*Original Article*

# Antepartum haemorrhage of unknown origin and maternal cigarette smoking beyond the first trimester

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**Background:** Antepartum haemorrhage of unknown origin (APHUO) is associated with preterm birth and perinatal mortality.
**Aim:** To determine whether smoking beyond the first trimester of pregnancy was an independent risk factor for APHUO.
**Methods:** Rates of APHUO were compared between non-smokers and smokers, and non-smokers and ceased smokers. Participants were healthy nulliparous women recruited to the Screening for Pregnancy Endpoints (SCOPE) prospective cohort study in New Zealand, Australia, Ireland and United Kingdom. Logistic regression was used to compare adjusted odds ratio, 95% confidence intervals (OR, 95% CI) of APHUO between continued smokers and non-smokers, adjusting for possible confounders.
**Results:** Of the 3513 participants, 77.9% ($n = 2737$) were non-smokers, 10.6% ($n = 371$) ceased in the first trimester and 11.5% ($n = 405$) continued smoking beyond the first trimester. APHUO rates were higher in smokers than non-smokers (7.4%, $n = 30$ vs 4.5%, $n = 122$; $P = 0.01$), but there was no difference between ceased smokers and non-smokers (4.3%, $n = 16$ vs 4.5%, $n = 122$; $P = 0.90$). Smoking was no longer significantly associated with APHUO after adjustment for confounders (adjusted OR = 1.28, 95% CI 0.76–2.14), but vaginal bleeding in early pregnancy (adjusted OR = 2.98, 95% CI 2.12–4.18) and overweight/obesity (adjusted OR = 1.43, 95% CI 1.02–1.99) were independent risk factors. First trimester folic acid use was associated with a reduced risk (adjusted OR = 0.44, 95% CI 0.25–0.77).
**Conclusion:** Smoking is not an independent risk factor for APHUO after adjustment for confounders, but other risk and protective factors have been identified.

**Key words**: antepartum haemorrhage of unknown origin, smoking, first trimester.

## Introduction

Antepartum haemorrhage (APH) or bleeding from the genital tract after 20 weeks gestation is associated with maternal and infant morbidity and perinatal mortality.[1–6] Well-established causes of APH include placenta praevia and placental abruption.[6] In more than 50% of cases of APH, the cause remains unexplained,[2,3,7] and this is referred to as antepartum haemorrhage of unknown origin

(APHUO). APHUO complicates approximately 2–6% of pregnancies[1,7–9] and is associated with an increased risk of fetal demise and preterm birth.[8]

Whilst smoking has been identified as a risk factor for APH secondary to placenta praevia and placental abruption,[10] the few studies investigating the relationship between smoking and APHUO have reported inconsistent findings.[1,9] Women who cease smoking in early pregnancy have similar rates of small-for-gestational-age (SGA) infants and spontaneous preterm birth (SPB) compared with non-smokers, whereas those who continue to smoke have an approximate twofold increase in SGA and SPB.[11] There are no data regarding the effect of ceasing smoking and the risk of APHUO.

The aim of this study was to determine whether APHUO was independently associated with smoking beyond the first trimester of pregnancy after adjusting for

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potential confounders. Our primary hypothesis was that rates of APHUO would be increased in smokers compared with non-smokers, and our secondary hypothesis was that rates of APHUO would be similar in non-smokers and ceased smokers.

## Materials and Methods

From November 2004 to August 2008, healthy nulliparous women with a singleton pregnancy were recruited from Auckland, New Zealand; Adelaide, Australia; Cork, Ireland; and London and Manchester in the United Kingdom to participate in the Screening for Pregnancy Endpoints (SCOPE) study. The aim of this prospective cohort study is to develop screening tests to predict SGA infants, pre-eclampsia and SPB.[12]

Ethical approval was obtained from local ethics committees, and all women provided written consent.[12] Women were not eligible if they were judged to be at high risk of SGA, pre-eclampsia, or SPB because of their gynaecological or medical history, or if they were on treatment that might modify pregnancy outcome.[12]

Participants were interviewed and examined by a research midwife at $15 \pm 1$ weeks of gestation, and detailed data, including demographic information, gynaecological, medical and family history, nutritional supplements, smoking, alcohol and recreational drug use, and early pregnancy complications, were entered into an internet-accessed central database with a complete audit trail (MedSciNet AB). Smoking status was self-reported at the interview and not confirmed using biochemical measures. Detailed methods have previously been documented.[13] Data regarding APHUO and other pregnancy outcomes were collected from all women by direct questioning at interview and review of the clinical records, usually within 72 h of birth. Interview and examination methods and data monitoring have also been described in detail previously.[13]

Women were categorised by their smoking status into three groups: Non-smokers, Ceased smokers and Smokers. Non-smokers were women who did not smoke during their pregnancy. Ceased smokers were women who smoked at some time during the first trimester, but had ceased by 12 weeks and six days. Smokers were women who were still smoking at $\geq 13$ weeks gestation. Smoking status at the end of the first trimester was utilised to classify smoking categories as this relates to the critical phase of trophoblast invasion,[14] and previous SCOPE data demonstrated that the majority of women who ceased smoking (94%) did so by 13 weeks.[11] Overweight or obesity was defined as a body mass index $\geq 25$ kg/m$^2$ (measured at $15 \pm 1$ weeks gestation) and binge alcohol as $\geq 6$ units of alcohol at any time point during the first trimester.

The primary outcome was APHUO, defined as vaginal bleeding after 20 weeks of gestation, but prior to birth that was not because of placental abruption (defined as evidence of retroplacental clot on scan or at time of delivery), placenta praevia, accreta, increta or percreta, vasa praevia, trauma, or from a vaginal or cervical cause.[9]

Data analysis was performed using the statistical software package SAS version 9.1. ANOVA tests were used to compare continuous variables between the three smoking groups with *post hoc* Tukey test for pair-wise comparisons. Chi-square or Fishers exact tests were used to compare categorical variables, as appropriate. Logistic regression analysis was used to compare the odds of APHUO between non-smokers and smokers. Multivariable analysis was performed adjusting for potential demographic confounders of age, ethnicity, socioeconomic index and single marital status; additional smoking-related confounders of binge drinking, marijuana use in the first trimester, previous miscarriage and overweight/obesity; and APHUO-related confounders of vaginal bleeding $\leq 16$ weeks of gestation and folic acid use in the first trimester. There were no missing data for any of the variables included in the multivariable analysis.

A power calculation indicated that to detect an increase in APHUO from 4% in non-smokers to 8% in smokers, at a $\beta$ 0.80 and 0.05 significance level, 309 smokers and 2163 non-smokers would be required.

## Results

Of the 3572 participants enrolled at $15 \pm 1$ weeks gestation, end of pregnancy data were available in 3513 (98.3%) women (Fig. 1). Overall, 168 (4.8%) women experienced APHUO of which 142 (84.5%) had one episode, 20 (11.9%) had two episodes and 6 (3.6%) had three or more episodes of bleeding. The median (interquartile range) for the first APHUO was 30 (25, 38) weeks gestation. Pregnancy outcomes were compared between women with and without APHUO. Those who experienced APHUO had lower mean (SD) birth weight (3152 (779) g vs 3401 (583) g, $P < 0.0001$), earlier gestation at delivery (37.9 (3.6) weeks vs 39.5 (2.2) weeks, $P < 0.0001$) and increased rate of SPB (12.5%
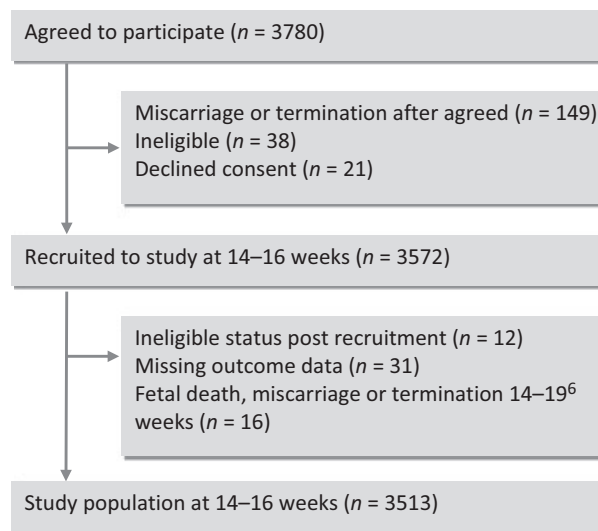


**Figure 1** Recruitment flow chart.

($n$ = 21) vs 4.4% ($n$ = 147), $P < 0.0001$). Although women with APHUO were more likely to experience a perinatal death (2.4% ($n$ = 4) vs 0.4% ($n$ = 13), $P = 0.007$), the median (interquartile range) for weeks of gestation when the death occurred did not differ significantly for those with APHUO versus no APHUO (26 (23, 31) vs 30 (24, 37) weeks, $P = 0.3$).

Of the final cohort, 77.9% ($n$ = 2737) reported they were non-smokers, 10.6% ($n$ = 371) had ceased smoking before 13 weeks of pregnancy and 11.5% ($n$ = 405) continued smoking beyond 13 weeks. Of the ceased smokers, 50.9% ($n$ = 189) had stopped before six weeks and 49.1% ($n$ = 182) between six and 13 weeks. Background characteristics differed between the three smoking groups (Table 1). Smokers were younger, more likely to be single, unemployed, with fewer years of education and to have had a previous miscarriage compared with non-smokers. Folic acid and multivitamin supplement intake during the first trimester were also less

common in smokers. The demographic characteristics of ceased smokers were generally intermediate between smokers and non-smokers. The rates of SGA babies and SPB were significantly higher amongst smokers, but did not differ between ceased smokers and non-smokers.

In univariable analysis, the rate of APHUO was significantly higher in smokers compared with non-smokers (7.4% of 405 vs 4.5% of 2737; $P = 0.01$). There was no difference in rates of APHUO between ceased smokers and non-smokers (4.3% of 371 vs 4.5% of 2737, respectively; $P = 0.90$), so multivariable analysis was not undertaken for this comparison. In the logistic regression model comparing smokers and non-smokers (ceased smokers excluded), the univariable results were adjusted for potential confounders (Table 2). Smoking was no longer independently associated with APHUO after multivariable analysis. Vaginal bleeding $\leq 16$ weeks of gestation (adjusted OR = 2.98, 95% CI: 2.12–4.18) and being overweight or obese (adjusted OR = 1.43, 95% CI:

**Table 1** Participant characteristics and pregnancy outcomes by smoking category at the end of the first trimester

| | Non-smokers N = 2737 (77.9%) | Ceased N = 371 (10.6%) | Smokers N = 405 (11.5%) | P |
|---|---|---|---|---|
| Age (years) | 29.2 ± 5.3 | 25.6 ± 6.0 | 23.2 ± 5.5 | <0.001*,**,*** |
| Ethnicity | | | | <0.001 |
| European | 2364 (86.4) | 322 (86.8) | 364 (89.9) | |
| Asian | 139 (5.1) | 9 (2.4) | 5 (1.2) | |
| Maori/Pacific Islander | 70 (2.6) | 23 (6.2) | 19 (4.7) | |
| Indian | 97 (3.5) | 2 (0.5) | 0 | |
| Other | 67 (2.4) | 15 (4.1) | 17 (4.2) | |
| Previous miscarriage | 380 (13.9) | 51 (13.8) | 81 (20.0) | 0.005 |
| Single marital status | 113 (4.1) | 62 (16.7) | 99 (24.4) | <0.001 |
| Employed | 2416 (88.3) | 283 (76.3) | 234 (57.8) | <0.001 |
| Total years of schooling | 12.5 ± 1.1 | 12.1 ± 1.2 | 11.5 ± 1.3 | <0.001*,**,*** |
| Body mass index (kg/m²) | | | | <0.001 |
| <18.5 | 37 (1.4) | 5 (1.3) | 17 (4.2) | |
| 18.5 to <25 | 1517 (55.4) | 179 (48.3) | 197 (48.6) | |
| 25 to <30 | 780 (28.5) | 112 (30.2) | 96 (23.7) | |
| ≥ 30 | 403 (14.7) | 75 (20.2) | 95 (23.5) | |
| Vaginal bleeding ≤ 16 weeks | 599 (21.9) | 78 (21.0) | 68 (16.8) | 0.06 |
| Folate intake† | 2626 (95.9) | 339 (91.4) | 344 (84.9) | <0.001 |
| Multivitamin intake† | 1543 (56.7) (52.6) | 174 (50.0) | 157 (38.9) | <0.001 |
| Binge alcohol† | 212 (7.8) | 100 (27.0) | 69 (17.0) | <0.001 |
| Marijuana use† | 40 (1.5) | 43 (11.6) | 91 (22.5) | <0.001 |
| Pregnancy outcomes | | | | |
| Gestation at delivery (weeks) | 39.5 ± 2.2 | 39.8 ± 2.1 | 39.0 ± 2.9 | <0.001*,*** |
| Birth weight (g) | 3410 ± 579 | 3478 ± 572 | 3172 ± 679 | <0.001*,*** |
| Small-for-gestational age | 267 (9.8) | 33 (8.9) | 76 (18.8) | <0.001 |
| Spontaneous preterm birth | 122 (4.5) | 14 (3.8) | 32 (7.9) | <0.01 |

$P$ values are for comparisons between the three smoking groups using chi-square or analysis of variance test with *post hoc* Tukey test for pair-wise comparisons. $P < 0.05$:
*Non-smokers versus smokers,
**Non-smokers versus ceased smokers,
***Ceased smokers versus smokers.
Data are $n$ (%) or mean ± standard deviation.
†Any in first trimester.

**Table 2** Final multivariable model: risk factors for antepartum bleeding of unknown origin

| | Odds ratio (95% confidence level)* | |
|---|---|---|
| Risk factor | Unadjusted | Adjusted |
| Smokers | 1.72 (1.14–2.60) | 1.28 (0.76–2.14) |
| Age (years) | 0.97 (0.95–1.00) | 0.99 (0.95–1.03) |
| European ethnicity | 1.07 (0.65–1.74) | 1.04 (0.63–1.71) |
| Socioeconomic index | 1.00 (0.99–1.01) | 1.00 (0.99–1.02) |
| Single marital status | 0.64 (0.37–1.12) | 1.21 (0.66–2.21) |
| Previous miscarriage | 1.21 (0.79–1.87) | 1.14 (0.73–1.78) |
| Binge alcohol† | 1.58 (0.97–2.57) | 1.42 (0.86–2.35) |
| Marijuana use† | 2.28 (1.25–4.14) | 1.86 (0.95–3.64) |
| Body mass index $\geq 25$ kg/m$^2$ | 1.02 (0.99–1.05) | 1.43 (1.02–1.99) |
| Vaginal bleeding $\leq 16$ weeks | 2.88 (2.06–4.02) | 2.98 (2.12–4.18) |
| Folic acid intake† | 0.40 (0.24–0.68) | 0.44 (0.25–0.77) |

*Ceased smokers excluded.
†Any use in first trimester.

1.02–1.99) were found to be associated with increased risk of APHUO, and folic acid use in the first trimester was protective for APHUO (adjusted OR = 0.44, 95% CI: 0.25–0.77). There was a trend to an increased risk in women who used marijuana in the first trimester $P = 0.07$ (adjusted OR = 1.86, 95% CI: 0.95–3.64).

## Discussion

Women who smoked at 13 weeks of gestation had an increased risk of APHUO on univariable analysis, but after adjustment for confounders, smoking was no longer independently associated with APHUO. Being overweight or obese and vaginal bleeding at $\leq 16$ weeks of gestation were both associated with an increased risk of APHUO. Our findings, therefore, suggest that the effect of smoking may be explained by other factors that are associated with women who smoke during pregnancy. Alternatively, a larger sample size may be required to demonstrate a smaller independent effect of cigarette smoking on risk of APHUO. Our study has also confirmed that APHUO is associated with a significantly increased risk of pregnancy complications, including SPB and perinatal death.

Consistent with our findings, Ananth *et al.*,[1] utilising data from a large prospective Canadian study, reported that smoking was not associated with APHUO after multivariable analysis. McCormack *et al.*[9] found a relationship between smoking and APHUO in univariable analysis, but did not undertake multivariable analysis.

Vaginal bleeding before 20 weeks of gestation is known to be associated with placental abruption and placenta praevia,[15,16] and a retrospective study, which did not adjust for confounders, identified early bleeding as a risk factor for APHUO.[9] Ours is the first report to confirm an independent relationship between vaginal bleeding in early pregnancy (at $\leq 16$ weeks of gestation) and APHUO and

is consistent with the findings of a recent metaanalysis, which reported that women with first trimester bleeding had an increased risk of APHUO (unadjusted OR(95% CI) 2.47 (1.5–4.0)).[17] This association is biologically plausible, given the likely similarity in placentation mechanisms leading to both early pregnancy bleeding and APHUO, and this association is of relevance to clinical practice.

A novel finding in the current study was that being overweight or obese was an independent risk factor for APHUO. Ahmed *et al.*[18] reported that obese women had an unadjusted relative risk of 3.18 for APH; however, they did not define APH nor adjust for possible confounders. In contrast, Denison *et al.*[19] did not find a difference in APH rates between BMI groups after linear regression analysis, but the prevalence of APH in this cohort was very low (0.4%).

We found that folic acid use in the first trimester was associated with more than a 50% reduction in risk of APHUO. Of the women using folic acid in the first trimester, 61% used a dosage of 800 μg and 60% also used it at pre-pregnancy. Our findings suggest that folic acid may have additional beneficial effects during pregnancy than just prevention of neurologic birth defects. A protective role of folic acid for placental abruption has been suggested in an earlier study by Nilsen *et al.*,[20] who found that the risk of abruption was reduced by almost 20% in women who used folic acid before or during pregnancy (OR 0.81, 95% CI: 0.68–0.98).

Folic acid has a pivotal role in homocysteine metabolism, and folate supplementation can lower levels of homocysteine.[21] Increased homocysteine is an independent risk factor for vascular disease[22] and has been associated with placental abruption,[23–25] These adverse vascular effects of homocysteine are a potential biological mechanism that could explain a beneficial effect of folic acid on risk of APHUO.[26]

Our multivariable analysis showed a trend for a relationship between APHUO and marijuana use in the first trimester. The overall rate of first trimester marijuana use in this study was 5%. Few studies have investigated the potential contribution of marijuana use in pregnancy to adverse pregnancy outcomes,[27,28] and there are no previous data investigating a potential relationship between marijuana use and APH. Further well-designed studies are needed to investigate the relationship between marijuana use and pregnancy complications as this habit, at least in participants in the SCOPE study, was quite common.

The strengths of this study are the prospective cohort design, the large number of participants and the high follow-up rate. The detailed data collected in the SCOPE study on smoking status, APH and a large number of potential confounding factors enabled multivariable analysis to be performed. The quality of the data was ensured by stringent data monitoring protocol.[13]

Our study was powered to detect a doubling in the rate of APHUO with smoking, but not adequately powered to

detect smaller differences. Larger studies are required to determine whether smoking has a smaller independent effect on risk of APHUO. Self-reported smoking status was not confirmed by measurement of cotinine levels. However, cotinine levels are highly correlated with self-reported smoking status and also have their own limitations.[29–31]

Smoking was not found to be an independent risk factor for APHUO, but smokers are at higher risk of this pregnancy complication because of other associated risk factors. We identified that early pregnancy bleeding and being overweight or obese were risk factors, whereas folic acid use in the first trimester was protective.

## Financial disclosure

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