Extremely preterm birth affects boys more and socio-economic and neonatal variables pose sex-specific risks

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ABSTRACT

Aim: The early identification of at-risk extremely preterm (EPT) children could improve long-term outcomes. This study sought to investigate sex differences in developmental outcomes and to identify sex-specific predictors at two and a half years of age.

Methods: We assessed 217 boys and 181 girls born before 27-week gestation using the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III), as a part of the Extremely Preterm Infants in Sweden Study. Sex-specific differences were calculated. Socio-economic, birth and neonatal factors were calculated separately for boys and girls using regression models.

Results: Girls scored significantly higher than boys on all Bayley-III indices. In both sexes, brain injury, long-term ventilator treatment and foreign-born mothers predicted lower scores. Receiving breast milk by hospital discharge predicted higher scores. Severe retinopathy of prematurity was the strongest predictor of cognitive and language deficits in boys. High parental education predicted higher cognitive and language scores in girls, whereas severe bronchopulmonary dysplasia was the strongest predictor of motor deficits.

Conclusion: Extreme prematurity seems to affect boys more than girls. Socio-economic and neonatal factors confer similar risks or protections on both sexes, but some variables pose sex-specific risks. An awareness of risk factors may provide the basis for treatment and follow-up guidelines.

INTRODUCTION

The survival of children born extremely preterm (EPT) has increased over the last few decades. EPT birth is associated with developmental delays, such as cognitive, language and motor deficits, as well as behavioural difficulties and attention problems during preschool and school years (1). Several studies have sought to identify predictors of these children's developmental outcomes. Neonatal risk factors for adverse outcomes include brain injury, such as intraventricular haemorrhage (IVH) and periventricular leukomalacia (2–4); bronchopulmonary dysplasia (BPD), long-term oxygen dependency (5,6) and intrauterine growth retardation (2). Socio-demographic factors, such as low parental educational background and low maternal age, are also associated with neurocognitive outcome (5,7–9). The absence of major neonatal morbidities, short neonatal hospitalisation, high socio-economic status and feeding with breast milk are all positively associated with good developmental outcomes (2,10,11). Male sex has been identified in many studies as a universal risk factor for neonatal complications and developmental delay. Boys have increased risks for brain injury and respiratory problems. Motor, cognitive, and communicative deficits and sensory impairments are more frequent among EPT boys than EPT girls (12,13).

Few studies have examined sex-specific predictors of neurodevelopmental outcome. Hintz et al. (14) investigated whether associations between certain perinatal factors and neurodevelopmental outcomes at 18–22 months differed with respect to gender in a group of extremely low-birthweight infants. They found several minor effects, although they could not explain the preterm male disadvantage. In a subcohort of the Extremely Preterm Infants in Sweden Study (EXPRESS), boys were found to be at greater risk than girls for moderate to severe disabilities at two and a half years of age (15). Sex differences could not be fully

Key notes

- Early identification of at-risk extremely preterm (EPT) children is critical and may improve long-term outcomes.
- EPT girls scored significantly higher than EPT boys on all indices of the Bayley Scales of Infant and Toddler Development, third edition, at a corrected age of two and a half years, and sex-specific predictors of these outcomes were identified.
- Awareness of sex-specific risk factors is important for anticipating developmental outcomes and special needs among preterm boys and girls.
explained by perinatal risk factors or by the delayed myelination that was observed more frequently in boys. Findings like these suggest that preterm boys are at an inherently greater risk for adverse outcomes. Certain domain specificity between risk factors and outcome has been suggested (16,17). Neonatal complications, such as cerebral injuries, retinopathy of prematurity (ROP) and prolonged duration of mechanical ventilation, are more critical determinants of motor impairments than social risk factors (16,18), whereas socio-economic disadvantages are associated with language difficulties and behavioural problems (19,20). However, language development is influenced by several neonatal risk factors as well, including BPD, days on ventilator treatment and feeding difficulties (21,22).

The aims of the present study were to investigate sex differences in cognitive, language and motor outcome among children born extremely preterm and to identify sex-specific predictors of developmental functioning at a corrected age of two and a half years. Our report is part of the population-based EXPRESS study, which includes all infants born before 27 gestational weeks in Sweden during a 3-year period. Data on survival after 1 year, prevalence of neonatal morbidities and neurodevelopmental outcome at a corrected age of two and a half years have been published (23–25). Early identification of at-risk EPT children is critical, because early diagnosis and intervention may improve long-term outcomes. By identifying early sex-specific determinants, clinicians may be better able to anticipate developmental outcomes and special needs among preterm boys and girls.

METHOD
Participants
All extremely preterm live-born infants (gestational age <27 weeks) and stillborn infants (gestational age 22 to <27 weeks) in Sweden from April 1, 2004 to March 31, 2007 were included, providing an incidence of 0.33% of all births (23). Data collection details are presented in Figure 1, partly adopted from the study by Serenius et al. (25). Children who survived to 1 year of age were recruited through a parent or guardian to follow-up at a corrected age of two and a half years (23,24). Information on children whose families declined participation has been published elsewhere (25). No significant differences were observed between the assessed children and those lost to follow-up in mean birthweight, gestational age, rate of male sex, severe neonatal morbidity (IVH ≥ 3), periventricular leukomalacia, ROP ≥ 3, necrotising enterocolitis (NEC), or severe BPD, foreign-born mothers and parental education level at the EPT birth.

Socio-economic factors
The socio-economic factors included were maternal age at delivery, parental education and foreign-born mother, mostly from the Middle East, North African, former East European and Nordic countries. Parental education was expressed as the highest academic achievement of either the mother or the father and was dichotomised into two categories: (i) <14 years, including 9 years of compulsory school and high school graduation, and (ii) ≥14 years, including a minimum 2-year university diploma. Parental education is a commonly used indicator for socio-economic

Figure 1 Follow-up from birth to two and a half years at a corrected age for the EXPRESS. Children included in the present study.
status, as are variables indicating ethnicity, such as first-generation immigrant status (26).

**Infant characteristics and postnatal events**

Predictors hypothesised to effect preterm outcomes, based on empirical findings in previous studies, were selected from the extensive EXPRESS database. Gestational age was expressed as completed weeks, based on ultrasound dating in 95% of the pregnancies (24). Infants with a birthweight of more than two standard deviation (SD) scores below the national mean were classified as small for gestational age (SGA). Neonatal morbidities were selected as in previous studies (23,24): severe ROP phase ≥3; severe BPD, oxygen dependence at an age corresponding to 56 weeks of gestational age; severe brain injury, including IVH grade ≥3, and periventricular leukomalacia. NEC was included as a binary variable, indicating NEC stage ≥2 (= 1) or NEC stage <2 (= 0), as was the variable mechanical ventilation >14 days (infant needed ventilation for >14 days = 1; ≤14 days = 0) – the same variable used by Neubauer, Voss and Kattner in their follow-up of ELBW infants (4). Breast milk ingestion was graded as full, partial or none by the time of hospital discharge.

**Developmental assessment**

The developmental examination was conducted using the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) (27). The Bayley scales have been widely used internationally for follow-up after preterm birth during infancy and early preschool years. The Bayley-III cognitive index assesses sensorimotor development, concept formation and memory. The language index is composed of the distinct receptive communication and expressive communication subtests. Receptive communication measures word comprehension, the ability to respond correctly to requests, and the understanding of morphological markers; the expressive communication scale measures preverbal communication, vocabulary and syntactic development. The motor index consists of the fine motor and gross motor subtests. The fine motor subtest assesses manipulation of objects and functional hand skills; the gross motor subtest assesses static positioning, movement of limbs and balance.

Norm-referenced scaled scores can be calculated for the Bayley-III subtests. The scaled scores are derived from the total raw scores and range from one to 19, with subtest means of 10 and SDs of three. Norm-referenced composite scores for the indices are derived from the sums of the subtest scale scores (a composite score equivalent is available for the cognitive index scale) and range from 40 to 160, with a mean of 100 and SD of 15. The Bayley-III has not yet been standardised in Sweden.

The Bayley-III has been reported to have psychometric weaknesses that can affect scores. Anderson et al. (28) found that using norm-referenced scores resulted in unexpectedly low rates of delay in a group of ELBW/EPT children. When using control group scores as reference, the proportions of delay rose to expectations. In the EXPRESS, EPT test scores have consistently been evaluated on the basis of the means and SDs of the controls (25,29). However, the inclusion of control group data is not relevant to the present analyses.

**Procedure**

Participating children were assessed by licensed psychologists at seven perinatal centres around Sweden. Prior to data collection, the psychologists were trained in the Bayley-III instrument by one of the authors (KS). The Bayley-III assessment was included in a clinical follow-up that was sometimes performed by the psychologist familiar to the infant. Thus, the examiners were not blinded to whether the child was born EPT or full term. Follow-up ended during 2010 when the last child in the cohort reached two and a half years. The study was approved by the Regional Ethics Review Board at Lund University (Dnr 469/2007). All parents provided written consent for the examination.

**Statistical analysis**

Student’s t-tests were used for comparisons of continuous data, and chi-square tests for comparisons of categorical data. To compare sex differences across the Bayley-III scores, effect sizes were calculated in terms of Cohen’s $d$, using the difference between two means divided by the pooled SD of those means. Cohen’s guidelines were followed to indicate the strength of the effect sizes, with a Cohen’s $d$ of 0.20, 0.50 and 0.80 referring to small, medium and large effect sizes, respectively (30). Bivariate correlation analyses were used to identify significant associations between the predictor variables and the Bayley-III composite scores.

To study the effect of socio-economic factors and neonatal data on the Bayley-III composite scores, hierarchical regression analyses were performed as described by Munk et al. (3). The variables were entered into the regression model in temporal order: socio-economic factors, infant characteristics at birth and postnatal events. In the first step of regression analysis, maternal age, foreign-born mother and parental education were entered as independent variables. Missing values in the parental education variable were included as a separate variable (parental education value missing). Gestational age and SGA were entered in the second step, and neonatal conditions (severe ROP, severe BPD, severe brain injury, NEC, mechanical ventilation >14 days, diet fully/partly breast milk) were added in the final step. Three equations were calculated using each of the Bayley-III composite scores as dependent variable. As they were performed for girls and boys separately, a total of six equations were performed. The IBM SPSS Statistics 22 (Armonk, NY, USA) was used for the analyses. A p-value of <0.05 was considered statistically significant.

**RESULTS**

There were no differences in the socio-economic data, infant characteristics, postnatal events and morbidities or
sensorimotor impairments of the boys and the girls. Data are presented in Table 1. Descriptive data regarding Bayley-III are presented in Table 2. Girls performed significantly better than boys in the cognitive, receptive, expressive communication and fine motor scale scores as well as in language and motor function composite scores of Bayley-III. The examiners were not able to successfully administer all subtests because of the child’s failure to understand Swedish (n = 5), the child’s inattention or fatigue (n = 2), cerebral palsy in the child (n = 6) or other motor difficulties (n = 1). Statistically significant differences were identified between boys and girls on the cognitive, receptive communication, expressive communication and fine motor scale scores, as well as in language and motor function composite scores. Effect sizes were small to medium, with the largest effect for fine motor performances. Bivariate correlation analyses identified factors that were associated with the Bayley-III cognitive, language and motor composite scores. Results are presented in Table S1.

Table S2 illustrates unstandardised coefficients (B), standardised beta weights (β), for each of the predictor variables, as well as the $R^2$, $R^2$ change ($ΔR^2$) and standard error (SE) for the final step of the regression analyses, separately for the boys and the girls.

For the EPT boys, severe ROP was the only variable that contributed significantly to their Bayley-III cognitive composite score ($\beta = -0.158$, $p = 0.031$). The total model accounted for 16.0% of the total score variation ($R^2 = 0.160$, $p = 0.005$). For the EPT girls, foreign-born mother ($\beta = -0.184$, $p = 0.011$), parental education ≥14 years ($\beta = 0.189$, $p = 0.020$), severe brain injury ($\beta = -0.168$, $p = 0.020$), mechanical ventilation >14 days ($\beta = -0.185$, $p = 0.037$) and breast milk (partly) at discharge ($\beta = 0.216$, $p = 0.006$) contributed significantly to their Bayley-III cognitive composite score. The total model accounted for 24.6% of the total score variation ($R^2 = 0.246$, $p = 0.000$).

For the EPT boys, foreign-born mother ($\beta = -0.162$, $p = 0.012$), parental education missing ($\beta = 0.090$, $p = 0.254$), severe ROP ($\beta = -0.231$, $p = 0.001$), mechanical ventilation >14 days ($\beta = -0.170$, $p = 0.026$) and breast milk (fully) at discharge ($\beta = 0.147$, $p = 0.045$) were significantly related to the Bayley-III language composite score. The total model accounted for 27.0% of the total score variation ($R^2 = 0.270$, $p = 0.00$). For the EPT girls, foreign-born mother ($\beta = -0.327$, $p = 0.000$) and parental education ≥14 years ($\beta = 0.188$, $p = 0.023$) were significantly related to the Bayley-III language composite score. The total model accounted for 25.5% of the total score variation ($R^2 = 0.235$, $p = 0.050$).

For the EPT boys, severe brain injury ($\beta = -0.138$, $p = 0.047$) and mechanical ventilation >14 days ($\beta = -0.214$, $p = 0.008$) contributed to the Bayley-III motor composite score. The total model accounted for 18.9% of the total score variation ($R^2 = 0.189$, $p = 0.002$). For the EPT girls, severe BPD ($\beta = -0.241$, $p = 0.002$) and severe brain injury ($\beta = -0.178$, $p = 0.019$) contributed to the Bayley-III motor composite score. The total model accounted for 19.6% of the total score variation ($R^2 = 0.196$, $p = 0.002$). All the steps of the hierarchical analyses are presented in Table S3 in the supplementary material.
DISCUSSION

This study examined differences in cognitive, language and motor functions, operationalised by Bayley-III scores, between boys and girls born EPT at a corrected age of two and a half years. Girls outperformed boys, scoring higher in the majority of the Bayley-III scores. No between-sex differences were found in the data on socio-economics, infant birth-characteristics, postnatal events and morbidities, or rates of sensorimotor impairments. It is therefore unlikely that these factors were responsible for the sex differences observed. Our findings concur with those of Peacock et al. (13) and Hintz et al. (14), as they emphasise the constitutional difference between boys and girls, with a specific male vulnerability causing the morbidities associated with EPT birth to have a greater effect, leading to worse outcomes in EPT boys. The present findings may also indicate a developmental lag in EPT boys in relation to girls at this early age, which underscores the need for further investigations during school years.

A detailed examination of the Bayley-III results showed that the statistically significant difference in fine motor functioning constituted the sex difference in the composite motor functioning scores. This points to the importance of the detailed characterisation of development facilitated by the Bayley-III assessment. The separation of fine and gross motor functioning and of receptive and expressive communicative skills allows a fine-tuned mapping of functions.

We found that both neonatal and socio-economic variables contributed significantly to variances on the Bayley-III cognitive and language scores and neonatal variables alone contributed to motor score variances. This result is congruent with other studies showing domain specificity in the associations between predictors and outcome (6). Gestational age was no longer a statistically significant predictor of developmental functioning after adding the neonatal variables to the model (Table S2). This suggests that the effects of the neonatal variables on developmental outcome exceeded the effect of immaturity.

Severe ROP contributed uniquely to the cognitive and language score variances in EPT boys, but not in girls. ROP is well known to be associated with neurosensory impairments and visual problems such as strabismus and refractive errors. Less is known about its distinct effect on mental outcomes. Despite being born EPT, the EXPRESS cohort’s prevalence of visual impairment is similar to that in previous Swedish cohorts of children born less prematurely. No visual impairment was recorded in 96% of the boys and 98% of the girls. The negative effect of ROP on cognitive and language development in EPT boys may be a consequence of its effect on the central nervous system rather than on visual acuity, and the adverse neurological disturbances caused by ROP may affect boys more than girls.

In the present study, BPD was associated with adverse motor outcome in girls, but not in boys. Wood et al. for the EPICure study (6) described how infants with chronic oxygen dependency experienced episodes of hypoxia, causing disturbances in neuronal organisation, myelination and consequent mental and psychomotor deficiencies. High levels of oxygen and long-term ventilator treatment are associated with BPD, a well-known risk factor for developmental delay in EPT children. Similar to the EPICure study, we found that indicators of respiratory dysfunction related to lower Bayley scores. Mechanical ventilation treatment >14 days was negatively associated with cognitive scores in girls and language and motor scores in boys. Along with reflecting high frequencies of apnoea and pulmonary deficiency, long-term ventilation could be considered a proxy for severity of illness or general weakness during the neonatal period.

The presence of severe brain injury contributed significant variance to the cognitive composite score in EPT girls. In this study, children with severe sensorimotor impairments and cerebral palsy were excluded from analyses. Still, brain injury predicted lower motor composite scores in both sexes. The relationship between cerebral abnormalities and developmental deficiencies has been documented in several studies. In the EPICure, it was demonstrated that white-matter damages were significantly related to cognitive delays and psychomotor impairments in preterm preschoolers (6), echoing findings in earlier EXPRESS subcohort studies using neonatal magnetic resonance imaging at two and a half years (15).
Consistent with previous reports (10), feeding with breast milk appeared as a protective factor in cognitive outcome in EPT girls and language outcome in EPT boys. Breast milk seems to contribute to more optimal development in several ways. The interaction between mother and child during nursing is positively associated with cognitive development and providing breast milk – not necessarily through nursing – may reflect maternal features such as parental ambition and attachment. Mothers providing breast milk for their children may be more socio-economically advantaged than mothers who do not (11). Also, the specific nutrients in breast milk have been shown to be significantly beneficial to neurological outcomes in preterm children (10). In our analyses, both breast-fed children and children receiving breast milk by tube or bottle were included. Therefore, the possibility that this socio-economic effect could be explained only by the relational act of breast-feeding was ruled out. Still, the variable is complex and more controlled investigations are warranted. Longer duration of breast milk ingestion is related to greater developmental advantages (10). We found that EPT girls fed even partially with breast milk by discharge had higher cognitive scores than infants fed only formula, and boys fed entirely on breast milk had higher language scores than formula-fed infants. We conclude that neonatal care should encourage feeding with breast milk and support mothers who prefer to nurse their EPT infants, but that the dose-response effect of breast milk ingestion needs further investigation.

Having a foreign-born mother was negatively associated with cognitive performance in girls and with language performance in both girls and boys. Walch et al. (9) found that parental bilingualism was associated with adverse cognitive and language development in children born with very low birthweight. In this study, data on bilingual background were not available, although it may be a central feature of the foreign-born mother variable. Parental education ≥14 years was related to higher cognitive and language scores in girls and we found a negative association between language score and the parental education missing variable, which we were unable to explore further, in boys. Our findings concur with others showing that having a parent with post-high school education was associated with higher developmental scores. Parents with higher education may provide a more intellectually stimulating environment for their children, use a more varied vocabulary and read more to their children than parents with limited academic achievements (8,26). Studies show that delayed development in EPT children occurs more often in socio-economically disadvantaged families. The negative effects may be explained by limited material and social resources and stress induced by everyday life conditions (5,26). On the basis of our findings, we agree with Wong and Edwards (26), who emphasised the importance of examining socio-economic status indicators as mediators of the cognitive development of preterm children. Social factors may become increasingly important predictors of developmental outcome as EPT children grow older (5,7). As this study included children at an early preschool age, an increasing impact of social factors in future EXPRESS follow-ups would be expected. Unlike other studies, we found no significant association between maternal age and mental development (5,26).

Mean values of our preterm group approached the normative means of 10 for scaled scores and 100 for composite scores. The EPT girls’ means were actually above the normative means on the language and fine motor scales. On the basis of the results of earlier EXPRESS publications using control group means and SDs as reference values, we conclude that these results are explained by the psychometric weaknesses of the Bayley-III reported earlier (28,29).

A potential limitation of the present study is that the total regression models modestly contributed to the Bayley-III total score variance. This indicates that we may have failed to include important variables as predictors, although we chose potential predictors based on the empirical findings of previous studies. It may also reflect the complexity of establishing the determinants and causes of EPT outcomes. In the research field of EPT sequelae, logistic regression analyses are often used to examine independent factors of categorical outcomes such as cognitive disability (2). As we used a hierarchical regression strategy to predict continuous Bayley-III scores, comparisons of results may be complicated. However, our results were similar to Munck et al. (5).

CONCLUSION
In our setting, EPT girls scored significantly higher than EPT boys on Bayley-III cognitive, receptive communication, expressive communication and fine motor subscales, as well as in language and motor function composite scores. There were some similarities between the sexes in the prediction of outcomes. Severe brain injury, long-term ventilator treatment and having a foreign-born mother predicted lower Bayley-III scores in both sexes; having received breast milk by hospital discharge predicted higher Bayley-III scores in both sexes. There were three apparent sex-specific predictors. Severe ROP was the strongest predictor of lower cognitive and language performances in boys, but not in girls, and severe BPD was the strongest predictor of poorer motor performance in girls, but not in boys. High parental education predicted higher cognitive and language scores in girls, but not in boys. We conclude that extreme prematurity seems to affect boys more than girls, indicating a specific male vulnerability. Socio-economic and neonatal factors confer similar incremental risks or protections to both sexes, but some variables pose certain risks for boys and for girls, respectively. Awareness of sex-specific risk factors is important for providing a basis for treatment and follow-up guidelines.

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Additional Supporting Information may be found in the online version of this article:

**Table S1** Univariate associations of socio-economic, birth characteristic and neonatal variables with Bayley-III composite scores.

**Table S2** The final step in the hierarchical regression analyses, performed to study sex-specific predictors of the Bayley-III cognitive, language, and motor index scale scores.

**Table S3** Results from hierarchical regression analyses, performed to study sex-specific predictors of the Bayley-III cognitive, language, and motor index scale scores. All steps included.