Using cluster analysis to describe phenotypical heterogeneity in extremely preterm infants: a retrospective whole-population study

Theodore Dassios, Emma E Williams, Christopher Harris, Anne Greenough

ABSTRACT

Objective To use cluster analysis to identify discrete phenotypic groups of extremely preterm infants.

Design Secondary analysis of a retrospective whole-population study.

Setting All neonatal units in England between 2014 and 2019.

Participants Infants live-born at less than 28 weeks of gestation and admitted to a neonatal unit.

Interventions K-means cluster analysis was performed with the gestational age, Apgar score at 5 min and duration of mechanical ventilation as input variables.

Primary and secondary outcome measures Bronchopulmonary dysplasia, discharge on home oxygen, intraventricular haemorrhage, death before discharge from neonatal care.

Results Ten thousand one hundred and ninety-seven infants (53% male) were classified into four clusters: Cluster 1 contained infants with intermediate gestation and duration of ventilation and an intermediate mortality and incidence of bronchopulmonary dysplasia. Cluster 2 contained infants with the highest gestation, a shorter duration of ventilation and the lowest mortality. Cluster 3 contained infants with the lowest Apgar score and highest mortality and incidence of intraventricular haemorrhage. Cluster 4 contained infants with the lowest gestation, longest duration of ventilation and highest incidence of bronchopulmonary dysplasia.

Conclusion Clinical parameters can classify extremely preterm infants into discrete phenotypic groups with differing subsequent neonatal outcomes.

INTRODUCTION

Preterm birth is a major cause of childhood morbidity and mortality with a global incidence of approximately 10.6%.

Infants born at less than 28 completed weeks of gestation (extremely preterm) suffer significant multi-system morbidity lasting into adolescence and adulthood, with important financial implications for health systems and an increased physical, emotional and financial burden for the families. The survival of these infants has increased over the past decades and the associated implications for patients and families are only expected to increase in the future.

Although many extremely preterm infants suffer significant perinatal and long-term morbidity, there is substantial variation in the severity of the neonatal outcomes with a sizeable proportion escaping major morbidity. For example, we have recently reported the overall mortality and incidence of bronchopulmonary dysplasia (BPD) of extremely preterm infants in England to be 18.9% and 57.5%, respectively, which means that 81.1% of those infants survived to discharge and 23.6% were free of BPD. Although the multifactorial origin of adverse neonatal outcomes has been well described, the heterogeneity in neonatal outcomes is poorly understood.

The use of unsupervised learning based on big-data analytics has been used in the clinical domain for the prediction of individual risk factors and for clinical decision support. Unsupervised learning could also be helpful...
to identify different phenotypic groups (clusters) corresponding to distinct clinical phenotypes of extreme preterm infants. Cluster analysis as a method of detecting subgroups within multidimensional data sets could also be helpful in identifying subgroups of patients that might benefit from targeted interventions. To our knowledge, such unsupervised learning approaches have not been previously employed to describe different phenotypic groups in extreme prematurity.

We aimed to derive and describe clusters of extremely preterm infants based on readily available clinical information and to explore whether these distinct phenotypes were associated with different neonatal outcomes.

MATERIALS AND METHODS

Study design and participants

All infants live-born before 28 completed weeks of gestation and admitted to a neonatal unit in England between 1 January 2014 and 1 January 2019 (study period) were included. This was an analysis of data previously acquired to investigate the relationship of growth impairment with the development of BPD in a retrospective, whole-population study. A predefined data set was acquired from the National Neonatal Research Database (NNRD), Imperial College London, UK. As the study used data held in an existing database, participation did not require approval from individual Trusts, but only from the NHS Trust holding the database (Chelsea and Westminster NHS Foundation Trust) which was obtained.

The following variables were collected: maternal age (years), administration of antenatal steroids (yes/no), gestational age at birth calculated from the last menstrual period or ultrasonographically (weeks), birth weight (kg), Apgar score at 5 min of age, sex (male/female), duration of invasive ventilation (days), BPD development defined as any need for respiratory support at 36 weeks postmenstrual age (PMA), administration of postnatal corticosteroids (defined as parenteral administration of dexamethasone or hydrocortisone for more than 5 consecutive days—yes/no), surgical intervention for necrotising enterocolitis (yes/no), surgical ligation of patent ductus arteriosus (yes/no), intraventricular haemorrhage (IVH) grade 3–4 (yes/no), periventricular leucomalacia (yes/no), death before discharge from neonatal care (yes/no), age at death (days), weight at 36 weeks PMA (kg), PMA at discharge (weeks), weight at discharge (kg), discharged on home oxygen (yes/no). We calculated the birth weight z-score (AWz) using the UK-WHO preterm reference chart and the Microsoft Excel add-in LMS Growth (V.2.77; www.healthforchildren.co.uk). The birth weight z-score was not calculated for infants born <23 completed weeks of gestation, as there were no reference data.

PPI statement

This is a retrospective study, so patients and public could not be involved.

Analysis

Cluster analysis was performed with the smallest possible number of continuous variables necessary to adequately characterise neonatal outcomes, since inclusion of a large number of variables might degrade the final classification. To our knowledge, such unsupervised learning approaches have not been previously employed to describe different phenotypic groups in extreme prematurity.

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the input parameters in the four clusters along with the birth weight a-score, the duration of PN and the PMA age at discharge are presented in figure 4.

Cluster 1 (intermediate) contained 27% of the population. The infants of cluster 1 had a lower median (IQR) gestational age (24.8 (24.1–25.2) weeks) compared with clusters 2 and 3 (27.0 (26.6–27.6) weeks and 25.4 (24.4–26.4) weeks respectively, p<0.001 for both). The median Apgar score for infants of cluster 1 was higher compared with the infants of clusters 3 and 4 (p<0.001 for both), but not of cluster 2 infants. Infants in cluster 1 were ventilated for a median (IQR) period of 21 (10–33) days, significantly longer that infants of cluster 2 and 3 (4 (2–10) and 9 (3–21) days, respectively, p<0.001 for both) but significantly shorter than the infants of cluster 4 (60 (52–73) days, p<0.001). Infants of cluster 1 developed more often BPD (59%) compared with infants of clusters 2 and 4 (48% and 89%, respectively, p<0.001) but less often than infants of cluster 3 (42%, p<0.001). Infants of cluster 1 had lower mortality (27%) compared with cluster 3 (40%, p<0.001) but higher mortality compared with cluster 2 (8%, p<0.001).

Cluster 2 (favourable) contained 47% of the population and the infants of this cluster had the highest median (IQR) gestational age (27.0 (26.6–27.6) weeks) compared with groups 1, 3 (25.4 (24.4–26.4) weeks, p<0.001) and 4 (24.4 (23.9–25.3) weeks, p<0.001). The median Apgar score for infants of cluster 2 was higher compared with the infants of clusters 3 and 4 (p<0.001 for both). Infants in cluster 2 had the shorter duration of ventilation with a median (IQR) period of 4 (2–10) days, compared with infants of cluster 1 (21 (10–33) days), cluster 3 (9 (3–21) days) and cluster 4 (60 (52–73) days, p<0.001 for both). Infants of cluster 2 developed less often BPD (48%) compared with infants of clusters 1 and 4 (59% and 89%, respectively, p<0.001 for both), but more often than infants of cluster 3 (42%, p<0.001). Infants of cluster 2 had the lowest mortality (8%) compared with all other clusters (p<0.001).

Cluster 3 (low Apgar) contained 17% of the population and the infants of this group had the lowest median (IQR) Apgar score (4 (2–5)) compared with all other groups (p<0.001 for all). Their median (IQR) gestation (25.4 (24.4–26.4) weeks) was higher than clusters 1 and 4, but lower than cluster 2 (p<0.001 for all). Infants of cluster 3 had a shorter median (IQR) duration of ventilation (9 (3–21) days), compared with infants of clusters 1 and 4 (p<0.001 for both), but longer than infants of cluster 2 (p<0.001). Infants of cluster 3 had the lower incidence of BPD (42%) compared with infants of clusters 1, 2 and 4 (59%, 48% and 89%, respectively, p<0.001 for all). Infants of cluster 3 had the highest mortality (40%) compared with all other clusters (p<0.001).
Table 1  Subject characteristics by cluster

<table>
<thead>
<tr>
<th></th>
<th>Whole population</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=10,197</td>
<td>N=2782</td>
<td>N=4773</td>
<td>N=1777</td>
<td>N=865</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>31 (26–35)</td>
<td>31 (26–35)</td>
<td>31 (26–35)</td>
<td>31 (26–35)</td>
<td>31 (26–35)</td>
<td>0.352</td>
</tr>
<tr>
<td>Tertiary neonatal unit at birth</td>
<td>7223 (71)</td>
<td>2093 (75)</td>
<td>3255 (68)</td>
<td>1180 (66)</td>
<td>695 (80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>9154 (90)</td>
<td>2501 (90)</td>
<td>4.371 (92)</td>
<td>1515 (85)</td>
<td>767 (89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>26.0 (24.9–27.1)</td>
<td>24.8 (24.1–25.4)</td>
<td>27.0 (26.6–27.6)</td>
<td>25.4 (24.4–26.4)</td>
<td>24.4 (23.9–25.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>5441 (53)</td>
<td>1464 (53)</td>
<td>2508 (53)</td>
<td>1022 (58)</td>
<td>491 (57)</td>
<td>0.001</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>7 (6–9)</td>
<td>8 (7–9)</td>
<td>8 (7–9)</td>
<td>4 (2–5)</td>
<td>6 (5–8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>0.81 (0.68–0.96)</td>
<td>0.70 (0.62–0.79)</td>
<td>0.94 (0.83–1.05)</td>
<td>0.77 (0.65–0.90)</td>
<td>0.64 (0.57–0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight z-score</td>
<td>−0.38 (-0.89–0.07)</td>
<td>−0.44 (-0.87 to -0.02)</td>
<td>−0.30 (-0.86 to 1.05)</td>
<td>−0.39 (-0.87 to 0.05)</td>
<td>−0.61 (-1.10 to -0.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of ventilation (days)</td>
<td>10 (3–26)</td>
<td>21 (10–33)</td>
<td>4 (2–10)</td>
<td>9 (3–21)</td>
<td>60 (52–73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postnatal corticosteroids</td>
<td>1995 (20)</td>
<td>771 (28)</td>
<td>338 (7)</td>
<td>287 (16)</td>
<td>599 (69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of PN (days)</td>
<td>15 (9–25)</td>
<td>17 (10–28)</td>
<td>13 (9–20)</td>
<td>13 (5–22)</td>
<td>36 (20–61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BPD at 36 weeks</td>
<td>5431 (53)</td>
<td>1629 (59)</td>
<td>2280 (48)</td>
<td>750 (42)</td>
<td>772 (89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Home oxygen</td>
<td>3010 (30)</td>
<td>937 (34)</td>
<td>1245 (26)</td>
<td>441 (25)</td>
<td>387 (45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Died before discharge</td>
<td>1985 (19)</td>
<td>753 (27)</td>
<td>366 (8)</td>
<td>705 (40)</td>
<td>161 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at death (days)</td>
<td>7 (2–25)</td>
<td>10 (3–24)</td>
<td>7 (2–23)</td>
<td>2 (1–10)</td>
<td>91 (60–144)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ROP treatment</td>
<td>1197 (12)</td>
<td>502 (18)</td>
<td>219 (5)</td>
<td>151 (8)</td>
<td>325 (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDA Ligation</td>
<td>389 (4)</td>
<td>134 (5)</td>
<td>62 (1)</td>
<td>40 (2)</td>
<td>153 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVH grade III–IV</td>
<td>1654 (16)</td>
<td>614 (22)</td>
<td>421 (9)</td>
<td>420 (24)</td>
<td>199 (23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PVL</td>
<td>399 (4)</td>
<td>110 (4)</td>
<td>143 (3)</td>
<td>60 (3)</td>
<td>86 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Operated for NEC</td>
<td>582 (6)</td>
<td>208 (7)</td>
<td>152 (3)</td>
<td>72 (4)</td>
<td>150 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight at 36 weeks (kg)</td>
<td>2.15 (1.89–2.41)</td>
<td>2.12 (1.88–2.38)</td>
<td>2.20 (1.95–2.45)</td>
<td>2.20 (1.93–2.47)</td>
<td>1.90 (1.63–2.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PMa at discharge†</td>
<td>39.6 (37.6–42.1)</td>
<td>40.7 (38.7–43.1)</td>
<td>38.5 (36.9–40.4)</td>
<td>40.0 (38.1–42.3)</td>
<td>44.9 (41.9–49.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Discharge weight (kg)†</td>
<td>2.68 (2.26–3.24)</td>
<td>2.85 (2.40–3.38)</td>
<td>2.50 (2.16–2.98)</td>
<td>2.80 (2.35–3.35)</td>
<td>3.41 (2.82–4.17)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR) or N (%).

*Comparison between clusters 1 and 4.
†Data for the alive at discharge only.

BPD, bronchopulmonary dysplasia; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; PMa, postmenstrual age; PN, parenteral nutrition; PVL, periventricular leucomalacia; ROP, retinopathy of prematurity.
Cluster 4 (prolonged ventilation) had the smallest number of subjects (8%), the lowest gestational age (24.4 (23.9–25.3) weeks) and the longest median (IQR) duration of ventilation (60 (52–73) days) compared with all other clusters (p<0.001 for all). The median Apgar score for infants of cluster 4 was 6 (5–8), which was higher compared with the infants of cluster 3 (p<0.001) but lower than clusters 1 and 2 infants (p<0.001 for both).

**Figure 2** Radial plots showing the clinical profiles for the four clusters. Data are standardised (expressed as z-scores referenced to the whole cohort) and the spike points represent medians.

**Figure 3** Discriminant two-dimensional plot of the clustering solution. The cluster centroids are presented as black circles.
Infants of cluster 4 had the highest rate of BPD (89%) compared with infants of all other clusters (p<0.001 for all). The mortality of the infants of cluster 4 (19%) was lower than clusters 1 and 3 (p<0.001 for both) and higher than cluster 2 (p<0.001).

DISCUSSION

Using unsupervised learning to detect and describe distinct phenotypical groups of extremely preterm infants, we demonstrated that survival to discharge from neonatal care and other important neonatal outcomes differed significantly across these groups. We used readily available parameters to classify the extremely preterm infants into the four clusters.

Cluster 1 (intermediate) comprised infants with a combination of a low gestational age and a relatively long duration of invasive ventilation whose resuscitation was overall straightforward as demonstrated by a median Apgar of 8 at 5 min after birth. Nevertheless, they had a relatively high mortality and incidence of BPD. Cluster 2 (favourable) was the largest group and exhibited the best outcomes. It contained infants with the highest gestational age and birth weight z-score and the briefest duration of ventilation. Not surprisingly, they also had the lowest mortality and a relatively modest incidence of complications such as BPD. They were also the earliest to be discharged from neonatal care. The major clustering characteristic of the infants in cluster 3 was the low Apgar score. The poor condition at birth might explain why they had the highest mortality and rate of IVH compared with all other groups, but their relatively larger gestation and brief duration of ventilation might explain why they had the lowest incidence of BPD. Cluster 4 (prolonged ventilation) consisted of the most premature infants, with the longest duration of mechanical ventilation and highest rates of BPD. These infants were also discharged home at a later PMA than all other cluster infants.

Our results highlight two distinct within-cluster associations. First, poor condition at birth with subsequent high rates of mortality and IVH (cluster 3) and, second, the association of prolonged ventilation with BPD (cluster 4). Both these associations have been previously individually described in population studies: Jensen et al studied 3343 extremely low-birth weight infants and reported that among the survivors, exposure to a greater number of mechanical ventilation episodes was associated with a progressive increase in the risk of BPD. Interestingly, the highest mortality in our study was not in the infants of the lower gestation at birth, but rather in the ones with the lowest Apgar score. The association of poor condition at birth with IVH and increased mortality is primarily attributed to the intrinsic fragility of the germinal matrix vasculature and the disturbance in the cerebral blood flow. It is also interesting to note that the infants of cluster 3, other than the highest mortality and lowest Apgar score, also had the lowest rate of antenatal steroids and the lowest incidence of birth in a tertiary centre. This is in agreement with previous studies that have
highlighted that delivery outside a tertiary centre is associated with higher mortality and a lower uptake of antenatal steroids.26 Our study complements the literature by presenting these associations within distinct groups of the same population rather than via a unilateral relationship that does not take into account the other outcomes.

The choice of our input variables (gestational age, Apgar score at 5 min and duration of mechanical ventilation) was made on clinical grounds and has evidently major implications for our conclusions. We selected to use these three parameters because they are continuous and reliably available at a population level, while they also represent relatively distinct pathophysiological processes that are associated with impaired later outcomes. It is well described, for example, that the Apgar score is influenced by gestation and cannot be generally considered as evidence of asphyxia nor can it be used at an individual level to predict mortality or neurological outcomes. 27

Alternative continuous variables to assess the condition at birth such as the cord pH or lactate could not be used, however, as these indices were not consistently inserted in the database and had a very high percentage of missing values. Similarly, the duration of ventilation was selected as a proxy for the severity of lung disease,28 which also can be assessed relatively early chronologically as the median duration of ventilation in our population was 10 days.

To our knowledge, non-supervised learning and cluster analysis have not been previously applied exclusively to extremely preterm infants. Souza et al used K-means cluster analysis to explore clinical conditions associated with preterm birth and reported that 4150 preterm births were clustered in three groups of women, yet although some maternal characteristics differed among the clusters, maternal and neonatal outcomes did not.29 This might be explained by population differences, as this was predominantly a maternity study in which the neonatal outcomes were not considered as input variables in the unsupervised models. Greenbury et al performed clustering on daily nutritional intakes in premature infants of less than 32 weeks of gestation and reported different nutritional clusters, which were heterogeneous in size, with some showing common interpretable clinical practices. They also identified a relationship between nutritional practice and outcomes such as BPD, with provision of human milk being a protective factor against developing BPD.30

Our classification could be clinically useful in further understanding the pathophysiology of extremely preterm morbidity and mortality and possibly in targeting selected subgroups of infants for specific interventions. For example, for cluster 4, such interventions might include administration of postnatal corticosteroids to assist earlier weaning from invasive respiratory support,31 more gentle resuscitation methods, avoidance of mechanical ventilation by newer non-invasive techniques32 and use of less invasive surfactant administration, which has been shown to reduce the incidence of BPD.33 The within-group association of increased mortality and birth outside a tertiary hospital in cluster 3 could also strengthen the argument for the centralisation of the care of extreme preterms concentrating on high-volume perinatal centres. 34

Our study has strengths and some limitations. To our knowledge, this is the first study to use unsupervised learning to classify extremely preterm infants in different phenotypical groups. A further strength of our study was that we used the whole population rather than a representative sample and therefore, by avoiding inclusion bias, our results are more generalisable. We should clarify that our analysis is not an early predictive tool that could be applied at birth, as we have included the duration of ventilation as an input parameter, which comes later in the life of an extremely preterm infant compared with the Apgar score or the gestational age. As such, our study is aiming to describe the interaction of different pathophysiological phenomena at a population level rather than provide individualised prediction of outcomes at birth. We should also note that our study only included infants from a recent 5-year period rather than a longer period that would extend more in the past. Our tighter time period, though, would better correspond to current standards of neonatal care. We did not include in our analysis information on whether preterm birth was spontaneous or on the duration of rupture of membranes as the amount of missing data in these categories was very high at a national level, even more so in non-tertiary units.

In conclusion, we have used readily available clinical parameters to classify extremely preterm infants into distinct phenotypic groups. The ensuing groups had differing neonatal outcomes such as survival to discharge from neonatal care and BPD.

Contributors TD: conceived the study, participated in data analysis, wrote the first version of the manuscript and is the guarantor of the manuscript, EEW: acquired and analysed the dataset and critically reviewed the manuscript, CH: participated in study design, supervised the project and critically revised the manuscript, AG: participated in study design, analysis and critically revised the manuscript.

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Competing interests Professor Greenough has held grants from various manufacturers (Abbot Laboratories, MedImmune) and ventilator manufacturers (SLE). Professor Greenough has received honoraria for giving lectures and advising various manufacturers (Abbot Laboratories, MedImmune) and ventilator manufacturers (SLE). Professor Greenough is currently receiving a non-conditional educational grant from SLE.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval A predefined dataset was acquired from the National Neonatal Research Database (NNRD), Imperial College London, UK. The NNRD is approved by the National Research Ethics Service (10/H0803/151), Confidentiality Advisory Group of the Health Research Authority (6-05/9/2010) and the Caldicott Guardians and Lead Clinicians of contributing hospitals. As the study used data held in an existing database, participation did not require approval from individual Trusts, but only from the NHS Trust holding the database (Chelsea and Westminster NHS Foundation Trust) which was obtained. The primary study was approved by the West Midlands—Edgbaston Research Ethics Committee (REC reference: 19/WM/0172)
REFERENCES


22 Milligan GW, Cooper MC. An examination of procedures for determining the number of clusters in a data set. Psychometrika 1985;50:159–79.


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<th>Item No</th>
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<tr>
<td><strong>Title and abstract</strong></td>
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<tr>
<td>1</td>
<td>(a) Indicate the study’s design with a commonly used term in the title or the abstract</td>
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<td>(b) Provide in the abstract an informative and balanced summary of what was done and what was found</td>
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<tr>
<td><strong>Introduction</strong></td>
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<td>2</td>
<td>Explain the scientific background and rationale for the investigation being reported</td>
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<td><strong>Objectives</strong></td>
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<td>3</td>
<td>State specific objectives, including any prespecified hypotheses</td>
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<td><strong>Methods</strong></td>
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<td>4</td>
<td>Present key elements of study design early in the paper</td>
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<td>5</td>
<td>Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection</td>
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<td>6</td>
<td>(a) <strong>Cohort study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <strong>Case-control study</strong>—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <strong>Cross-sectional study</strong>—Give the eligibility criteria, and the sources and methods of selection of participants</td>
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<td>(b) <strong>Cohort study</strong>—For matched studies, give matching criteria and number of exposed and unexposed <strong>Case-control study</strong>—For matched studies, give matching criteria and the number of controls per case</td>
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<td>Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable</td>
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<td>8*</td>
<td>For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group</td>
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<td>9</td>
<td>Describe any efforts to address potential sources of bias</td>
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<td>10</td>
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<td>11</td>
<td>Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why</td>
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<tr>
<td>12</td>
<td>(a) Describe all statistical methods, including those used to control for confounding</td>
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<td>(b) Describe any methods used to examine subgroups and interactions</td>
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<td>(c) Explain how missing data were addressed</td>
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<td>(d) <strong>Cohort study</strong>—If applicable, explain how loss to follow-up was addressed <strong>Case-control study</strong>—If applicable, explain how matching of cases and controls was addressed <strong>Cross-sectional study</strong>—If applicable, describe analytical methods taking account of sampling strategy</td>
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<td>(e) Describe any sensitivity analyses</td>
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### Results

#### Participants
- **13**
  - (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
  - (b) Give reasons for non-participation at each stage
  - (c) Consider use of a flow diagram

#### Descriptive data
- **14**
  - (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
  - (b) Indicate number of participants with missing data for each variable of interest
  - (c) **Cohort study**—Summarise follow-up time (eg, average and total amount)

#### Outcome data
- **15**
  - **Cohort study**—Report numbers of outcome events or summary measures over time
  - **Case-control study**—Report numbers in each exposure category, or summary measures of exposure
  - **Cross-sectional study**—Report numbers of outcome events or summary measures

#### Main results
- **16**
  - (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
  - (b) Report category boundaries when continuous variables were categorized
  - (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

#### Other analyses
- **17**
  - Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

### Discussion

#### Key results
- **18**
  - Summarise key results with reference to study objectives

#### Limitations
- **19**
  - Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias

#### Interpretation
- **20**
  - Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

#### Generalisability
- **21**
  - Discuss the generalisability (external validity) of the study results

### Other information

#### Funding
- **22**
  - Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.