Risk of miscarriage following amniocentesis and chorionic villus sampling – systematic review of the literature and updated meta-analysis

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ABSTRACT

Objectives: To estimate the procedure-related risks of miscarriage after amniocentesis and trans-abdominal chorionic villus sampling (CVS) based on a systematic review of the literature and an updated meta-analysis.

Methods: A search of MEDLINE, EMBASE, and The Cochrane Library was carried out to identify studies reporting complications following CVS or amniocentesis. The inclusion criteria for the systematic review were studies reporting results from large controlled studies and those reporting data for pregnancy loss prior to 24 weeks' gestation. Study authors were contacted when required to identify additional necessary data. Data for cases that had invasive procedure and controls groups were inputted in contingency tables and risk of miscarriage was estimated for each study. Summary statistics based on a fixed and random effects model were calculated after taking into account the weighting for each study included in the systematic review. Procedure-related risk of miscarriage was estimated as a weighted risk difference from the summary statistics for cases and controls. A subgroup analyses according to the similarity risk levels in the invasive testing and control groups was performed. Heterogeneity was assessed using Cochrane's Q and I^2 statistic. Egger Bias was estimated to assess reporting bias in published studies. Summary statistics for procedure-related risk of miscarriage were graphically represented in Forest plots.

Results: The electronic search from the databases yielded 2,943 potential citations, from which, we selected 20 controlled studies for inclusion in the systematic review to estimate the procedure-related risk of miscarriage from invasive procedures. There were a total of 580 miscarriages from 63,273 amniocentesis procedures with a weighted risk of pregnancy loss of 0.91% (95%CI: 0.73 to 1.09). In the control group, there were 1,726 miscarriages in 330,469 pregnancies with a loss rate of 0.58% (95CI%: 0.47 to 0.70). The weighted procedure-related risk of miscarriage was 0.30% (95%CI: 0.11 to 0.49, I^2=70.1%). There were a total of 163 miscarriages from 13,011 CVS procedures with a risk of pregnancy loss of 1.39% (95%CI: 0.76 to 2.02). In the control group, there were 1,946 miscarriages in 232,680 pregnancies with a loss rate of 1.23% (95CI%: 0.86 to 1.59). The weighted procedure-related risk of miscarriage following CVS was 0.20% (95%CI: -0.12 to 0.52, I^2=51.9%). However, when only studies with similar risk profiles between the intervention and control groups were considered, the procedure related risk for amniocentesis became 0.03% (95%CI -0.08 to 0.14, I^2=0%) and for CVS -0.38 (95% CI -1.12 to 0.36, $I^{2=}$0%).

Conclusion: The procedure-related risks of miscarriage following amniocentesis and CVS are lower than currently quoted to women. The risk appears to be negligible when these interventions are compared to control groups of the same risk profile.

Introduction

There is considerable evidence suggesting that the procedure-related risks of miscarriage following amniocentesis and chorionic villus sampling (CVS) are much lower than are currently quoted by professional bodies.[1-3] The pooled summary statistics of these procedure-related risks based on data reported in large controlled cohort studies published until January 2014 were reported in a systematic review and meta-analysis.[4,5] There have been further large studies published in the last few years reporting the procedure-related risks of miscarriage following invasive procedures from large cohort, population and randomised controlled studies with data from more than 20,000 procedures.[6-11] We aimed to derive updated procedure-related risks of miscarriage after including data from all studies published till 31st January 2019 in order to provide clinicians with most recent estimates which can be used to counsel women.

Methods

Eligibility criteria

The articles eligible for inclusion in our study were randomised controlled trials, prospective or retrospective cohort or case-control studies reporting on the pregnancy outcomes in women who had invasive prenatal testing and that of control pregnancies that did not have an invasive procedure. In view of the improvements and advances in ultrasound resolution and the subsequent improvements in the techniques of performing CVS/amniocentesis over the last couple of decades, we only included studies published from year 2000 onwards to ensure uniformity in comparing results of studies using similar equipment and techniques. In case of studies reporting data spanning years before and after 2000, we only included cases that underwent invasive testing from 2000 onwards. We only included studies reporting their data in English language.

Types of participants: We included studies reporting their data on invasive procedures carried out in singleton pregnancies. Studies reporting results from both singleton and multiple pregnancies were deemed eligible if data from multiple pregnancies were <5% of the total sample size.

Types of interventions: We compared women who underwent invasive prenatal testing (CVS or amniocentesis) to those that did not have any invasive procedure. When

separate data were reported for transabdominal and transcervical CVS, only the former group was entered in the analyses.

Outcome measures: Miscarriage, defined as fetal loss before 24 or 22 weeks[12]. In studies reporting data for miscarriage due to various causes, we only included procedure-related losses, i.e. those not associated with structural anomalies or other factors likely to cause miscarriage independently from invasive testing.

Data sources and search strategy

This systematic review and meta-analysis was undertaken based on an *a-priori* designed study protocol and was registered in advance with the PROSPERO International Prospective Register of Systematic Reviews (Registration number: CRD42019130495). An electronic search of MEDLINE, EMBASE, and The Cochrane Library was carried out on 31st January 2019 utilising combinations of the relevant Medical Subject Heading (MeSH) terms, key words, and word variants for "Amniocentesis"," Chorionic Villus Sampling (CVS)", "miscarriage", "pregnancy loss" and "procedure-related risk". The search and selection criteria were restricted to studies reported in English language. The citations retrieved following this search strategy were examined for relevance to this study based on the type of invasive prenatal procedure, study design, sample size of the study, study period and gestational age at assessing pregnancy outcome. We complemented the searches by perusing the references of retrieved articles and the studies included in previous systematic reviews on the topic.

Study selection and data extraction

Search results were screened by two of the authors (RA and LJS) and the full text of all relevant studies was reviewed. The citations were examined to produce a list of relevant studies after excluding studies that were duplicates, those that did not fit selection criteria after review of title and abstract and those that were case-reports, letters, or review articles. These two authors independently assessed for inclusion all the potential studies identified from the search strategy. Data were extracted using a pre-specified form. We resolved any disagreement through discussion or, if required, we consulted a third author (AS).

For each study, we recorded information about the authors, country of origin, years of enrolment for the intervention and control groups, indications for invasive testing, technique of CVS/amniocentesis, experience of the operators, characteristics of control

women and risk level for the two groups. We recorded the number of terminations of pregnancy and we subtracted these cases from the denominator. We contacted the authors of primary study if further details or clarifications were required.

Risk of bias of individual studies

The methodological quality of studies included in the systematic review was assessed using the Newcastle-Ottawa Scale (NOS)[13]. Briefly, NOS assess the quality of cohort or case-control studies across three domains, i.e. selection (including representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure and demonstration that the outcome of interest was not already present at the start of the study), comparability and outcome (including assessment of outcome and adequacy of follow-up length). The assessment of the domains is performed based on a standardized checklist and indicators of high quality are awarded a star; the number and combination of stars expresses the overall quality of a study in an AHRQ-compliant way (good, fair or poor).

We reported our study as per PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) (Supplementary Table 1).[14]

Summary measures and synthesis of the results

The data from each study was extracted to note the type of procedure, study design, sample size, and miscarriage rate in each study group. Study authors were contacted when required to identify additional necessary data. Data were entered in contingency tables and miscarriage rate (95% confidence interval [CI) was estimated in the invasive procedure and the control group, for each study as well as a pooled estimate, weighted by sample size of each study. The procedure-related risk for amniocentesis and CVS for each study were estimated as a risk difference (RD) based on the miscarriage rates in the invasive and control group, which were then used to calculate the weighted pooled summary estimate (95% CI). Given the non-randomized design and the anticipated heterogeneity of the studies, we calculated the summary effect sizes using random effects models. The random effects model assumes that the true effect size varies between the studies, and that included studies represent a random sample of effect sizes that could have been observed. Therefore, we opted to use this model as it allows for not just for variation within studies but also variation between studies, thus providing a conservative estimate of the summary statistics with wider CIs.[15] The procedure-related

risks for amniocentesis and CVS were graphically expressed in Forest plots. Pooled proportions were calculated using the *metaprop* command. The heterogeneity between studies was assessed by estimation of Cochrane's Q and I^2 statistic; Egger's meta-regression test was used to assess reporting bias in studies when 10 or more studies were available.[16-18]

Subgroup analyses

We planned subgroup analyses according to the similarity risk levels in the invasive testing and control groups, as extracted by their description in the primary studies (similar or dissimilar risk), as this could be related to confounders that can affect the procedure-related risk, and therefore the risk difference (RD).

We carried out statistical analyses in Stata 14.0 software (StataCorp College Station, TX) using the *metan* and *metaprop* commands.

Results

Data search results

The electronic search from the databases yielded 2,943 potential citations of which 2,907 were excluded as they were duplicates, or a review of the title or abstract did not meet the inclusion criteria, leaving 36 studies for full-text review. After the full manuscript review, we finally considered 12 studies for amniocentesis[6,7,9-11,19-25] and 8 studies for CVS[6-8,10,11,26-28] Of those, one study (Lau *et al.,* 2005) was eventually excluded[26], as it gave cumulative data for miscarriage and stillbirth, leaving 12 studies for amniocentesis and 7 for CVS. The raw data for the Wulff *et al.,* 2016[6] study were calculated from the published adjusted estimates and were complemented with additional information provided by the authors. Similarly, the authors of Malan et al., 2018 were contacted to obtain data additional data for amniocentesis and CVS.[10] The characteristics of the included studies are shown in Supplementary Table 2.

Assessment of the quality of included studies

The methodological quality of studies was assessed using the NOS. The rating of the included studies according to the NOS based on study type, selection, comparability and outcome are shown in Supplementary Table 3.

Amniocentesis group

There were a total of 580 miscarriages from 63,723 amniocentesis procedures with a pooled risk of pregnancy loss of 0.91% (95%CI 0.73 to 1.09, I^2=88.2%). In the control group, there were 1726 miscarriages in 330469 pregnancies with a pooled loss rate of 0.58% (95%CI 0.47 to 0.70, I^2=96.1%). The pooled procedure-related risk of miscarriage was 0.30% (95%CI: 0.11 to 0.49), I^2=70.1% (Figure 2).

Chorionic villus sampling group

There were a total of 163 miscarriages from 13,011 CVS procedures, with a pooled risk of pregnancy loss of 1.39% (95%CI 0.76 to 2.02, I^2=89.1%). In the control group, there were 1,946 miscarriages in 232,680 pregnancies with a pooled loss rate of 1.23% (95%CI 0.86 to 1.59, I^2=98.1%). The pooled procedure-related risk of miscarriage following CVS was non-significant (0.20, 95%CI: -0.12 to 0.52, I^2=51.9%) (Figure 3).

Subgroup analyses

For each invasive test (amniocentesis or CVS) we examined the procedure-related risk of miscarriage separately for studies with similar or dissimilar risk level for chromosomal abnormalities. The pooled risk RD (i.e. procedure related risk) for amniocentesis was 0.46% (95%CI 0.25 to 0.67, I^2=38.1%) when studies with dissimilar risk level for the two groups were synthesized. When studies with similar risk levels for the amniocentesis and control groups were synthesized, there was no significant procedure-related risk (RD 0.12 95%CI -0.05 to 0.30%, I^2=44.1%) (Figure 4).

The procedure-related risk for CVS was non-significant when studies with similar level of risk between the two groups were compared (pooled RD -0.011, 95%CI -0.29 to 0.08, I^2=0%). In contrast, CVS was associated with a pooled prodedure-related risk of 0.48% (95%CI 0.17 to 0.78, I^2=0%) when studies with dissimilar level of risk between the intervention and control group were synthesized (Figure 5).

Publication bias

We were able to assess the potential for publication bias for amniocentesis only, as the number of CVS studies was less than 10. The Egger's meta-regression test did not demostrate the presence of small-study effects (p=0.179) (Supplemental figure 1.)

Discussion

Main findings

The results of our study demonstrate the following facts: first, the procedure-related risk of miscarriage is considerably lower than is currently quoted in guidelines from professional bodies and is 0.30% following amniocentesis, whereas there is no significant procedure-related risk associated with CVS, which may be a safer procedure than amniocentesis;; second, our results highlight that the point-estimates for miscarriage are even substantially lower with no significant increase in risk of miscarriage for both, amniocentesis and CVS, when the analysis is restricted to studies in which the control population has a similar risk profile for chromosomal abnormalities as the women who underwent invasive prenatal testing.

Strengths and limitations

This is an updated version of our previous meta-analyses, adding the only new randomized trial[10] in three decades and following a new approach to address the issue of heterogeneity between reported studies.

The published studies have used different indications for invasive testing and different selection criteria for the control women, culminating in different background risk levels for the compared groups both within and across studies. The resulting heterogeneity has been the major argument against quantitative synthesis of such studies. To this end, we have taken the following measures. First, we excluded terminations of pregnancy from both the intervention and control groups. Second, we excluded cases of miscarriage directly attributable to structural defects or obstetric complications unrelated to invasive testing, if data were available. Third, we only analysed invasive procedures performed from 2000 onwards, if such data were available, to account for the progress in ultrasound resolution and sampling techniques. Fourth, we stratified the intervention and control groups according to their risk profiles, as extracted from their inclusion criteria, and we separately analysed studies where the two groups had similar risk profiles. This analysis highlighted the impact of dissimilar background risks as a source of risk inflation and statistical heterogeneity. Fifth, anticipating that the studies do not represent random samples from the same population, we used the random effects model, which does not assume a common underlying effect size and produces more conservative estimates. In

any case, as the control groups have usually more favourable risk profile, any bias would be against invasive procedures, which means that the latter may be even safer than they appear in an aggregate analysis.

There are some limitations to our study which could not be overcome despite our robust methodology of well-defined eligibility criteria, inclusion of controlled studies with large sample size, as well as the strict approach to minimising heterogeneity. A limitation of our study is that when we compared the risks of miscarriage in the intervention and control pregnancies with similar risk profile and background risks, these were mainly in high-risk populations and the results of our study therefore cannot comment on risk estimates of miscarriage, if these were performed in large low-risk populations. In our study, we did not examine other pregnancy complications such as preterm birth and stillbirth nor did we examine the serious but rare outcomes such as maternal septicaemia or amniotic fluid embolism as they were not consistently reported by included studies. Another limitation refers to lack of analyses of data with regard to operator experience as it is potentially an important factor associated with procedure-related loss. Unfortunately, with the exception of two recent studies with conflicting results (Akolekar *et al.,* 2011 and Bakker *et al.,* 2017)[7,27], the rest of the studies do not provide data about the effect of operator experience on the risk of miscarriage, and we were unable to account for this in our analyses.

Interpretation of the findings

The first question we aimed to address is whether invasive prenatal diagnosis is a safe procedure. Our results suggest that amniocentesis is associated with a procedure-related risk of 1:300 at most, or more likely, no significant increase in risk if we considered the results from our analysis which only included studies with comparable risk profile in the intervention and control groups. With regard to CVS, our results demonstrate that, there is no significant procedure-related risk associated with undertaking this procedure.

A second, related question is whether, which is a safer procedure to undertake, CVS or amniocentesis. There is no statistically appropriate way to answer this through either a direct or network meta-analysis, as the two methods do not have a common comparator. The closest approximation to a valid answer to this question is to estimate a pooled procedure-related risk from those studies which reported results for both amniocentesis and CVS by comparison with a control group. There were four such studies (Wulff *et al.,*

2016, Bakker *et al.,*2017, Malan *et al.,*2018 and Beta *et al.,* 2019)[6,7,10,11] comparing both CVS and amniocentesis to their corresponding control groups; their pooled procedure-related risk was 0.11% (95%CI -0.28 to 0.50, I^2=42.1%) for CVS and 0.55% (95%CI 0.29 to 0.81, I^2=0%) for amniocentesis. There are a few hypotheses than can potentially explain the reasons for these differences and the apparent greater safety of CVS compared to amniocentesis. Firstly, CVS is usually performed by specialist and experienced Fetal Medicine operators compared to amniocentesis; secondly, performing a CVS involves introduction of the needle into the placental tissue, which is highly vascular tissue with blood flow of about 90-100 mL/minute/kg at 12 weeks' gestation[29,30], as opposed to introduction of a needle into the amniotic sac, which is a closed cavity and therefore has a higher chance of a potential infection introduced into a confined intraamniotic space and lastly, the option to reschedule the procedure and undertake amniocentesis if there are technical limitations.

The quest to accurately quantify procedure-related risks for invasive procedures may seem trivial. However, we consider it to be quite important and relevant to current clinical practice. There is a recent systematic review in 2017 attempting to quantify procedure-related risks of miscarriage from published RCTs with the conclusion that second trimester amniocentesis increases the risk of procedure-related loss but it is not possible to quantify this increase precisely from one study.[31] Such conclusions are unhelpful and do not provide the women nor their clinicians any clear evidence-based estimates of risks for decision making. These invasive procedures are still routinely carried out for prenatal diagnosis but instead of accurate and recent estimates of risks from expert operators, these are rather based on historical and inflated estimates of risks. There are significant advances in cytogenetics analysis and genomic sequencing which are progressing at a rapid pace, and pregnant women must receive appropriate counselling to enable them to make informed choices about their options for prenatal testing, without being deterred by falsely exaggerated rates of procedure-related risks of miscarriage. Until such a time that non-invasive testing is not diagnostic and as comprehensive as cytogenetics techniques, the questions about the safety of invasive procedures and the factors affecting it remain topical.[32]

<u>Comparison with previous studies</u>

In comparison to our previous meta-analyses[4,5], this update includes the first RCT published in the last three decades and it addresses the issue of heterogeneity by

carefully excluding cases potentially affected by confounders and by accounting for the effect of different background risks. In terms of numerical estimates, the procedure related risk of amniocentesis seems to stabilize around 1:300, whereas the risk difference between CVS and controls still fails to reach significance. The most important novelty is the synthesis of studies in which the intervention and control groups had similar background risks for chromosomal abnormalities, as derived from their description. It appears that, when comparing women at a similar risk level (be it high, intermediate or low, Table 1), the procedure-related risk for amniocentesis also fails to reach significance, whereas the statistical heterogeneity substantially decreases. This is a significant finding, supporting the concept that, all other things being equal, an invasive procedure is not associated with a significant increase in the rate of miscarriage. This does not imply that a miscarriage following an invasive procedure cannot occur, but this is more likely to be related to either operator-independent maternal factors (Akolekar *et al.,* 2011)[27], or to the experience and technique of the operator, rather than the procedure itself. In a large UK study, the authors reported that there was no significant increase in overall procedural risk of miscarriage, regardless of whether the procedure was carried out by fetal medicine experts or trainees under direct supervision of an expert (Akolekar *et al.,* 2011), whereas the point estimates of procedure-related miscarriage appeared to decrease with operators' experience, though in a non-significant way in a Dutch study (Bakker *et al.,* 2017).[7,27] As neither of these studies were randomised, there is no reliable way to ascertain how experience may impact on outcomes or the effects are subject to modifications by confounders like negative selection bias.

In terms of raw numbers and sample size of studies, the evidence is dominated by a large Danish registry study, which shows that invasive procedures themselves do not carry a significant miscarriage risk (Wulff *et al.,* 2017).[6] The random effects model we used reduces the weight (and therefore the dominance) of this single trial, which in any case is in line with the aggregate findings. Similarly, the French multicentre RCT, which aimed to show that there would be a reduction in the risk of miscarriage in the group that was offered invasive testing only for positive cfDNA results (n=1,034) as opposed to those with direct invasive testing (n=1,017), also failed to show a significant difference (RD -0.03%).[10]

Conclusion

The results of this systematic review and meta-analysis demonstrate that the procedure-related risks of miscarriage from invasive procedures are low, or negligible if groups with similar background risks are compared. In terms of safety of a prenatal diagnostic procedure, it appears that CVS is potentially safer compared to amniocentesis. Women should be reassured that invasive procedures carried out by experienced operators in specialist centres are not associated with a significant increase in miscarriage rate as compared to those women not undergoing these procedures.

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Figure legends

Figure 1. Flowchart of study selection of studies included in the meta-analysis.

Figure 2. Procedure-related risk for miscarriage after amniocentesis, expressed as risk difference (RD, 95% CI) to controls (Random effects model)

Figure 3. Procedure-related risk for miscarriage after chorionic villus sampling (CVS), expressed as risk difference (RD, 95% CI) to controls (Random effects model)

Figure 4. Subgroup analysis for the procedure-related risk of miscarriage after amniocentesis, expressed as risk difference (RD, 95% CI) to controls (Random effects model). Subgroup 1 included studies in which the intervention and control groups had similar risk profile (i.e. they were both of high-, intermediate- or low risk). Subgroup 2 included studies in which the intervention and control groups had similar risk profile

Figure 5. Subgroup analysis for the procedure-related risk of miscarriage after amniocentesis, expressed as risk difference (RD, 95% CI) to controls (Random effects model). Subgroup 1 included studies in which the intervention and control groups had similar risk profile. Subgroup 2 included studies in which the intervention and control groups had similar risk profile (i.e. they were both of high-, intermediate- or low risk).

Supplemental figure. Egger's meta-regression test for the presence of small-study effects.