer an opportunity for early detection of this condition and more effective intervention, in a period of rapid changes in brain development and increased neuroplasticity[6]. Current evidence shows that early and appropriate interventions contribute to reducing the core deficits of ASD and improving adaptive functioning, reducing the degree of disability[7,8].

The diagnosis of ASD can be reliably made by trained professionals at around 2 years of age, reaching stability close to 80%[9,10]. Although the age at diagnosis of ASD has shown a decreasing trend in recent years, it remains around 4 years[2], with marked disparities in relation to factors such as sex, severity of symptomatology, mental retardation/intellectual disability comorbidities, lower parental education, socioeconomic status, and lower access to education and health services[11,12].

There is little information on age at diagnosis in low- and middle-income countries. In Venezuela, it has been reported that parental concerns appear early, at 17 months, however, the age of diagnosis is 53-54 months[13]. In Chile, the first parental concerns occur at 29 months on average, but the age of diagnosis is estimated to be around 59 months, that is, thirty months later[14]. It is therefore a priority to improve the age of diagnosis and to allow early access to therapeutic interventions[15]. To this end, the American Academy of Pediatrics has recommended universal screening at 18 and 24 months of age, ideally in primary care, and seeking more accurate and culturally sensitive screening approaches[16].

The gold standard assessments for ASD diagnosis include clinical assessment according to the DSM-5 classification[17], the Autism Diagnostic Observation Schedule-second edition (ADOS-2), a semi-structured and standardized observation[18], and the Autism Diagnostic Interview-Revised (ADI-R)[19]. However, these assessments are expensive, time-consuming, and require a high level of training, which makes them less feasible to administer in routine clinical practice.

The ADOS-2 is the instrument with the highest sensitivity and specificity for the diagnosis of autism[20]. It requires to be administered by highly trained professionals and its certification costs approximately US$1,000. In addition, the instrument costs US$2,500 plus shipping expenses. An additional difficulty is the lack of adaptation and validation of the ADOS-2 for non-English-speaking low- and middle-income populations[21]. In such circumstances, clinical assessment, and especially mental examination, remain essential for diagnosis.

In the literature, there is a lack of publications on the mental examination of ASD persons. Questionnaires such as the Modified Checklist for Autism in Toddlers (M-CHAT)[22], the Social Communication Questionnaire (SCQ)[23], and the Social Responsiveness Scale (SRS)[24] are based on parental reports and do not provide the opportunity to integrate clinical observations. Therefore, for the early identification of cases, ideally in Primary Health Care, it is necessary to look for screening instruments that combine reporting and observation, that are brief and simple to administer, and that do not involve expensive training and materials[25].

The Autism Mental Status Exam (AMSE)[26] is a brief, free-to-use tool to be administered by the clini-

cian which is composed of eight items that measure the social, communicative, and behavioral functioning of persons with a suspected diagnosis of ASD. It is based on direct clinical observation and parent reports, and it was designed to address the lack of a standardized observational instrument in contexts of lower health resources. Its diagnostic capacity has been evaluated in different age groups, from 18 months to adulthood, compared with DSM-5, CARS, ADOS-2, and ICD-10, with promising results[26,27,28,29,30,31,32,33], which justify its adaptation and validation in Chile.

The objective of this study was to adapt and validate the AMSE, comparing its psychometric characteristics with the ADOS-2, an instrument considered the gold standard for the diagnosis of ASD, in Chilean children and adolescents. In addition, the study aimed at obtaining conceptual and empirical evidence of the discriminant/diagnostic capacity of the AMSE, in order to provide an observational instrument that supports timely, accurate, and efficient diagnosis of ASD, especially in low-income settings.

Subjects and Method

The participants were children and adolescents, aged between 15 months and 17 years, who consulted spontaneously or who maintained medical routine check-ups in the Departments of Child Psychiatry or Pediatric Neurology of the *Clínica Las Condes* or in the Department of Child and Adolescent Psychiatry of the Psychiatric Clinic of the University of Chile, whose reasons for consultation or check-ups were: (a) concerns in language or communication areas; (b) unusual behaviors not classified in a precise diagnosis; (c) complex cases in which the diagnosis of ASD was considered; and (d) cases with a strong clinical suspicion of ASD. Exclusion criteria were defined as the presence of severe visual or hearing deficits or diagnosis of a genetic syndrome with neuropsychiatric manifestations. Informed consent was obtained from all parents or responsible caregivers and assent was obtained from participants older than 11 years of age. The families and participants received face-to-face information about the aims and characteristics of the study and a written document containing a summary and objectives.

Based on practical assumptions made in the literature, which suggest a range of five to ten subjects per item to estimate a minimum sample value, a sample size of 64 subjects was defined[34,35]. This number is consistent with previous experiences of AMSE validation studies[28,30,32,33].

This study was approved by the Human Research Ethics Committee, Faculty of Medicine, University of Chile, and by the Research Ethics Committee of the *Clínica Las Condes*.

Instruments used

- *Autism Mental Status Exam (AMSE):* It is an instrument designed for the screening of ASD in routine clinical evaluation. The instrument structures the observation and documentation of signs and symptoms of ASD[26]. It consists of 8 items, reflecting the current conceptualization of ASD (DSM-5). It combines signs and symptoms from both diagnostic domains: (A) Social Communication/Interaction and (B) Repetitive Behaviors, Restricted and Unusual Interests, and/or Sensory Hyper/Hypo-Reactivity. Each item is assigned a score from 0 to 2, with a maximum total score of 14 points. Three items are completed by direct observation and five items by observation or report. Scoring of items 4 and 5 is for exclusive use. Table 1 shows the AMSE items and scoring guidelines. A higher score implies greater severity of autistic symptomatology. Its administration is brief and easily integrated into routine clinical care and electronic medical records. The training is available online and is free to use[36].
- *Autism Diagnostic Observation Schedule (ADOS-2):* This is a semi-structured, observational instrument that evaluates social interaction, communication, play, and behavior. It provides situations with social pressure that in a standardized context generate spontaneous behaviors associated with ASD [37]. It consists of five modules that are selected according to the language level and age of the individual. Modules T, 1, and 2 are administered from 12 months of age, in children with independent walking, from those with absence of oral language to those who do not achieve fluent language, while modules 3 and 4 are applied to children, adolescents, and adults who achieve verbal fluency[38,39]. Each module contains an algorithm made up of specific items that when summed and compared with the corresponding cut-off points result in the classification of "Autism" (AUT), "Autism Spectrum Disorder" (ASD), or "Non-Autism Spectrum Disorder" (Non-ASD)[40,41,42]. Its administration takes between 45 and 60 minutes. This instrument presents the highest sensitivity and specificity for the diagnosis of autism[20].

Procedure

In the first stage, the AMSE was translated and adapted. This process included the following steps: the translation from English to Spanish, then, the original English version and the Spanish translation were delivered to an expert with experience in reviewing and translating psychological instruments who compared the fidelity of the translation with the original, its semantic equivalence, and its suitabili-

ty for the Chilean population. Second, this translated version was reviewed by two professionals with several years of experience in ASD assessment, who evaluated the content, technical, and criteria equivalence of the instrument. A native English-speaker translator, proficient in Spanish, performed a reverse translation into English, finding no significant discrepancies between this version and the original one. Third, a pilot test was performed on a sample of 10 evaluators (psychiatrists, pediatric neurologists), confirming that the adaptation of the items did not present any type of comprehension difficulty. Finally, training and assessment of inter-rater concordance were carried out until an agreement was reached on what was observed and the scores assigned.

The validation stage of the AMSE included the recruitment of the sample, the application of the instruments, and the collection of information for the analysis of the psychometric results. During the routine clinical check-up or first consultation evaluation, the pediatric neurologist or pediatric psychiatrist proposed to the children/adolescents and their parents join the study. If they agreed, the professional obtained informed consent/assent and administered the AMSE. In the following period, no longer than one month, the ADOS-2 was administered by an independent evaluator, who was blind to the AMSE score and clinical diagnosis. All ADOS-2 administrations were performed by trained and certified raters. Recruitment and instrument administration was conducted between 2018 and 2019.

Finally, the analysis of the diagnostic ability of AMSE included: (a) descriptive statistics of both instruments, according to total scores for AMSE and diagnostic categories for ADOS-2 (AUT, ASD, Non-ASD), considering the total group and groups according to ADOS-2 modules, (b) reliability analysis of AMSE through internal consistency using Cronbach's Alpha, (c) validity analysis of AMSE through construct and concurrent criterion validity, and (d) diagnostic accuracy, referred to the ability of AMSE to discriminate between AUT, ASD, and Non-ASD categories, using the receiver operating characteristics (ROC) curve analysis, estimation of the area under the curve (AUC), cut-off point, sensitivity and specificity for the total group, and for the groups according to the ADOS-2, language level (modules T, 1, 2 and modules 3, 4)[43]. The analysis was performed using SPSS 21.

Table 1. AMSE items and scoring Guidelines (© 2011 David M. Grodberg, M.D. and The Mount Sinai School of Medicine) (36)

Item/score	0	1	2
1. Eye contact[a]	≥ 3 seconds	Fleeting (<3 seconds)	None
2. Interest in others[a]	Initiates interaction with examiner	Only passively responds	No interest
3. Pointing skilss[a]	Can point/gesture to object	Only follows point	None
4. Language[b]	Can speak about another time or place	Single words Phrases (≤ 3 words) Undeveloped sentences	Nonverbal
5.Pragmatics of Language[c]	Not impaired	Cannot manage turns or topics Unvaried or odd intonation	
		Reported	Observed
Articulation Problems[e]			
6.Repetitive Behaviors/ Stereotypy[d]	None	Compulsive-like behaviours/Insists on routines	Motor stereotypy or vocal stereotypy Echolalia Stereotyped speech
7.Unusual or encompassing preoccupations[c]	None	Present, describe:	
		Reported	Observed
8. Unusual sensitivities[c]	None	Heightened sensitivities High Pain Threshold	
		Reported	Observed

[a]Social items (items 1,2,3) must be based on the examiner observation. [b]Language item is based equally in reported or observed data. [c]Communication an Behavior (items (5, 7, 8) are based on reported or observed data. [c]Pragmatics language (item 5) is not applicable if item 4 is scored 1 or 2. [d]Repetitive Behaviors/Stereotypy (item 6) is based equally on reported or observed data. [e]Articulation Problems item does not contribute to the score.

Results

The sample consisted of 64 participants, 55 were males (86%), with a mean age of 6.4 years (SD: 3.6; range: 1.3-17; median: 5.8). The clinical diagnoses made by the pediatric neurologist or pediatric psychiatrist were: suspected diagnosis of ASD (81.3%), behavioral difficulties (48.4%), psychomotor retardation/intellectual disability (39.1%), language disorder (39.1%), attention deficit hyperactivity disorder (ADHD) (32.8%), sensory hyper/hypo-reactivity (21.9%), developmental coordination disorder (18.8%), and stereotypies (6.3%).

According to the results of the ADOS-2 administration, 51.6% of the participants were in the AUT category (n:33), 29.7% in ASD (n:19), and 18.8% in Non-ASD (n:12). Figure 1 shows the ADOS-2 modules administered and diagnostic categories obtained.

The total AMSE scores for the group studied had a mean of 6.3 (SD ± 2.4; range 2-13; median and mode: 6). Figure 2 shows the mean AMSE scores for the 3 groups according to the categories determined by the ADOS-2: for AUT: 7.42 (SD±2.41), ASD: 6.0 (SD±1.29), and Non-ASD:3.58 (SD±1.24). There were significant differences in the mean AMSE score between the three groups, according to the ADOS-2 categories Non-ASD, ASD, and AUT; Kruskal-Wallis ($p < 0.0001$), and Mann-Whitney (Non-ASD/ASD: $p<0.001$; Non-ASD/AUT: $p < 0.0001$; ASD/AUT: $p < .01$) (figure 2).

The evaluation of internal consistency was carried out by eliminating item 5 "Pragmatics of Language" since it only corresponded to be evaluated in 30 of the 64 cases. Cronbach's alpha was 0.61. When the "Encompassing Preoccupations" and "Unusual Sensitivities" items were removed, Cronbach's Alpha increased to 0.67 and 0.68, respectively.

A ROC curve analysis was performed to determine the optimal cut-off point for AMSE compared with diagnosis by ADOS-2. With a 6-point cut-off point, the curve showed an accuracy of 91%, with a sensitivity of 0.79, a specificity of 0.92, and an AUC of 0.91. The positive predictive value (PPV) was 0.98 and the negative predictive value (NPV) was 0.5 (figure 3, table 2).

Considering the 6-point cut-off point, there were eleven false negatives and one false positive. The eleven false negatives corresponded to participants in whom Module 1 (n = 1), Module 2 (n = 1), Module 3 (n = 8), and Module 4 (n = 1) were administered. The only false positive corresponded to a participant in Module 3.

Consequently, the ROC curve analysis was performed by dividing the sample according to the ADOS-2 modules administered, forming a group with less language development (module T, 1, and 2) (n = 36) and a group with better language development (module 3 and 4) (n = 28). For the first group (ADOS-2 MT, M1, M2), the ROC curve had an accuracy of 99% for a 6-point cut-off point, with a sensitivity of 0.93, a specificity of 1.0, and an AUC of 0.99 (figure 4, table 3) and for the second group (ADOS-2 M3, M4), the accuracy was 77%, keeping the cut-off point at 6, with a sensitivity of 0.59, a specificity of 0.83, and an AUC of 0.77 (figure 4, table 3).

Discussion

This study, the first to support the clinical utility of AMSE in a sample of at-risk Spanish-speaking children and adolescents, opens the real possibility of using this low-cost tool that allows a structured examination of mental status in search of signs and symptoms of ASD.

In this sample of children at risk of autism, AMSE showed an adequate discrimination capacity, with significant differences in its total score between groups, according to the ADOS-2 classification criteria. These differences are not only observed when comparing the Non-ASD group with the categories ASD and AUT but also in the comparison between the latter, raising the possibility of advancing in the study of another cut-off point that allows differentiating mild/moderate cases from severe cases, as suggested by Galdino[31].

In a blinded evaluation using the gold standard as a comparison instrument, the assessment of the efficacy and capacity of AMSE revealed that a total score of ≥ 6 points serves as the optimal cut-off point for distinguishing cases that meet ASD criteria (ASD and AUT categories of ADOS-2) from those that do not. The same cut-off point of ≥ 6 points has been reported by Grodberg for an at-risk sample without verbal fluency between 18 months and 5 years using the ADOS-2 and ADI-R as a comparison instrument, although the reported sensitivity and specificity were higher[28]. Recently, Yang et al obtained the same result in a high-risk sample of children aged 2-11 years[33]. Other studies have found cut-off points of 4 or 5 points, in samples including control groups with other neuro-developmental, anxiety, or behavioral disorders[31], neurotypical control subjects without associated symptomatology[30], or children, adolescents, and adults with higher levels of language and cognitive functioning[26,27,44].

In this study, although only one of the cases that equaled or exceeded the 6-point cut-off point did not correspond to the ADOS-2 ASD or AUT categories, the detection of eleven false negatives, nine of them with fluent language, led us to the separate ROC curve analysis of the two groups according to their language level. In both groups, the cut-off point remained at ≥ 6 points. However, we observed an increase in sensitivity and specificity in the lower language group (modules T, 1, and 2), with values that closely mirror those re-

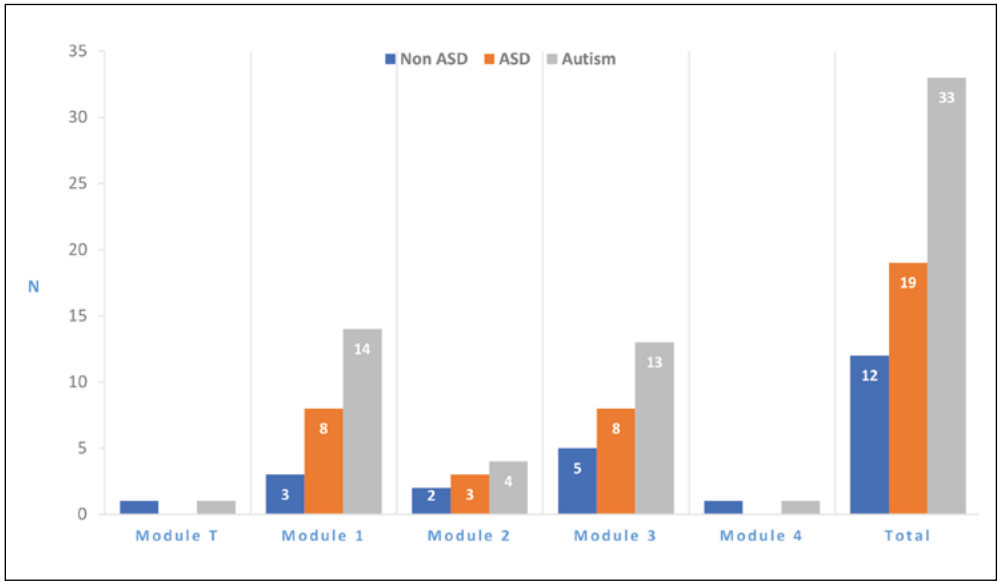

Figure 1. Diagnostic categories according to ADOS-2 modules in 64 participants

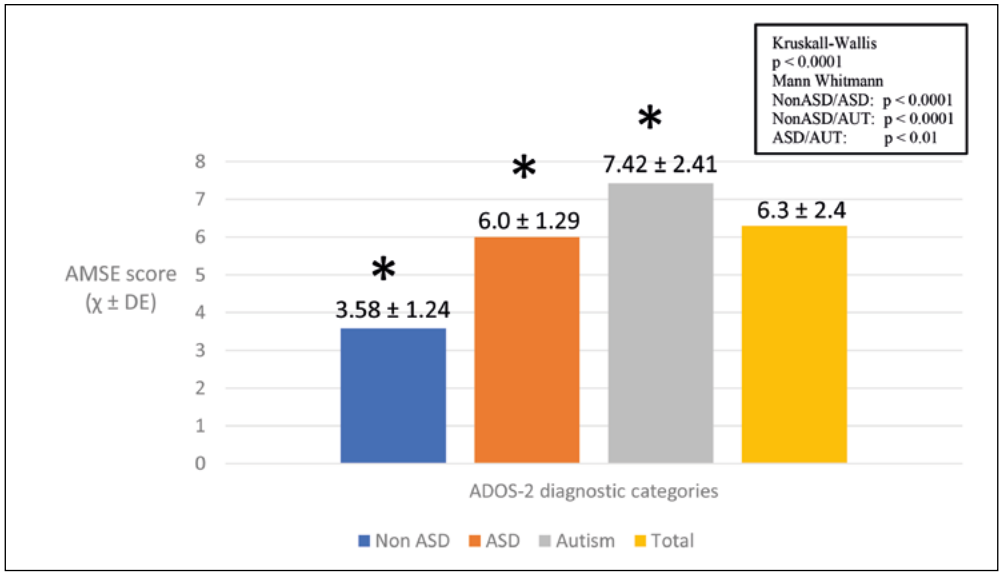

Figure 2. AMSE total scores (X±SD) according to ADOS-2 diagnostic categories in 64 participants.

ported by Grodberg in a similar group[28].

The lower diagnostic accuracy of AMSE in participants with higher language level and possibly higher level of functioning could be explained by the fact that some of the observation items that assess the communication and social interaction domain, such as "1. Eye contact" and "3. Pointing skills", offer coarse alternatives that do not allow detecting their alteration during the interview. Besides, other items that point to the domain of repetitive or restricted behaviors, such as "7. Encompassing preoccupations" and "8. Unusual sensitivities", are less frequently observed during the interview in participants without intellectual disability or good language skills, who often learn to compensate or suppress these behaviors in public[45]. Thus, they often score only 1 as "reported" rather than 2 as "observed" on these items.

These considerations raised previously in a study of high-functioning verbal adults[27] point to the possibility of re-evaluating some AMSE items according to the mentioned variables. In contrast, in non-verbal, lower-functioning individuals or young children, observable manifestations in the above items tend to be more frequent. Although the numbers of this study are insufficient to generate definitive conclusions, the data suggest

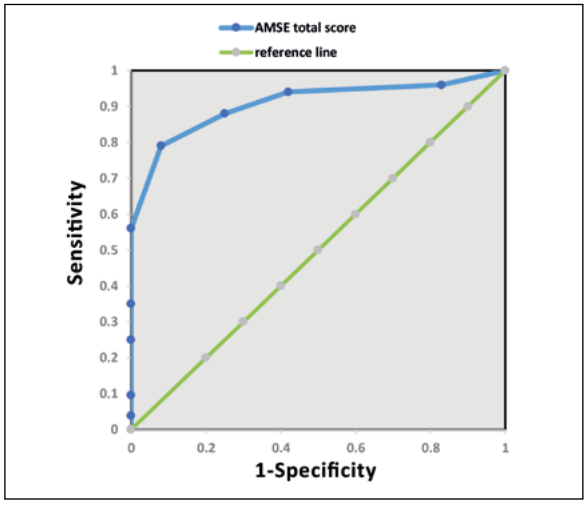

Figure 3. Receiver operating characteristic curve (ROC curve) for AMSE considering the total group assessed with ADOS-2

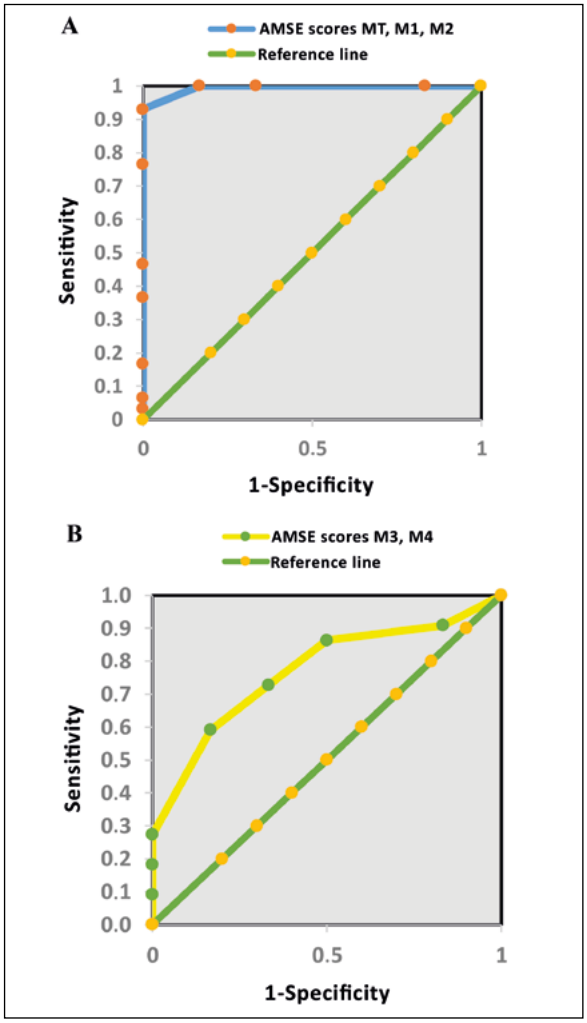

Figure 4. Receiver operating characteristic curve (ROC curve) for AMSE; Lower language group (ADOS-2: Modules T, 1 and 2) (A). Higher language group (ADOS-2: Modules 3 and 4) (B)

Table 2. Sensitivity, Specificity, PPV, NPV and cut-off points of AMSE for the total sample assessed with ADOS-2.

Cut-off points AMSE	Sensitivity	Specificity	PPV	NPV
1	-	-	-	-
2	1	0	0.81	0
3	0.96	0.17	0.83	0.5
4	0.94	0.58	0.91	0.7
5	0.88	0.75	0.94	0.6
6	0.79	0.92	0.98	0.5
7	0.56	1	1	0.34
8	0.35	1	1	0.26
9	0.25	1	1	0.235
10	0.096	1	1	0.2
11	0.038	1	1	0.19
12	-	-	-	-
13	0	1	1	0.19
14	-	-	-	-

PPV: Positive Predictive Value, NPV: Negative Predictive Value

Table 3. AMSE sensitivity, specificity and cut-off points for groups according to language level (lower language: Modules T, 1 and 2, higher language: Modules 3 and 4).

Cut-off points AMSE	Sensitivity MT,M1,M2	Specificity MT,M1,M2	Sensitivity M3. M4	Specificity M3, M4
1	-	-	-	-
2	1.00	0.00	1.00	0.00
3	1.00	0.17	0.91	0.17
4	1.00	0.67	0.86	0.50
5	1.00	0.83	0.73	0.67
6	0.93	1.00	0.59	0.83
7	0.77	1.00	0.27	1.00
8	0.47	1.00	0.18	1.00
9	0.37	1.00	0.09	1.00
10	0.17	1.00	0.00	1.00
11	0.07	1.00	-	-
12	0.03	1.00	-	-
13	0.00	1.00	-	-
14	1.00		-	-

that AMSE would be an instrument with greater diagnostic capacity and accuracy in cases with less language development, and possibly in younger age groups, versus people with high functioning and good verbal skills.

Several projections derive from this study. First, the data provided are in addition to previous works and support the early and necessary development of further research on the psychometric properties of the AMSE in broader and well-characterized groups. It is relevant to deepen the greater relative usefulness in some specific groups, for example, younger children, especially for its projections of application at the Primary Health Care level and aimed at achieving an early diagnosis. Secondly, the levels of sensitivity and specificity found depend on the reliable use of AMSE. The teams that participated in this study are experienced in the clinical evaluation of ASD and achieved 100% inter-rater accuracy. Our false negative rate of 17% emphasizes that AMSE should be administered by professionals who have experience in the diagnosis of ASD or by those who work regularly with the pediatric population and are trained in the use of the instrument, being aware that in cases of complex clinical presentation, it should not replace a more complete diagnostic evaluation. Its eventual use in pediatric consultation or Primary Care would require training in clinical aspects of ASD and the administration of the AMSE, in addition to regular supervision of its use.

Although we present an adequate AMSE classification accuracy, several limitations must be addressed. The results may be influenced by being a high-risk ASD sample recruited from two centers with specialized autism teams, and with a risk probably higher than the rest of the population. This could generate a bias towards borderline cases, with only some clinical manifestations or subtle manifestations of ASD that make a definitive clinical diagnosis difficult. In fact, both mean and mode of AMSE scores were at 6 points, 48% of cases clustered between 5-7 points, and total AMSE scores did not exceed (with 2 exceptions) a score of 10. It is possible that the study of larger samples, heterogeneous in their characteristics and severity, including participants with other developmental difficulties, or as complements to population screening in healthy controls, may generate AMSE scores with a distribution closer to normal so that the shape of the ROC curve reflects a more gradual change in sensitivity and specificity as the cut-off point approaches or moves away from a score of 6. Importantly, while the nature of the cohort would modify the shape of the ROC curve, it would not affect the ultimate clinical utility of the 6-point cut-off point. In addition, the sample studied is relatively small, with a wide age range, and belongs to middle- and high socioeconomic levels, which limits the projections of the results, requiring future studies examining the use of AMSE in larger samples stratified according to age, level of cognitive and adaptive functioning, and socioeconomic level.

Conclusions

This study shows that AMSE offers a standardized, observational diagnostic assessment that can quickly and accurately support the diagnosis of ASD in children and adolescents consulting due to concerns related to or suspected of ASD, psychomotor retardation, learning difficulties, attention difficulties, language disorders, and/or behavioral difficulties. The results encourage the prompt development of new studies aimed at implementing the administration of AMSE in Primary Health Care and disadvantaged contexts, contributing to an earlier diagnosis and rapid referral to treatment, which counteracts the large gap that currently exists, limiting the opportunities to achieve the best development and level of adaptive functioning.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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