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Original Article

Longitudinal study for the early detection of autism in children with very preterm birth

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ABSTRACT

Introduction: Very preterm birth is an important risk factor for autism spectrum disorder (ASD). The aim of this study is the early detection of ASD risk, using a follow-up protocol, in children weighing less than 1500 g at birth or born before 32 weeks of gestation.
Methods: This is a prospective longitudinal study in which a total of 133 very premature babies were monitored to the age of 2 years with the M-CHAT autism screening test and, in the event of a positive result, the Autism Diagnostic Observation Schedule (ADOS-2).
Results: 53 cases (4 out of 10) screened positive, and the rest negative. Among the positives, the ADOS-2 was administered in 50 cases, of which 24 scored above the ASD cutoff point. The average age of detection was 25.39 months. The results suggest an estimated prevalence of ASD in the very premature population of 18.46 %.
Conclusions: The application of the follow-up protocol in the very premature population is effective for early detection of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a neurological development disorder characterised, according to the Diagnostic and Statistical Manual of Mental Disorders [1], by deficits in communication and social interaction, and restrictive and repetitive behaviour patterns. Recent publications indicate that while the current prevalence is around 2.7 % in the general population [2], this is increasing, making it a global public health issue [3]. However, premature babies are particularly vulnerable to neurological morbidity. This vulnerability increases as gestational age and birth weight decrease [4], with ASD prevalence considerably higher, up to 6 %, in the premature population [5], and up to 20.8 % in the very premature population (less than 32 weeks of gestation) [6].

Due to the strong association between extreme prematurity and the risk of ASD, the Spanish Neonatology Society [7] recommends applying a follow-up protocol for children up to 32 weeks of gestation and/or up to a birth weight of 1500 g. This consists of common action guidelines that aim to standardise activities according to good practice criteria, ideally up to 7 years of age, or even until adolescence [7]. The protocol recommends specialised ASD screening with the Modified Checklist for Autism in Toddlers (M-CHAT) for all premature babies between 18 and 24 months of age and, if positive, performing a formal assessment.

Currently, the age of ASD detection varies a great deal, although a recent meta-analysis places it at an average of 60 months [8]. Lowering the age at detection to 24 months is essential, as early interventions can improve developmental and social adaptation prospects for people with ASD [9]. The main goal of our study is to identify subjects at high risk of ASD around 24 months of age using the Spanish Neonatology Society follow-up protocol [7] for children weighing less than 1500 g at birth or born before 32 weeks of gestation. As a secondary objective, we aim to determine the estimated prevalence of ASD in very premature babies.

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2. Material and methods

2.1. Study design and participants

This is a prospective longitudinal study in which premature babies were followed up from birth until 4 years old. The study was approved by the Clinical Research Ethics Committee at Vall d'Hebron Hospital, Barcelona in October 2019.

Between 2019 and 2020, neonates born before 32 weeks gestation or weighing less than 1500 g at birth and admitted to the hospital's Neonatal Intensive Care Unit (NICU), were included in the study. Children with severe motor impairment were excluded.

Taking the findings of previous meta-analyses [10,11] into account, potentially relevant prenatal, perinatal, and postnatal data were collected during admission (Table 1). Subsequently, the data were supplemented with the hospital discharge report and recorded in a comprehensive database. At 24 months, the M-CHAT-R/F was administered to the parents or primary caregivers face-to face at the hospital or, in some cases, by telephone due to restrictions in place during the COVID-19 pandemic. Children who screened positive for ASD were formally assessed using the ADOS-2 T Module at the hospital by a researcher clinically certified in ADOS with extensive clinical experience. They were recorded on video, with the prior authorisation of the families, and scores for all 41 items were obtained. The range of concern was established and a written report with the results and intervention recommendations was given to the parents. Finally, subjects who screened negative were monitored and assessed at 48 months with the Childhood Autism Spectrum Test (CAST) with the aim of detecting possible false negatives. Informed consent was obtained individually from the parents or guardians of all study participants.

Table 1
Characteristics of the study population.

Variables	Number of participants (%)
Gestational age (days), mean (SD)	204.96 (19.58)
Birth weight (grams), mean (SD)	1139.89 (343.77)
Sex: male	68 (51.13)
Mother born abroad	67 (50.38)
Maternal age at pregnancy (years), mean (SD)	33.48 (5.55)
Paternal age at pregnancy (years), mean (SD)	36.22 (6.86)
Substance misuse - Mother (including tobacco and alcohol)	18 (13.53)
Substance misuse - Father (including tobacco)	28 (21.37)
Mother's education: up to secondary	44 (33.08)
Father's education: up to secondary	50 (38.17)
Assisted reproductive technology	38 (28.57)
Dystocic delivery	111 (83.46)
Length of stay in NICU (days), mean (SD)	69.55 (36.02)
Twins	54 (40.60)
Preeclampsia	32 (24.06)
Incomplete corticosteroid maturation	21 (15.79)
IUGR	38 (28.57)
Chorioamnionitis	38 (28.57)
Abnormal Presentation (breech, transverse, buttocks)	59 (44.36)
Apgar 5 min < 7 ($n = 131$)	33 (25.19)
Apgar 10 min < 7 ($n = 118$)	9 (7.63)
Umbilical artery pH <7.20 ($n = 103$)	9 (8.74)
Umbilical vein pH <7.28 ($n = 108$)	50 (46.30)
Length (centimetres), mean (SD)	36.88 (4.14)
Head circumference (centimetres), mean (SD)	26.23 (2.88)
Severe CNS abnormality	23 (17.29)
Ductus	31 (23.30)
BPD	37 (27.82)
ROP ≥ 2	26 (19.55)
IVH ≥ 2	15 (11.28)

Abbreviations: CNS = Central Nervous System. BPD = Bronchopulmonary Dysplasia. ROP = Retinopathy of Prematurity. IVH = Intraventricular Haemorrhage. IUGR = Intrauterine Growth Restriction.

2.2. Definition of the study variables

2.2.1. Modified Checklist for Autism with Follow-Up Interview (M-CHAT-R/F)

The M-CHAT [12] is an autism screening questionnaire applicable from 16 to 30 months of age. It consists of 23 items and the interview is considered to be a screen positive if the child fails any two items on the follow-up. The M-CHAT-R/F includes a follow-up interview [13] that significantly improves the predictive values of ASD [14], with a pooled sensitivity of 83 % and a pooled specificity of 94 % [15].

2.2.2. Autism Diagnostic Observation Schedule (ADOS-2)

The ADOS-2 [16] is a structured and standardised assessment tool for autism through direct observation of the subjects' behaviour while they perform different activities. The Toddler Module [17] is designed to assess children between 12 and 30 months. It collects scores on 41 items, based on language, social communication and relevant behaviours, and establishes three ranges of concern: from little-to-no, from mild-to-moderate, and from moderate-to-severe. For the clinical diagnosis of autism, the most accurate cutoff is 12 points in nonverbal children and 10 points in children with some words. The area under the curve (AUC) of these cutoff points is 0.92 and 0.96 respectively, indicating excellent diagnostic validity [18].

2.2.3. Childhood Autism Spectrum Test (CAST)

The CAST [19] is an ASD screening tool applicable from 48 months onwards consisting of 37 questions addressed to parents or caregivers. In the Spanish version, the most accurate cutoff for a positive screen is set at 15 points, with a sensitivity of 83.9 % and a specificity of 92.5 % [20].

2.3. Statistical analysis

2.3.1. Descriptive statistics

The R programming language version 4.2.2 (R Core Team, 2022) was used for statistical analysis, and descriptive statistics were performed to summarise the characteristics of the sample, as well as to present measures of central tendency, dispersion, and frequency of the variables of interest.

2.3.2. Justification of sample size

For the determination of sample size, we performed power statistical calculations using the function pwr.p.test in R. These analyses demonstrated that our sample was adequate to identify a significant difference in the prevalence of ASD in very premature babies. With a power of 90 % and a significance level of 5 %, we assumed as a unilateral hypothesis that this prevalence exceeds that of the general population. This provided a solid basis and increased the robustness of our statistical findings.

3. Results

3.1. Characteristics of the participants

A total of 133 neonates weighing less than 1500 g or born before 32 weeks of gestation were included. Fig. 1 shows the study flow diagram.

Of the 133 infants, 68 were male and 65 were female, with a gestational age of 204.96 ± 19.58 days and a birth weight of 1139.89 ± 343.77 g. All the characteristics of the participants are listed in Table 1.

3.2. M-CHAT-R/F

The M-CHAT-R/F screening of 133 very premature infants resulted in 80 (60.15 %) negatives and 53 (39.85 %) positives. Of the negatives, 38 were girls (47.5 %) and 42 were boys (52.5 %); of the positives, the distribution was 27 girls (50.94 %) and 26 boys (49.06 %). The average chronological age at test administration was 22.72 ± 3.01 months, and

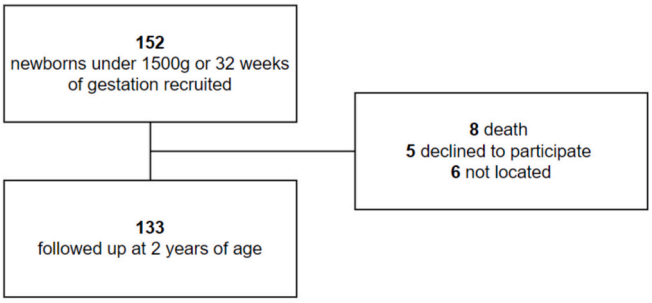

Fig. 1. Participants.

20.22 ± 3.06 months corrected age.

3.3. ADOS-2

Of the 53 cases identified as positive, the assessment was completed with ADOS-2 Module T in 50 (94.34 %) (Fig. 2), 35 of which were administered to preverbal children or children with few words, and 15 to children with regular use of some words. There were 3 total losses, all of them male children, which will be considered for the calculation of descriptive statistics from this point onwards, reducing the total sample from 133 to 130 subjects. On average, the test was applied at 25.39 ± 3.78 months in chronological age, or 22.73 ± 3.64 months in corrected age.

3.3.1. Ranges of concern

The results of the 50 evaluable ADOS-2 cases showed that 20 subjects (40 %) fell within the range of concern from little-to-no, 7 (14 %) from mild-to-moderate, and 23 (46 %) from moderate-to-severe (Table 2).

Thus, of the total sample, 15.38 % obtained a range from little-to-no, 5.38 % from mild-to-moderate, and 17.69 % from moderate-to-severe concern. Regarding sex differences, of the total number of girls in the sample, 20 % are in the lowest concern range, 7.70 % in the intermediate range, and 13.85 % in the highest concern range. In contrast, of the total number of boys, the percentages are 10.29 %, 2.94 %, and 20.59 % respectively.

3.3.2. ASD cutoff point

Of the 50 subjects assessed, subjects exceeded the most accurate cutoff point for the diagnosis of ASD, which represents 18.46 % of the total sample, while the remaining 26 were not at risk for ASD. This group scores lower on the M-CHAT-R/F, with a mean of 3.92 ± 3.39, compared

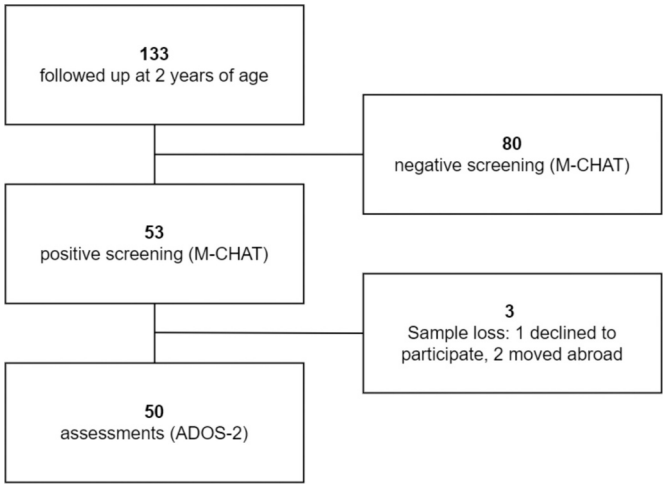

Fig. 2. General scheme of M-CHAT administration and ADOS-2T Module.

Table 2
ADOS-2 module T results (%) classified by gender and rank of concern.

	Little-to-no concern	Mild to moderate concern	Moderate to severe concern
Boys	7	2	14
Girls	13	5	9
Total	20 (40 %)	7 (14 %)	23 (46 %)

to children who exceed the cut-off point, with a mean of 8.75 ± 4.31. By sex, 14 boys (21.54 % of the total) and 10 girls (15.38 % of the total) are above the cutoff (Table 3).

3.4. CAST

Only 36 responses were obtained in the follow-up of negative screenings with the CAST, (45 % of the sample), 34 of which confirmed the negative result and the remaining 2 cases were positive, both with borderline scores (exactly 15 points). According to these data, 94.44 % of the subjects without ASD were correctly screened with the M-CHAT-R/F.

4. Discussion

This study was designed to detect ASD early through the implementation of a follow-up protocol for very premature babies in the healthcare system, as multiple previous studies identified prematurity and low birth weight as risk factors for neurodevelopmental disorders. The results suggest, as hypothesised, that the application of the follow-up protocol is effective, as it allows the identification of subjects with a high probability of ASD diagnosis around the age of two years, reducing the average age of detection found in the literature by about three years [8]. Interventions commencing before the age of 3 can have a greater positive impact than those initiated after the age of 5 [21], making early diagnosis (at 18–24 months) crucial in improving outcomes in children with ASD, especially in cognitive, linguistic and socio-emotional functioning [22]. It is important to note that early intervention has positive effects not only on the individual but also on society, and it is ethical that all children have access to such programmes [9]. The public health system should provide support for diagnosis and treatment [2].

The results of our study suggest that 26 subjects, corresponding to 52 % of the sample who screened positive, are false positives. This is in line with previous findings indicating that positive results on the M-CHAT occur more frequently in very premature infants than in those born at term [23], due to cognitive, visual, motor or auditory deficits.

However, regarding subjects who screened negative and were reassessed at 48 months with the CAST, results showed that 94.4 % received a new negative result. As such, the data suggest, in accordance with previous studies [15], that the M-CHAT-R/F is a good screening tool, especially for detecting negative cases. Nonetheless, since only 45 % of the families responded to the CAST, these results must be interpreted with caution.

To estimate the prevalence of ASD in our sample, we used the cut-off point from the ADOS-2 Module for young children as it has demonstrated excellent clinical validity [18]. The results suggest that 24 subjects are at high risk of ASD diagnosis, representing an estimated prevalence of 18.46 % in very premature babies. This is consistent with the prevalence found in other studies with very premature populations

Table 3
Results of the 50 ADOS-2 Module T assessments by cut-off point.

	Preverbal children with a score ≥ 12 ($N = 35$)	Verbal children with a score ≥ 10 ($N = 15$)
Boys	12	2
Girls	9	1
Total	21	3

[6], significantly higher than that found in premature populations and the general population [2]. However, a complete assessment in later stages is required to confirm the diagnoses and verify the prevalence in our sample.

Our study results revealed sex differences regarding proportion and phenotype. With respect to the ratios between the sexes, there are differences depending on diagnostic criteria. Specifically, when considering the highest concern range, the ratio of boys to girls is 1.8:1. However, at the cutoff point for the diagnosis of ASD [18], the ratio is slightly more even at 1.4:1. These results are similar to those found in previous publications [6] and seem to indicate a more balanced sex ratio in the very premature population than in the general population, which is 3:1, respectively [24].

As for phenotype, the results obtained with the ADOS-2 Module T indicate that it is more severe in males than in females, as the majority of the boys evaluated (60.87 %) are in the most severe concern range, while the majority of the girls (48.15 %) are in the lowest concern range. This differs from the general population, in which girls diagnosed with ASD present more severe symptoms than boys [25]. On the whole, it is postulated that girls require a higher genetic load to be diagnosed with ASD, hence there is a lower prevalence but greater severity [26]. We hypothesis that prematurity, as an environmental factor, impacts the brain development of girls and boys in different ways from the genetic factors implied in children with mainly primary ASD. Further research on these differences is warranted.

Some limitations of the study should be considered when interpreting the results. Firstly, although the sample size calculation indicated that the number of subjects was adequate, future studies should examine larger samples to enhance statistical power and representativeness. Moreover, as indicated in the Spanish protocol, appropriate follow-up until adolescence [7] and a thorough assessment of ASD to confirm the diagnosis would be advisable. Although the precision of the ADOS-2 cutoff point is high [18], it is possible that some cases may eventually present altered neurodevelopment without meeting the criteria for ASD. Lastly, only 45 % of the families responded in the follow-up of subjects who screened negative. The low response may have been due to parents' relief at receiving a negative result.

The present study has laid the groundwork for a range of future research areas. Exploring the interactions between the variables in Table 1 and the risk of ASD is essential. Detailed analysis of patient backgrounds could help identify premature infants at high risk of developing ASD. This knowledge would inform families of potential risks when leaving the NICU, aiding in the early detection of ASD.

Knowledge on the early signs of ASD in premature newborns is scarce and this is the first prospective longitudinal study on the matter [27]. This design allows the earlier detection of warning signs, which is essential for designing individualised interventions before a formal diagnosis, and better understanding their phenotype. Moreover, in our sample, there is an equitable representation of the sexes, which reinforces the validity of our findings in relation to differences between boys and girls. Lastly, the administration of the M-CHAT and the ADOS-2 was carried out by the same rater in all cases, which helps to ensure rater reliability.

The main strength of our study lies in the fact that the implementation of a follow-up protocol in very premature babies, with a particularly high prevalence of ASD, could lower the age of detection of the disorder to around 24 months, which is essential for early diagnosis and intervention. These would significantly improve the developmental prospects and quality of life for people with ASD.

Author contributions

All authors contributed to the study design and implementation, to the analysis of results and to the drafting of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] American Psychiatric Association. Diagnostic and statistical manual of mental disorders [Internet]. In: DSM-5-TR. American Psychiatric Association Publishing; 2022. https://doi.org/10.1176/appi.books.9780890425787 [cited 2024 Apr 28]. Available from:.
[2] Maenner MJ, Warren Z, Williams AR, Amoakohene E, Bakian AV, Bilder DA, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2020. MMWR Surveill Summ 2023;72:1–14.
[3] Li YA, Chen ZJ, Li XD, Gu MH, Xia N, Gong C, et al. Epidemiology of autism spectrum disorders: global burden of disease 2019 and bibliometric analysis of risk factors. Front Pediatr 2022;10:972809.
[4] Soria S, Leire E, Junqué C. Prematuridad y bajo peso al nacer. In: Neuropsicología pediátrica. "Prematurity and low birth weight" [translated from Spanish by the present author] Madrid: Editorial Síntesis; 2015. p. 63–82. Spanish.
[5] Laverty C, Surtees A, O'Sullivan R, Sutherland D, Jones C, Richards C. The prevalence and profile of autism in individuals born preterm: a systematic review and meta-analysis. J Neurodev Disord 2021;13:41.
[6] Nagai Y, Mizutani Y, Nomura K, Uemura O, Saitoh S, Iwata O. Diagnostic rate of autism spectrum disorder in a high-survival cohort of children born very preterm: a cross-sectional study. Int J Dev Neurosci 2022;82:188–95.
[7] Pallás Alonso C, García González P, Jimenez Moya A, Loureiro González B, Martín Peinador Y, Soriano Faura J, et al. Follow-up protocol for newborns of birthweight less than 1500 g or less than 32 weeks gestation. An Pediatr (Engl Ed) 2018;88:229.e1–229.e10.
[8] Van 'T Hof M, Tisseur C, Van Berckelear-Onnes I, Van Nieuwenhuyzen A, Daniels AM, Deen M, et al. Age at autism spectrum disorder diagnosis: A systematic review and meta-analysis from 2012 to 2019. Autism 2021;25:862–73.
[9] Koegel LK, Koegel RL, Ashbaugh K, Bradshaw J. The importance of early identification and intervention for children with or at risk for autism spectrum disorders. Int J Speech Lang Pathol 2014;16:50–6.
[10] Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics 2011;128:344–55.
[11] Wang C, Geng H, Liu W, Zhang G. Prenatal, perinatal, and postnatal factors associated with autism: a meta-analysis. Medicine (Baltimore) 2017;96:e6696.
[12] Robins DL, Fein D, Barton ML, Green JA. The modified checklist for autism in toddlers: an initial study investigating the early detection of autism and pervasive developmental disorders. J Autism Dev Disord 2001;31:131–44.
[13] Robins DL, Casagrande K, Barton M, Chen CMA, Dumont-Mathieu T, Fein D. Validation of the modified checklist for autism in toddlers, revised with follow-up (M-CHAT-R/F). Pediatrics 2014;133:37–45.
[14] Guy A, Seaton SE, Boyle EM, Draper ES, Field DJ, Manktelow BN, et al. Infants born late/moderately preterm are at increased risk for a positive autism screen at 2 years of age. J Pediatr 2015;166:269–275.e3.
[15] Wieckowski AT, Williams LN, Rando J, Lyall K, Robins DL. Sensitivity and specificity of the modified checklist for autism in toddlers (original and revised): a systematic review and Meta-analysis. JAMA Pediatr 2023;177:373.
[16] Lord C, Rutter M, Goode S, Heemsbergen J, Jordan H, Mawhood L, et al. Autism diagnostic observation schedule: a standardized observation of communicative and social behavior. J Autism Dev Disord 1989;19:185–212.
[17] Luyster R, Gotham K, Guthrie W, Coffing M, Petrak R, Pierce K, et al. The autism diagnostic observation schedule—toddler module: a new module of a standardized diagnostic measure for autism spectrum disorders. J Autism Dev Disord 2009;39:1305–20.
[18] Hong JS, Singh V, Kalb L, Ashkar A, Landa R. Replication study of ADOS-2 toddler module cut-off scores for autism spectrum disorder classification. Autism Res 2021;14:1284–95.
[19] Scott FJ, Baron-Cohen S, Bolton P, Brayne C. The CAST (childhood asperger syndrome test): preliminary development of a UK screen for mainstream primary-school-age children. Autism 2002;6:9–31.
[20] Morales-Hidalgo P, Roigé-Castellví J, Vigil-Colet A, Canals Sans J. The childhood autism spectrum test (CAST): Spanish adaptation and validation. Autism Res 2017;10:1491–8.
[21] Zwaigenbaum L, Bauman ML, Fein D, Pierce K, Buie T, Davis PA, et al. Early screening of autism spectrum disorder: recommendations for practice and research. Pediatrics 2015;136:S41–59.
[22] Okoye C, Obialo-Ibeawuchi CM, Obajeun OA, Sarwar S, Tawfik C, Waleed MS, et al. Early diagnosis of autism spectrum disorder: a review and analysis of the risks and benefits. Cureus 2023;15:e43226.

[23] Gray PH, Edwards DM, O'Callaghan MJ, Gibbons K. Screening for autism spectrum disorder in very preterm infants during early childhood. Early Hum Dev 2015;91:271–6.
[24] Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2017;56:466–74.
[25] Dworzynski K, Ronald A, Bolton P, Happé F. How different are girls and boys above and below the diagnostic threshold for autism spectrum disorders? J Am Acad Child Adolesc Psychiatry 2012;51:788–97.
[26] Ferri SL, Abel T, Brodkin ES. Sex differences in autism spectrum disorder: a review. Curr Psychiatry Rep 2018;20:9.
[27] Hernandez-Fabian A, Canal-Bedia R, Magan-Maganto M, de la Fuente G, Ruiz-Ayucar de la Vega I, Bejarano-Martin A, et al. Autism spectrum disorder and prematurity: towards a prospective screening program. Rev Neurol 2018;66:S25–9. Spanish.