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Abstract

Objective: Abnormal prenatal biochemical screening for trisomies and birth defects, completed among millions of women, is also related to a higher risk of preeclampsia. Preeclampsia is linked to premature cardiovascular disease (CVD). It is unknown if abnormal prenatal biochemical screening is associated with premature CVD after pregnancy, the goal of the current study.

Design: Population-based cohort study.

Setting and Participants: The entire province of Ontario, Canada, where healthcare is universally available. Included were women aged 12-55 years, without preexisting CVD, and who underwent prenatal screening between 1993-2011. One pregnancy was randomly selected per woman. Abnormal cut-points were ≤ 5th percentile multiple of the median (MoM) for serum total chorionic gonadotropin (hCG), unconjugated estriol (uE3) and pregnancy-associated plasma protein A (PAPP-A), and ≥ 95th percentile MoM for alphafetoprotein (AFP) and dimeric inhibin-A (DIA).

Main Outcome Measures: The primary CVD outcome was a composite of hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia ≥ 365 days after pregnancy.

Results: There were 14,666,867 person-years of follow-up among 1,209,690 pregnancies. Each of the five prenatal biochemical screening analytes was associated with a higher risk of CVD, especially DIA. Those with an abnormally high DIA ≥ 95th percentile had the highest rate of CVD (30 events; 8.3 per 10,000 person-years) vs. < 95th percentile (251 events; 3.8 per 10,000 person-years) (aHR 2.0, 95% CI 1.4-3.0). Relative to those without any abnormal biochemical measure (5.6 per 10,000 person-years), the rate of the composite CVD outcome was 7.3 per 10,000 person-years with 1, 8.9 per 10,000 person-years with 2, and 14.4 per 10,000 person-years with ≥ 3 abnormal analytes (aHR 2.3, 95% CI 1.1-4.8).

Conclusions: Women with abnormal prenatal biochemical screening, especially DIA, may be at higher risk of CVD. If these findings are replicated elsewhere, then there presently exists a massive amount of data that could aid in identifying women at higher risk of premature CVD, and which could be conveyed to her or her healthcare provider.

Keywords: Prenatal biochemical; screening; Coronary artery disease; stroke; peripheral arterial disease; cardiovascular disease; heart failure; dysrhythmia; preeclampsia, placenta; cohort study.

What is already known on this subject

- Abnormal prenatal biochemical screening for trisomies and birth defects has been completed among millions of women.
- A detailed search of PubMed and Google Scholar from 1980 to January 2018 did not reveal any prior study examining the risk of maternal cardiovascular disease (CVD), or any sub-type of CVD, in relation to prenatal biochemical screening.
- This search included a broad array of terms related to “prenatal screening” or “maternal serum screening”, and the intersection of each with a broad array of terms reflective of heart disease or cerebrovascular disease.

What this study adds

- Women with abnormal prenatal biochemical screening were found to be at modestly higher risk of a broad premature CVD composite outcome, as well as the secondary outcome of major adverse cardiovascular events.
- As millions of women worldwide have completed prenatal biochemical screening, there now exists a massive amount of data that might be applied to better estimate a woman’s long-term risk of CVD..

BACKGROUND

A healthy pregnancy depends on the successful linkage of a woman's physiology with that of her fetus, via the placenta. Placental hormones arise from the syncytiotrophoblast[1], and in combination with cytokines and growth factors, alter various maternal physiological systems as a means to sustaining pregnancy[2]. It is within the temporary interface of the trophoblast and endometrial decidua that placental vascular disease likely arises, resulting in adversity for mother and fetus, including preeclampsia, placental abruption, poor fetal growth and preterm birth[3].

Several risk factors for placental vascular disease, especially for preeclampsia[3,4], are shared with those for cardiovascular disease (CVD) (see Supplementary file 1). A maternal placental syndrome in pregnancy appears to forecast a woman's cardiovascular health in the years that follow, including premature onset of coronary artery disease[5,6], heart failure and dysrhythmias[7], and death after coronary revascularization[8]. Different guidelines for the prevention of CVD recommend screening for CVD risk factors in women with a prior maternal placental syndrome[9].

Starting around the year 1993, maternal serum screening was made freely available to all pregnant women in Ontario, to screen for trisomy 21 and 18, and neural tube defects. Triple-screening comprised maternal serum alphafetoprotein (AFP), total human chorionic gonadotropin (hCG) and unconjugated estriol (uE3), collected in the second trimester at 15^{+0} to 20^{+6} weeks' gestation. Thereafter, dimeric inhibin-A (DIA) was added. By the year 2000, serum pregnancy-associated plasma protein A (PAPP-A) measured at 11^{+0} to 13^{+6} weeks' were further added. Although maternal serum screening has been used primarily to detect anomalies in the fetus, a particular pattern of prenatal biochemical screening results -- a high serum AFP, hCG or DIA, and low uE3 or PAPP-A – have been found to have a high specificity for identifying those at risk of preeclampsia in the index pregnancy[10].

Given that certain prenatal biochemical screening hormones are associated with preeclampsia, and that preeclampsia is associated with a higher future risk of CVD in women, one ensuing question is whether abnormal prenatal hormone concentrations are associated with a higher risk of CVD after pregnancy, the goal of the current study -- whose conceptual framework is summarized in Supplementary file 1.

METHODS

This population-based cohort study was completed in Ontario, Canada, where there is universal healthcare, including prenatal screening and obstetrics care. All prenatal biochemical screening records were eligible and aggregated within the Ontario Maternal Multiple Marker Screening Database, 1993 to 2011. The uptake of prenatal screening varies geographically, between 28% and 80%[11]. Screened pregnancies in the Ontario Maternal Multiple Marker Screening Database were deterministically linked to administrative health databases at the Institute for Clinical Evaluation Sciences (ICES), by each woman's unique encoded identifiers. Specifics about the ICES databases are described elsewhere[6-8], and shown in Supplementary file 2. The study was approved by the institutional review boards at Sunnybrook Health Sciences Centre and the North York General Hospital, Toronto, Canada.

Patient involvement

Patients were not involved in the development or design of this study, participant recruitment or study dissemination.

Participants

Included were females aged 12 to 55 years who underwent prenatal biochemical screening at 11 to 20 weeks' gestation, in the years 1993 to 2011. They were included regardless of pregnancy outcome (i.e., miscarriage or ectopic pregnancy at 11 to < 20 weeks', induced abortion at 11 to < 20 weeks', stillbirth ≥ 20 weeks', or livebirth ≥ 20 weeks' gestation). Excluded were women diagnosed with any cardiac, cerebrovascular or peripheral arterial disease ≤ 5 years prior to the prenatal biochemical screening in the index pregnancy (Supplementary file 2). Also excluded were non-Ontarian residents, and those without a valid OHIP health card number (Supplementary file 2). Of all remaining deliveries, we randomly selected one pregnancy per woman as the index pregnancy in order to simplify the data analyses. These women formed the screened cohort. Those with a recognized pregnancy, but without prenatal biochemical screening, were assembled within a non-screened cohort, and analysed in a supplementary manner, as outlined below.

Exposures and outcomes

The *exposure of interest* was each prenatal biochemical screening analyte -- AFP, hCG, uE3, DIA and PAPP-A. As DIA and PAPP-A were added to prenatal screening in later years, the number of different analytes could vary per woman. The unit of analysis for each analyte was its multiple of the median (MoM), a convention used commonly in clinical reporting that standardizes test results between different labs, describing how far an individual test result deviates from the median concentration at a given gestational age. Derivation of the MoM cut-points used to define "abnormal" for each prenatal biochemical screening analyte is described below.

The *primary outcome* was a CVD composite of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy ("time zero") (Supplementary file 2). To establish a common starting point for the follow-up period for each participant, "time zero" was calculated by subtracting the gestational age (in days) at prenatal screening from the date at prenatal screening – equivalent to the estimated first day of the last menstrual period -- and then adding 365 days to that date. Starting follow-up at 365 days ensured that a woman was well past her index pregnancy, and avoided including within the CVD composite an event that was a direct consequence of a pregnancy complication, such as peripartum stroke or heart failure due to preeclampsia, for example. Censoring was on death or arrival at the end of the study (March 31, 2016).

The *secondary outcome* was a major adverse cardiovascular event (MACE), comprising all-cause mortality or any hospitalization for myocardial infarction or stroke, arising ≥ 365 days after the start of the index pregnancy, without censoring on death.

All study outcomes were identified using the International Classification of Diseases (ICD) coding system (ICD-9 before 2002 and ICD-10-CA thereafter). Several outcomes have been validated under this approach (Supplementary file 2, last column). Maternal mortality was identified from the Ontario Ministry of Health and Long Term Care's Registered Persons Database. Dissemination area income quintile and rurality were based on Statistics Canada census data.

Statistical analysis

We explored the shape of the association between each continuous analyte (in MoM) and the log hazard of CVD using univariable fractional polynomial regression and the RA2 selection algorithm, which selected the best fitting out of 44 regression models with different combinations of power transformations of the explanatory variable[12,13]. Inspection of the derived plots showed extreme outliers of MoM well beyond the 0.2nd or 99.8th percentiles, likely related to pregnancies affected by an anomaly[14], and which was not resolved by various mathematical transformations of the MoM[15,16]. After removing livebirths or stillbirths with a congenital or chromosomal anomaly diagnosis on the maternal or newborn hospital record, as well as outliers of MoM beyond the 0.2nd or 99.8th percentiles[17] (Supplementary file 3), the best-fit plots were more interpretable (Supplementary file 4). Inspection of each plot, while considering the existing literature related to placental disease, such as preeclampsia[10,18], together facilitated setting the abnormal cut-points for hCG, uE3 and PAPP-A each at ≤ 5th percentile MoM, and at ≥ 95th percentile MoM for AFP and DIA. The comparative referent for each analyte was a MoM > 5th percentile for hCG, uE3 and PAPP-A, and < 95th percentile for AFP and DIA.

The main model assessed the primary CVD composite outcome in relation to each analyte for the screened cohort, with censoring at a woman's death or arrival at the end of study period of March 31, 2016, allowing for a maximum follow-up of 22 years. Time-to-event analyses were conducted using multivariable Cox regression models, to derive a hazard ratio (HR) and 95% confidence interval (CI) for each study outcome. Assessment of the secondary outcome of MACE was not censored on death. HRs were adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (Asian, "Oriental", Black, Caucasian, Hispanic, other, missing) and gestational age (continuous) – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days before or after the start of the index pregnancy (i.e. within 2 years preceding time zero). In some years, "Asian" and "Oriental" were classified together, as were "Hispanic" and "other". As maternal weight at the time of screening was missing for about 10%

of pregnancies, it was adjusted for in an analysis restricted to those with non-missing weight (Additional analysis 1). The proportional hazards assumption was assessed by a Wald test for interaction between the exposure and a function of survival time, which did not detect a significant departure.

The CVD composite outcome was further assessed in relation to the number of abnormal prenatal biochemical screening analytes: 0 (referent), 1, 2, or ≥ 3 (Additional analysis 2). To increase the specificity of the exposure, the main model for the CVD composite outcome was also run using abnormal cut-points of ≤ 1st percentile MoM for hCG, uE3 and PAPP-A, and of ≥ 99th percentile MoM for AFP and DIA (Additional analysis 3).

In Additional analysis 4, each biochemical analyte was re-evaluated in the absence or co-presence of each of four factors known to be associated with maternal CVD: i) any chromosomal or congenital anomaly, at the time of a livebirth or stillbirth – a reflection of a higher likelihood of having abnormal biochemical screening, chronic maternal stress, and unmeasurable genetic factors in both mother and child; ii) preterm birth before 37 weeks' gestation, at the time of a livebirth[2]; iii) a pregnancy ending in a non-livebirth[6]; and iv) a maternal placental syndrome -- preeclampsia, gestational hypertension, or placental abruption or infarction[6,7], at the time of a livebirth or a stillbirth. In each model therein, the referent was a normal biochemical measure in the absence of the given perinatal or maternal factor.

A woman who undergoes prenatal screening may differ from one who does not. To address this point, from among all women in the non-screened cohort (and who also met the same criteria as for the screened cohort [Supplementary file 2]), one woman was randomly selected and 1:1 matched by year of pregnancy to a counterpart in the screened cohort. Baseline variables were compared between screened vs. non-screened cohorts using standardized differences, and the primary CVD composite outcome expressed as an adjusted HR, accounting for the matching in the aforementioned Cox regression model (Additional analysis 5).

All statistical analyses were performed using SAS version 9.4 for UNIX (SAS Institute Inc.).

RESULTS

There were 1,380,840 identified pregnancies in the Ontario Maternal Multiple Marker Screening Database during the study period, of which 1,210,146 (87.6%) formed the screened cohort (Supplementary file 3). Serum AFP was the most frequent analyte (1,120,363 pregnancies), whereas, DIA was only available in the later years (98,160 pregnancies) (Table 1). In the index pregnancy, the mean maternal age was about 30 years, 35% of participants were non-Caucasian, and 40% were primigravid. Nearly 97% of recognized pregnancies ended in a livebirth (Table 1). The rate of diabetes mellitus was about 5.5%, chronic hypertension 3.3%, dyslipidemia 1.1%, renal disease 0.3% and drug dependence/tobacco use 1.2%. The selected 95th and 99th percentile MoM cut-points for each analyte are listed at the bottom of Table 1. The median (IQR) duration of follow-up varied from 12.5 (7.9 to 17.4) years in the hCG group to 7.2 (5.3 to 9.5) years in the PAPP-A group. There were 14,666,867 person-years of follow-up among all pregnancies, largely related to AFP, hCG and uE3 (Table 1).

The primary composite CVD outcome was typically about 1.2 to 1.3 times more likely to occur in a pregnancy with an abnormal biochemical analyte, even after adjusting for other covariates (Table 2). Women with an abnormally elevated DIA had a more pronounced rate of CVD (8.3 per 10,000 person-years) than those below the cut-point (3.8 per 10,000 person-years) – equivalent to a crude HR of 2.2 (95% CI 1.5 to 3.2) and an adjusted HR of 2.0 (95% CI 1.4 to 3.0) (Table 2). Adding maternal weight to the main model did not alter these findings (Additional analysis 1, Supplementary file 5).

Women with an abnormal serum analyte were more likely to experience the secondary outcome of MACE (Table 3). Those with a high DIA had an adjusted HR of 1.9 (95% CI 1.3 to 2.6), and women with a low PAPP-A had an adjusted HR of 1.6 (95% CI 1.3 to 1.9) (Table 3).

The rate of the primary composite CVD outcome was 7.3 per 10,000 person-years with 1 abnormal analyte, 8.9 per 10,000 person-years with 2 abnormal analytes, and 14.4 per 10,000 person-years with 3 or more abnormal analytes – the latter equivalent to an adjusted HR of 2.3 (95% CI 1.1 to 4.8) relative to those with no abnormal biochemical measure (5.6 per 10,000 person-years) (Additional analysis 2, Table 4). Re-setting the respective abnormal cut-points to

the 1st or 99th percentile in the main model did not appreciably change the results (Additional analysis 3, Supplementary file 6).

The risk of the primary composite CVD outcome was notably higher in the co-presence of a recognized congenital or chromosomal anomaly at birth and either an abnormal uE3 (adjusted HR 2.0, 95% CI 1.5 to 2.7) or DIA (adjusted HR 5.3, 95% CI 2.2 to 13.0) (Additional analysis 4, Figure 1a). Women with a preterm livebirth were at significantly higher risk of CVD than those with a term livebirth, and the risk was highest in the co-presence of each abnormal analyte, though confidence limits overlapped with the exception of serum AFP (Additional analysis 4, Figure 1b, upper). The HR for CVD was only marginally higher for pregnancies resulting in non-livebirth outcome and an abnormal biochemical measure (Additional analysis 4, Figure 1c, upper). In contrast, a livebirth or stillbirth pregnancy affected by a maternal placental syndrome had a higher risk of CVD, especially with a concomitantly abnormal DIA (adjusted HR 4.7, 95% CI 2.4 to 9.4) (Additional analysis 4, Figure 1d, lower).

The non-screened and screened cohorts each contain 750,742 women (Supplementary file 7). Comparing the non-screened and screened cohorts, important standardized differences above 0.10 were only seen for maternal age, rural residence and gravidity. The primary CVD composite outcome was only marginally more likely in the non-screened vs. screened pregnancies (adjusted HR 1.1. 95% CI 1.0 to 1.1) (Additional analysis 5, Supplementary file 7).

DISCUSSION

Women with abnormal prenatal biochemical screening were found to be at modestly higher risk of a broad premature CVD composite outcome, especially those with an elevated serum DIA. The hazard for the secondary outcome of MACE was particularly higher in those with an elevated serum DIA or low PAPP-A. CVD risk was more pronounced as the number of abnormal screening analytes increased, particularly, in the co-presence of a newborn congenital anomaly, preterm birth or a maternal placental syndrome.

Limitations and strengths

Of all recognized pregnancies, about 49% had prenatal biochemical screening offered within a universal free healthcare setting. This apparently low rate may be partly explained by our inclusion of some pregnancies that ended before 20 weeks' gestation. Those who underwent screening differed minimally at baseline from those who did not, and their respective incidence rates of CVD were similar (Supplementary file 7).

One pregnancy was randomly selected per woman, which should have produced an unbiased estimate of her risk of CVD in relation to her prenatal screening test result. At the same time, we cannot comment on the effect of repeat pregnancies with abnormal prenatal screening results. Nevertheless fairly consistent between-pregnancy correlations in serum markers have been reported previously[19,20]. The study lacked direct data about the relation between each prenatal biochemical analyte and its prediction of a fetal anomaly, but did further analyze the risk of CVD by the co-presence or absence of an anomaly at birth, assuming that the related diagnostic codes were accurate. Some screened pregnancies may have also ended in a miscarriage or an induced abortion missed by the administrative datasets. Not all analytes showed a direct relation with CVD (Supplementary file 4), and while the abnormal MoM cut-point for each was derived using the best possible approach, that method has not been previously validated. At a more discrete cut-point, such as the 1st or 99th percentile, the main findings did not change, however (Supplementary file 6).

The CVD outcomes were confined to hospitalizations, arising well after pregnancy, and many of the core diagnostic codes have been shown to be valid and accurate (see final column in Supplementary file 2). While some out-of-hospital CVD events leading to death would be missed, the secondary outcome of MACE included fatalities. Potential confounders between an abnormal analyte and the risk of CVD, including diabetes mellitus, chronic kidney disease and ethnicity[21-23], were each accounted for in the models. Still, about 10% of pregnancies lacked information on maternal weight, while height and menopausal status were entirely unknown. Certainly, the relation between an abnormal analyte and maternal CVD risk can be explained by a series of factors (Supplementary file 1).

Other studies

No prior data exist about the use of prenatal biochemical screening to estimate the long-term cardiovascular health of a woman. In non-pregnant adults, a high serum PAPP-A has been evaluated in relation to plaque instability in acute coronary syndrome, but its clinical utility remains uncertain[24]. While there is some understanding of the functional effects of DIA in human reproduction and pregnancy, its role in CVD is largely unknown[25]. Hence, it remains to be determined if one or more of the abnormal analytes evaluated herein are merely reflective of placental vascular disease[3], or are persistently abnormal outside of pregnancy, as a reflection of, or contributor to, vascular injury.

Clinical and policy relevance

To date, prenatal biochemical screening has focused on fetal screening, and perhaps, placenta-related pregnancy outcomes[2,10,14]. A practical issue raised herein is which prenatal biochemical analyte, or combination of analytes, is predictive of future CVD. Furthermore, given that the ratio of serum soluble fms-like tyrosine kinase 1 to placental growth factor has recently been validated as a biochemical predictor of preeclampsia[26], it too can be evaluated as a marker of persistent endothelial dysfunction and CVD risk after pregnancy[27,28]. Regardless, we require better data about whether prenatal biochemical screening offers additive information over that provided by conventional CVD risk factors[4] and adverse events in pregnancy[5-8], such as the maternal placental syndromes or preterm delivery.

International guidelines for the prevention of stroke[29] and CVD[30] now recommend screening for CVD risk factors in women with prior maternal placental syndromes, such as preeclampsia. However, maternal recall of a hypertensive disorder in a prior pregnancy lacks sensitivity[31]. Tens of millions of women worldwide have completed prenatal biochemical screening, and although the original intent was to screen for certain congenital and chromosomal anomalies, there now exists a massive amount of data that might be applied to better estimate a woman's long-term risk of CVD. The latter need not only be done prospectively: In the age of data mining and machine learning[32] it would seem possible to identify a woman who previously had abnormal prenatal biochemical screening, and to convey that information about her higher CVD risk to her

or her healthcare provider. However, before giving consideration to these points, the current findings should be replicated in other populations of women who have undergone prenatal biochemical screening, including an evaluation of various combinations of analytes.

AUTHOR CONTRIBUTIONS

JGR, ALP: Study concept, analysis and interpretation of the data, drafting of manuscript, manuscript revision, approval of final version.

JGR, ALP, TH, WM: Interpretation of the data, manuscript revision, approval of final version.

EC: Manuscript revision, approval of final version.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

COMPETING INTERESTS

No authors have any competing interests.

ETHICS STATEMENT

The Research Ethics Boards of the Sunnybrook Health Sciences Centre and the North York General Hospital granted ethics approval.

TRANSPARENCY DECLARATION

Joel Ray, the lead author and manuscript's guarantor, affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; no important aspects of the study have been omitted; any discrepancies from the study as originally planned have been explained.

DATA SHARING STATEMENT

No additional unpublished data from the study are available. Only Alison Park is permitted to access the data.

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Table 1. Characteristics of pregnancies with prenatal biochemical screening, by analyte. One pregnancy was allowed per woman. All data are presented as number (%) unless otherwise indicated.

	Biochemical serum screening analyte (number of exclusive pregnancies)				
Characteristic	**Alphafetoprotein (N = 1,120,363)**	**Unconjugated estriol (N = 1,110,624)**	**Total human chorionic gonadotropin (N = 1,087,933)**	**Pregnancy-associated plasma protein A (N = 499,790)**	**Dimeric inhibin-A (N = 98,160)**
At the time of maternal serum screening					
Mean (SD) age, years	29.8 (5.2)	29.7 (5.1)	29.7 (5.1)	30.9 (5.1)	29.0 (5.6)
Advanced maternal age, 40 to 44 years	24,329 (2.2)	22,612 (2.0)	22,063 (2.0)	17,321 (3.5)	2,492 (2.5)
Advanced maternal age, 45 to 55 years	766 (0.1)	673 (0.1)	657 (0.1)	588 (0.1)	106 (0.1)
Ethnicity[a]					
Caucasian	715,262 (63.8)	716,494 (64.5)	696,712 (64.0)	319,036 (63.8)	49,427 (50.4)
Asian	254,898 (22.8)	250,126 (22.5)	248,412 (22.8)	119,473 (23.9)	29,345 (29.9)
Black	65,913 (5.9)	64,621 (5.8)	64,165 (5.9)	24,750 (5.0)	8,840 (9.0)
Other	20,339 (1.8)	20,188 (1.8)	19,533 (1.8)	14,930 (3.0)	4,438 (4.5)
Hispanic	1,399 (0.1)	1,388 (0.1)	1,388 (0.1)	< 6 (0.0)	0 (0.0)
Oriental	2,025 (0.2)	1,964 (0.2)	1,965 (0.2)	0 (0.0)	0 (0.0)
Unknown	60,527 (5.4)	55,843 (5.0)	55,758 (5.1)	21,600 (4.3)	6,110 (6.2)
Income quintile (Q)					
Q1 (lowest)	248,305 (22.2)	246,332 (22.2)	241,826 (22.2)	92,884 (18.6)	29,147 (29.7)
Q5 (highest)	185,690 (16.6)	183,163 (16.5)	179,033 (16.5)	96,309 (19.3)	10,929 (11.1)
Missing	3,902 (0.3)	3,870 (0.3)	3,778 (0.3)	1,316 (0.3)	618 (0.6)
Rural residence	78,552 (7.0)	80,865 (7.3)	77,186 (7.1)	26,471 (5.3)	9,416 (9.6)
Median (IQR) gravidity	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)	2 (1 to 2)	2 (1 to 3)
Primigravid	442,638 (39.5)	437,418 (39.4)	428,924 (39.4)	198,967 (39.8)	39,799 (40.5)
Gravidity unknown	14,244 (1.3)	14,112 (1.3)	13,581 (1.2)	12,536 (2.5)	1,954 (2.0)
Mean (SD) maternal weight, kg	65.3 (17.1)	65.4 (17.1)	65.2 (17.1)	59.7 (20.9)	63.8 (19.8)
Missing maternal weight	106,262 (9.5)	100,328 (9.0)	100,097 (9.2)	81,626 (16.2)	13,203 (13.5)
Type of pregnancy					
Singleton	1,068,078 (95.3)	1,068,420 (96.2)	1,045,731 (96.1)	427,389 (85.5)	96,992 (98.8)
Multi-fetal	12,057 (1.1)	6,346 (0.6)	6,347 (0.6)	158 (0.0)	152 (0.2)
Unknown	40,228 (3.6)	35,858 (3.2)	35,855 (3.3)	72,243 (14.5)	1,016 (1.0)

Mean (SD) gestational age at screening, weeks	17 (1.1)	17 (1.1)	17 (1.1)	13 (0.5)	17 (1.3)
Year of screening					
1993 to 2002	549,137 (49.0)	541,735 (48.8)	541,662 (49.8)	18,483 (3.7)	0 (0.0)
2003 to 2011	571,226 (51.0)	568,889 (51.2)	546,271 (50.2)	481,307 (96.3)	98,160 (100.0)
Outcome of the index pregnancy					
Livebirth	1,083,248 (96.7)	1,074,121 (96.7)	1,052,200 (96.7)	477,888 (95.6)	94,792 (96.6)
Stillbirth	6,291 (0.6)	6,054 (0.5)	5,974 (0.5)	2,425 (0.5)	613 (0.6)
Miscarriage	3,961 (0.4)	3,867 (0.3)	3,796 (0.3)	3,395 (0.7)	380 (0.4)
Induced abortion	3,386 (0.3)	3,310 (0.3)	3,223 (0.3)	3,546 (0.7)	421 (0.4)
Unknown outcome	23,477 (2.1)	23,272 (2.1)	22,740 (2.1)	12,536 (2.5)	1,954 (2.0)
Conditions ≤ 365 days before, or up to 365 days after, the start of pregnancy					
Diabetes mellitus	61,053 (5.4)	60,092 (5.4)	59,092 (5.4)	27,358 (5.5)	5,552 (5.7)
Chronic hypertension	36,616 (3.3)	36,216 (3.3)	35,267 (3.2)	19,695 (3.9)	3,730 (3.8)
Dyslipidemia	12,790 (1.1)	12,484 (1.1)	12,326 (1.1)	7,228 (1.4)	1,534 (1.6)
Renal disease	2,801 (0.3)	2,759 (0.2)	2,686 (0.2)	1,585 (0.3)	339 (0.3)
Drug dependence/tobacco use	13,767 (1.2)	13,831 (1.2)	13,342 (1.2)	6,365 (1.3)	1,610 (1.6)
Conditions at the livebirth or stillbirth delivery					
Congenital or chromosomal anomaly	41,178 (3.8)	40,774 (3.8)	39,961 (3.8)	14,331 (3.0)	2,645 (2.8)
Preeclampsia or eclampsia	20,974 (1.9)	20,528 (1.9)	20,283 (1.9)	5,146 (1.1)	1,021 (1.1)
Gestational hypertension	33,194 (3.0)	32,874 (3.0)	31,856 (3.0)	19,222 (4.0)	3,605 (3.8)
Placental abruption	9,973 (0.9)	9,813 (0.9)	9,632 (0.9)	3,674 (0.8)	815 (0.9)
Placental infarction	6,560 (0.6)	6,462 (0.6)	6,373 (0.6)	1,810 (0.4)	279 (0.3)
Conditions at the livebirth delivery					
Preterm birth < 37 weeks' gestation	73,204 (6.7)	69,601 (6.4)	68,245 (6.4)	31,419 (6.5)	6,379 (6.7)
Prenatal biochemical serum screening analyte[b]					
Number of pregnancies	1,055,118	1,045,859	1,024,401	473,091	93,518
95th percentile MoM cut-point	1.83	0.49	0.51	0.38	2.20
99th percentile MoM cut-point	2.52	0.32	0.34	0.25	3.21
Median (IQR) number of years of follow-up, from ≥ 365 days after the start of the index pregnancy	12.3 (7.7 to 17.4)	12.2 (7.6 to 17.3)	12.5 (7.9 to 17.4)	7.2 (5.3 to 9.5)	7.6 (5.7 to 9.4)
Total number of person-years of follow-up, from ≥ 365 days after the start of the index pregnancy	14,041,650	13,86,0187	13,748,671	3,751,052	740,020

[a]In some years, "Asian" and "Oriental" were classified together, as were "Hispanic" and "other"

[b]Cut-points were derived from pregnancies resulting in a livebirth or stillbirth without a diagnosis of a congenital or chromosomal anomaly at the time of the index birth.

IQR, Inter-Quartile Range; MoM, Multiple of the Median; SD, Standard Deviation

Table 2. Risk of the cardiovascular disease composite outcome of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy, in association with an abnormal cut-point of the 5th or 95th percentile of the multiple of the median (MoM) for a given serum analyte.

		Cardiovascular disease composite outcome		
Abnormal serum analyte	**Cut-points used to define normal and abnormal**	**No. (incidence rate per 10,000 person years)**	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)[a]**
High alphafetoprotein	**Normal**: ≤ 95th percentile MoM (n = 763,716)	5600 (5.9)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 43,576)	420 (7.4)	1.2 (1.1 to 1.3)	1.2 (1.1 to 1.3)
Low total human chorionic gonadotropin	**Normal**: ≥ 5th percentile MoM (n = 741,491)	5670 (6.0)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 43,136)	224 (5.8)	1.3 (1.2 to 1.4)	1.2 (1.1 to 1.4)
Low unconjugated estriol	**Normal**: ≥ 5th percentile MoM (n = 756,958)	5332 (5.8)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 42,405)	585 (8.5)	1.3 (1.1 to 1.5)	1.3 (1.2 to 1.4)
High dimeric inhibin-A	**Normal**: ≤ 95th percentile MoM (n = 87,097)	251 (3.8)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 4729)	30 (8.3)	2.2 (1.5 to 3.2)	2.0 (1.4 to 3.0)
Low pregnancy-associated plasma protein A	**Normal**: ≥ 5th percentile MoM (n = 371,097)	990 (3.6)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 22,302)	84 (5.1)	1.4 (1.1 to 1.8)	1.3 (1.1 to 1.7)

[a]Adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighborhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (Asian, Black Caucasian, Hispanic, Oriental, other, missing) and gestational age (continuous) – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored on death or arrival at the end of study date of March 31, 2016.

Table 3. Risk of the secondary major adverse cardiovascular event (MACE) outcome, comprising all-cause mortality or any hospitalization for myocardial infarction or stroke, arising ≥ 365 days after the start of the index pregnancy, in association with an abnormal cut-point of the 5th or 95th percentile of the multiple of the median (MoM) for a given serum analyte.

		MACE composite outcome		
Abnormal serum analyte	**Cut-points used to define normal and abnormal**	**No. (incidence rate per 10,000 person years)**	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)[a]**
High alphafetoprotein	**Normal**: ≤ 95th percentile MoM (n = 763,716)	7183 (7.5)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 43,576)	589 (10.3)	1.3 (1.2 to 1.4)	1.3 (1.2 to 1.4)
Low beta human chorionic gonadotropin	**Normal**: ≥ 5th percentile MoM (n = 741,491)	7291 (7.7)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 43,136)	291 (7.5)	1.3 (1.1 to 1.4)	1.2 (1.0 to 1.3)
Low unconjugated estriol	**Normal**: ≥ 5th percentile MoM (n = 756,958)	6859 (7.4)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 42,405)	756 (11.0)	1.3 (1.2 to 1.4)	1.3 (1.2 to 1.4)
High dimeric inhibin-A	**Normal**: ≤ 95th percentile MoM (n = 87,097)	306 (4.7)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 4729)	37 (10.3)	2.2 (1.6 to 3.1)	1.9 (1.3 to 2.6)
Low pregnancy-associated plasma protein A	**Normal**: ≥ 5th percentile MoM (n = 371,097)	1179 (4.3)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 22,302)	117 (7.0)	1.6 (1.4 to 2.0)	1.6 (1.3 to 1.9)

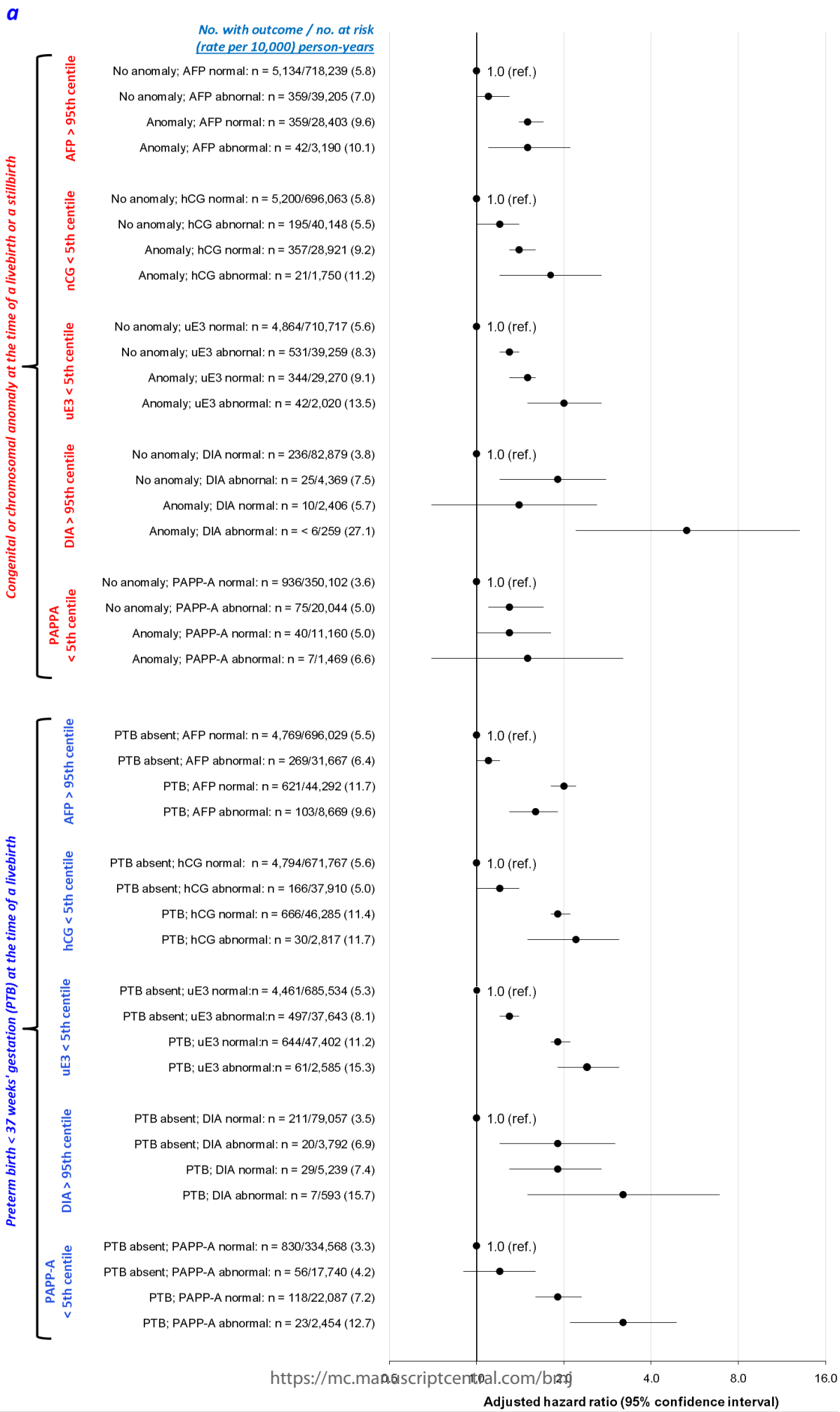
[a]Adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (Asian, Black Caucasian, Hispanic, Oriental, other, missing) and gestational age (continuous) – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored only on arrival at the end of study date of March 31, 2016.

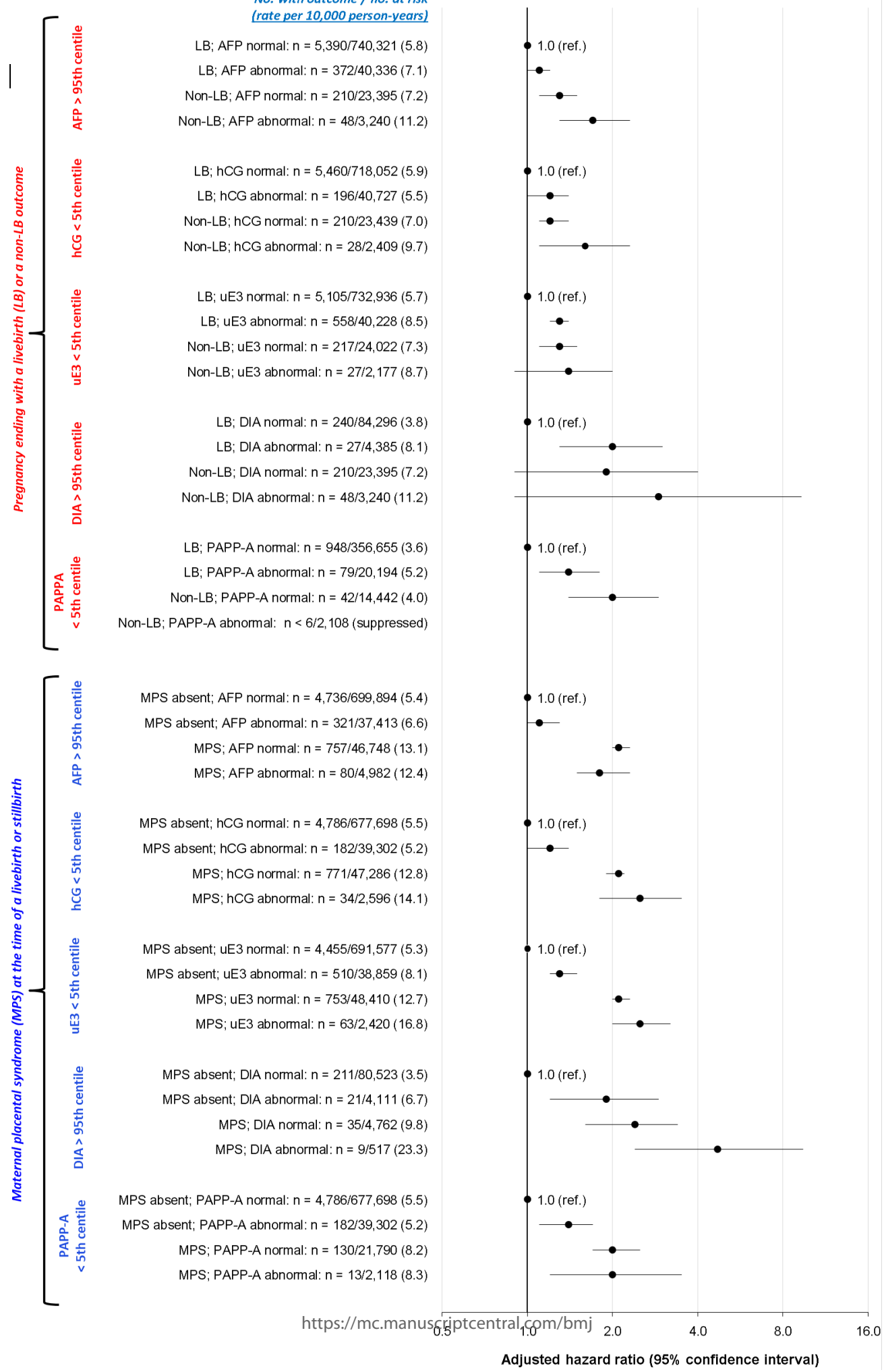
Table 4 (Additional analysis 2). Risk of the cardiovascular disease composite outcome of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy, in association with the number of abnormal serum analytes, based on the 5th or 95th percentile of the multiple of the median (MoM) cut-points.

	Cardiovascular disease composite outcome		
Number of abnormal serum analytes	**No. (incidence rate per 10,000 person years)**	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)[a]**
0 (n = 715,367)	4897 (5.6)	1.0 (Ref.)	1.0 (Ref.)
1 (n = 132,664)	1,168 (7.3)	1.3 (1.2 to 1.4)	1.3 (1.2 to 1.3)
2 (n = 7038)	65 (8.9)	1.7 (1.4 to 2.2)	1.6 (1.2 to 2.0)
≥ 3 (n = 467)	7 (14.4)	2.8 (1.3 to 5.9)	2.3 (1.1 to 4.8)

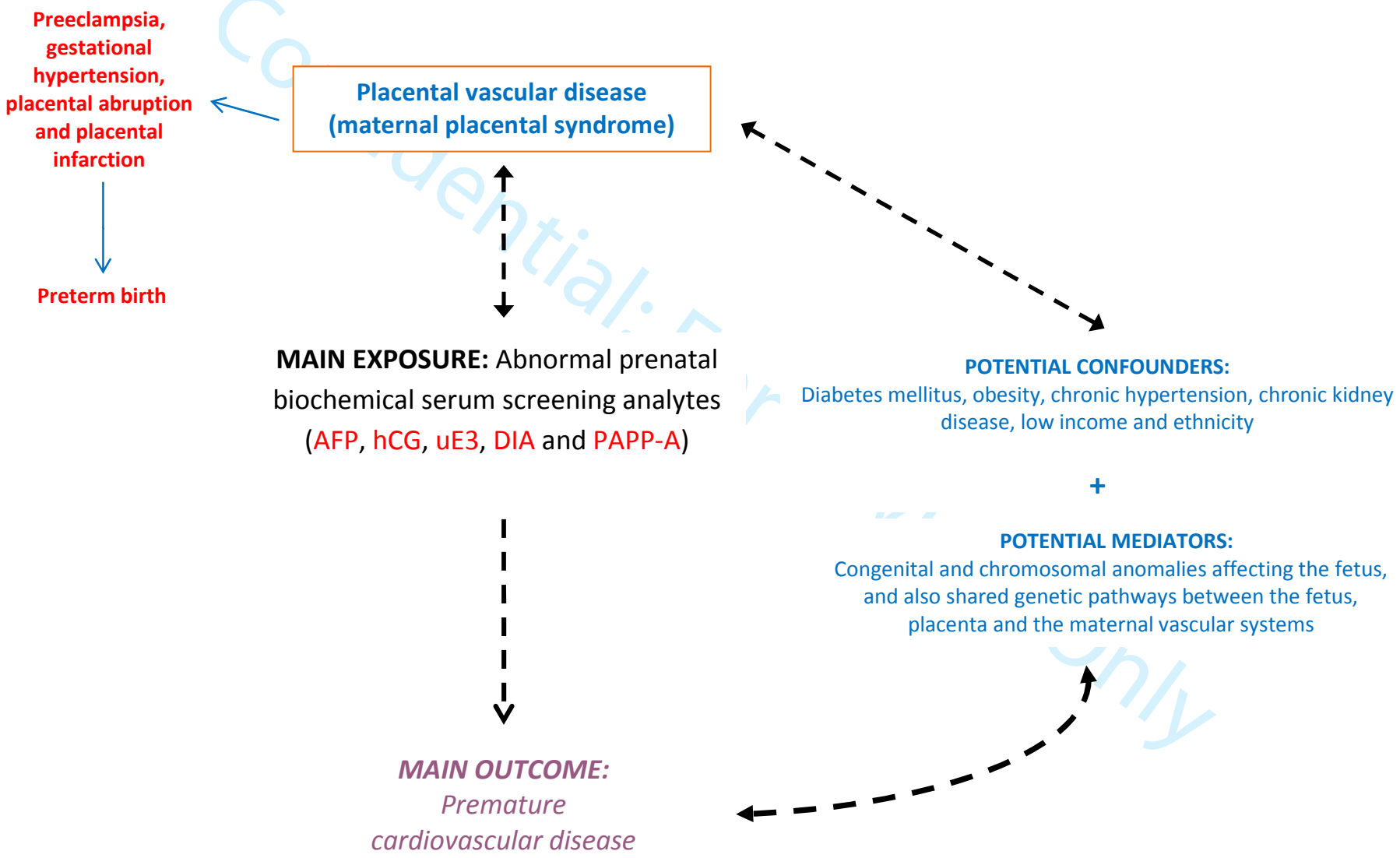
[a]Adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (Asian, Black Caucasian, Hispanic, Oriental, other, missing) and gestational age (continuous) – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored on death or arrival at the end of study date of March 31, 2016.

Figure 1 (Additional analysis 4). Evaluation of each biochemical analyte in the absence or presence of a chromosomal or congenital anomaly at the time of a livebirth or a stillbirth (*a*, upper), preterm birth before 37 weeks' gestation at the time of a livebirth (*a*, lower), a pregnancy ending with a non-livebirth (*b*, upper), and a maternal placental syndrome -- preeclampsia, gestational hypertension, or placental abruption or infarction -- at the time of a livebirth or a stillbirth (*b*, lower). In each model, the referent was a normal biochemical measure in conjunction with the absence of the perinatal or maternal factor. Models are adjusted for maternal age, gravidity, neighbourhood income quintile, rural residence and gestational age – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days before or 365 days after the start of the index pregnancy.




b
No. with outcome / no. at risk (rate per 10,000 person-years)
Pregnancy ending with a livebirth (LB) or a non-LB outcome
AFP > 95th centile
LB; AFP normal: n = 5,390/740,321 (5.8)
LB; AFP abnormal: n = 372/40,336 (7.1)
Non-LB; AFP normal: n = 210/23,395 (7.2)
Non-LB; AFP abnormal: n = 48/3,240 (11.2)
hCG < 5th centile
LB; hCG normal: n = 5,460/718,052 (5.9)
LB; hCG abnormal: n = 196/40,727 (5.5)
Non-LB; hCG normal: n = 210/23,439 (7.0)
Non-LB; hCG abnormal: n = 28/2,409 (9.7)
uE3 < 5th centile
LB; uE3 normal: n = 5,105/732,936 (5.7)
LB; uE3 abnormal: n = 558/40,228 (8.5)
Non-LB; uE3 normal: n = 217/24,022 (7.3)
Non-LB; uE3 abnormal: n = 27/2,177 (8.7)
DIA > 95th centile
LB; DIA normal: n = 240/84,296 (3.8)
LB; DIA abnormal: n = 27/4,385 (8.1)
Non-LB; DIA normal: n = 210/23,395 (7.2)
Non-LB; DIA abnormal: n = 48/3,240 (11.2)
PAPPA < 5th centile
LB; PAPP-A normal: n = 948/356,655 (3.6)
LB; PAPP-A abnormal: n = 79/20,194 (5.2)
Non-LB; PAPP-A normal: n = 42/14,442 (4.0)
Non-LB; PAPP-A abnormal: n < 6/2,108 (suppressed)
Maternal placental syndrome (MPS) at the time of a livebirth or stillbirth
AFP > 95th centile
MPS absent; AFP normal: n = 4,736/699,894 (5.4)
MPS absent; AFP abnormal: n = 321/37,413 (6.6)
MPS; AFP normal: n = 757/46,748 (13.1)
MPS; AFP abnormal: n = 80/4,982 (12.4)
hCG < 5th centile
MPS absent; hCG normal: n = 4,786/677,698 (5.5)
MPS absent; hCG abnormal: n = 182/39,302 (5.2)
MPS; hCG normal: n = 771/47,286 (12.8)
MPS; hCG abnormal: n = 34/2,596 (14.1)
uE3 < 5th centile
MPS absent; uE3 normal: n = 4,455/691,577 (5.3)
MPS absent; uE3 abnormal: n = 510/38,859 (8.1)
MPS; uE3 normal: n = 753/48,410 (12.7)
MPS; uE3 abnormal: n = 63/2,420 (16.8)
DIA > 95th centile
MPS absent; DIA normal: n = 211/80,523 (3.5)
MPS absent; DIA abnormal: n = 21/4,111 (6.7)
MPS; DIA normal: n = 35/4,762 (9.8)
MPS; DIA abnormal: n = 9/517 (23.3)
PAPP-A < 5th centile
MPS absent; PAPP-A normal: n = 4,786/677,698 (5.5)
MPS absent; PAPP-A abnormal: n = 182/39,302 (5.2)
MPS; PAPP-A normal: n = 130/21,790 (8.2)
MPS; PAPP-A abnormal: n = 13/2,118 (8.3)
1.0 (ref.)
0.5
1.0
2.0
4.0
8.0
16.0
Adjusted hazard ratio (95% confidence interval)

Supplementary file 1. Conceptual framework. The main study exposure is an abnormal prenatal biochemical screening analyte (alphafetoprotein [AFP], total human chorionic gonadotropin [hCG], unconjugated estriol [uE3], dimeric inhibin-A [DIA] and pregnancy-associated plasma protein A [PAPP-A]) The main study outcome is cardiovascular disease. Potential confounders and mediators of the relation between placental vascular disease or an abnormal prenatal biochemical screening analyte and subsequent cardiovascular disease are also shown.



Supplementary file 2. Variables used to define cohort entry and exclusion criteria, study exposures, outcomes and adjustment variables.

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
Cohort entry criterion	May 20, 1993 to December 29, 2011	Women aged 12 to 55 who underwent maternal serum screening from 11 to 20 weeks' gestation	--	{Ontario Maternal Serum Screening (OMSS) database}	
Exclusion criteria	Within ≤ 5 years before the maternal serum screening date in the index pregnancy	Coronary artery disease	410, 411, 413, 414.0, 429.2 [I20, I21, I24, I25.0, I25.1, I51.3] 48* [1HZ80*, 1IJ50*, 1IJ55*, 1IJ57*, 1IJ76*, 1IJ80*, 1IK80*, 1IK87*, 1IL35*, 2IL70*, 3IP10*]	410, 412, 413, 429	
	Same	Cardiac dysrhythmia	427.3 [I48], 427.1 [I47.2], 427.4 [I49.0], 427.2 [I47.2]	427	
	Same	Heart failure	428 [I50]	428	
	Same	Pericardial disease, endocarditis, myocarditis, cardiomyopathy or peripartum cardiomyopathy, valvular heart disease	420-425 [I30-I43], 674.5 [O90.3], 390-392 [I00-I02, I05-I09]	398	
	Same	Congenital heart disease	745-746, 7470, 7471, 7472, 7473, 7474 [Q20-Q26]	745-747	
	Same	Cerebrovascular disease	433, 434, 436, 437.0, 437.1, 437.8, 437.9 [G46, I63.0-I66.9, I67.2, I67.8] 50.11*, 50.12* [1JE57*, 1JW57*, 1JX57*]	432, 436, 437	
	Same	Peripheral arterial disease	440.0, 440.2, 444 [I70.0, I70.2, I74], 50.18*, 50.28*, 50.38*, 51.24*, 51.25*, 51.26*, 51.29* [1JM76*, 1JW76*, 1JX76*, 1KA76*, 1KE76*, 1KG57*, 1KR76*, 1KR87LA*, 1KT76*, 1ID76MU*, 1KG76*, 1KG87*]	443	

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
	At the time of the maternal serum screening	Non-Ontario resident or invalid OHIP number	--	{Registered Persons Database (RPDB) contains demographic information and encrypted healthcare numbers for all individuals eligible for OHIP}	
	Same	Maternal serum screening results missing, duplicated or outside of 11^{+0}-13^{+6} weeks (pregnancy associated plasma protein A) or 15^{+0}-20^{+6} weeks (all other analytes)	--	{OMSS}	
`	Same	Date of last contact was prior to or on the date of the maternal serum screening	--	{RPDB}	
	Same	Implausibly low total human chorionic gonadotropin ≤ 0.1^{st} percentile Multiple of the Median (MoM)	--	{OMSS}	
Main study exposures	At the time of the maternal serum screening	Abnomal serum alphafetoprotein (AFP)	--	{OMSS}	
	Same	Abnormal serum total human chorionic gonadotropin (hCG)	--	{OMSS}	
	Same	Abnormal serum unconjugated estriol (uE3)	--	{OMSS}	

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
	Same	Abnormal serum dimeric inhibin-A (DIA)	--	{OMSS}	
	Same	Abnormal serum pregnancy-associated plasma protein A (PAPP-A)	--	{OMSS}	
Study outcomes	Starting at 365 days after the start of the index pregnancy (January 27, 1994 to March 31, 2016) = "t_0"	#1: Cardiovascular disease composite of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, or any hospitalization for heart failure or dysrhythmia. Censored on death or at the end of follow-up.	Coronary artery disease: 410, 411, 413, 414.0, 429.2, [I20, I21, I24, I25.0, I25.1, I51.3], 48* [1HZ80*, 1IJ50*, 1IJ55*, 1IJ57*, 1IJ76*, 1IJ80*, 1IK80*, 1IK87*, 1IL35*, 2IL70*, 3IP10*] Cerebrovascular disease: 431, 433, 434, 436, 437.0, 437.1 [G46, I61, I63.0-I66.9, I67.2, I67.8], 50.11*, 50.12* [1JE57*, 1JW57*, 1JX57*] Peripheral arterial disease: 440.0, 440.2, 444 [I70.0, I70.2, I74], 50.18*, 50.28*, 50.38*, 51.24*, 51.25*, 51.26*, 51.29* [1JM76*, 1JW76*, 1JX76*, 1KA76*, 1KE76*, 1KG57*, 1KR76*, 1KR87LA*, 1KT76*, 1ID76MU, 1KG76*, 1KG87*] Heart failure: 428 [I50] Cardiac dysrhythmia: 427.3 [I48], 427.1 [I47.2], 427.4 [I49.0], 427.2 [I47.9]	--	https://www.ncbi.nlm.nih.gov/pubmed/12177647 https://www.ncbi.nlm.nih.gov/pubmed/27426016 https://www.ncbi.nlm.nih.gov/pubmed/12177647 https://www.ncbi.nlm.nih.gov/pubmed/12177647

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
	Same	#2: Major Adverse Cardiovascular Events (MACE) composite outcome of myocardial infarction, stroke or death. Censored on end of follow-up only.	Myocardial infarction: 410 [I21] Stroke: 431, 433, 434 [I61, I63, I64]	{Death in RPDB}	
Covariates	At the time of the maternal serum screening	Maternal age	--	{OMSS}	
	Same	Maternal area-level income quintile	--	{Statistics Canada census data}	
	Same	Maternal rural residence	--	{Statistics Canada census data}	
	Same	Maternal ethnicity	--	{OMSS}	
	At time of the maternal serum screening in the index pregnancy	Maternal weight (kg)	--	{OMSS}	
	At the time of the maternal serum screening (if available), otherwise calculated from all pregnancies in	Gravidity	Calculated based on all pregnancy outcomes in DAD, SDS, NACRS and OHIP	{OMSS}	
	Up to 2 years before "t_0" (i.e. 365 days before the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy)	Diabetes mellitus	250, 648.8 [E10, E11, E13, E14, O244]	250 or {OMSS}	https://www.ncbi.nlm.nih.gov/pubmed/11874939

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
	Same	Chronic hypertension	401, 405, 642.0-642.2, 642.7 [I10, I15, O10, O11]	401	https://www.ncbi.nlm.nih.gov/pubmed/19858407
	Same	Dyslipidemia	272.0, 272.1, 272.3, 272.4, 272.5 [E78]	272	
	Same	Renal disease	584.5-584.9, 669.3, 958.5 634.3, 635.3, 636.3, 637.3, 638.3, 639.3, 250.4x, 274.1x, 403.xx, 404.xx, 405.01, 405.11, 405.91, 440.1, 446.21, 581.xx, 582.xx, 583.xx, 585.x, 586, 587.x, 588.0, 588.8x, 588.9, 590.0x, 593.7x, 791.0, 794.4 [N17.x, O08.4, T79.5, O90.4, E10.20, E10.21, E10.23, E11.20, E11.21, E11.23, M10.39, I12, I13, I15.0, I70.1, M31.0, N01.x, N03.x, N04.x, N05.x, N06.x, N07.x, N08.x, N11.x, N12, N13.7, N13.8, N13.9, N14.x, N15.x, N16.x, N18.x, N19.x, N25.0, N25.8, N25.9, N26, R80, R94.4]	403, 581, 585	https://www.ncbi.nlm.nih.gov/pubmed/23560464
	Same	Drug dependence or tobacco use	291, 292, 2940, 303, 304, 305, 648.3, 649.0, 6555, 980 [F10-F19, F55, G312, O354, O355, T51, T652, Z720, Z721, Z722]	291, 292, 303, 304, 305	
Censoring variables	Starting at 365 days after the start of the index pregnancy (January 27, 1994 to March 31, 2016) = "t_0"	Death (for study outcome #1 only)	--	{RPDB}	
	March 31, 2016	End of study	--	--	

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
Stratification variables	Determined by the end of the pregnancy	Livebirth	M_STILLBIRTH='F' in MOMBABY (see https://datadictionary.ices.on.ca/Applications/DataDictionary/Library.aspx?Library=MOMBABY)	--	
	Same	Stillbirth[a]	M_STILLBIRTH='T' in MOMBABY (see https://datadictionary.ices.on.ca/Applications/DataDictionary/Library.aspx?Library=MOMBABY)	--	
	Same	Induced abortion[a]	635 [O04, O08] AND 81.01*, 87.0*, 87.1*, 87.21*, 87.29* [5CA89*, 5CA88*, 5CA20FK*, 5CA24*] AND prsuff not in 8, 9	Fee Code: S785, A920, P001 and ICD-9: 635, 895; OR Fee Code: S752 and ICD-9: 635, 895	
	Same	Spontaneous abortion (miscarriage) or ectopic pregnancy[a]	632, 633, 634 [O00, O021, O03]	Fee Codes: A920, P001 and ICD-9: 632, 633, 634, 640; OR Fee Code: A922; OR Fee Codes: S752, S785 and ICD-9: 632, 633, 634, 640; OR Fee Codes: S756, S768, S784, S770	
	Same	Unknown[a]	Pregnancy outcome not documented in the CIHI-DAD, SDS or NACRS.	Pregnancy outcome not documented in OHIP.	
Stratification variables	At the time of the index livebirth or stillbirth delivery hospitalization	Preeclampsia or eclampsia[b]	642.4-642.7 [O11, O14, O15]	--	https://www.ncbi.nlm.nih.gov/pubmed/19527567

Assessment	Timing	Disease or procedure or condition	ICD-9 [ICD-10-CA] codes	OHIP ICD-9 diagnostic codes or fee codes {or other source if in parentheses}	PubMed link to related validation studies for some codes
	Same	Gestational hypertension[b]	642.3, 642.9 [O13]	--	
	Same	Placental abruption[b]	641.2 [O45]	--	
	Same	Placental infarction[b]	656.7 [O43.1, O43.801, O43.803, O43.809, O43.811, O43.813, O43.819, O43.9]	--	
	Same	Congenital or chromosomal anomaly	648.9, 655.0, 655.1, 74, 75 [O28, O35.0, O35.1, Q]	--	
	At the time of the index livebirth delivery hospitalization	Preterm live birth < 37 completed weeks' gestation	Before FY2002: 644.2, 765 [O60, P07.2, P07.3] FY2002 onward: M_GESTWKS_DEL or B_GESTWKS_DEL < 37 in MOMBABY (https://datadictionary.ices.on.ca/Applications/DataDictionary/Library.aspx?Library=MOMBABY)	--	

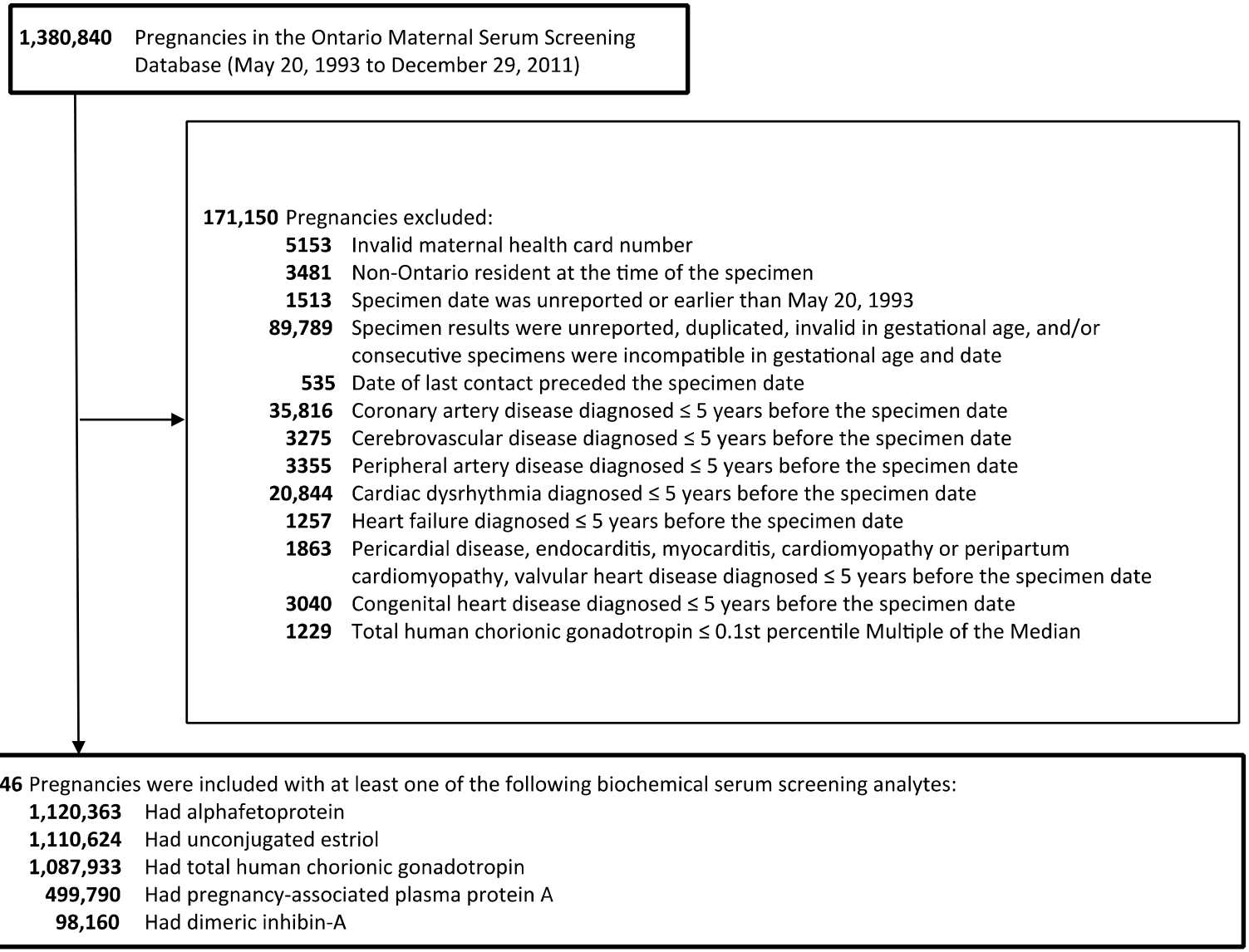
*Indicates coding by Canadian Classification of Procedures (corresponding to ICD-9 years) or Canadian Classification of Interventions (corresponding to ICD-10-CA years)

[a]Each is a pregnancy outcome used to broadly define non-livebirth.

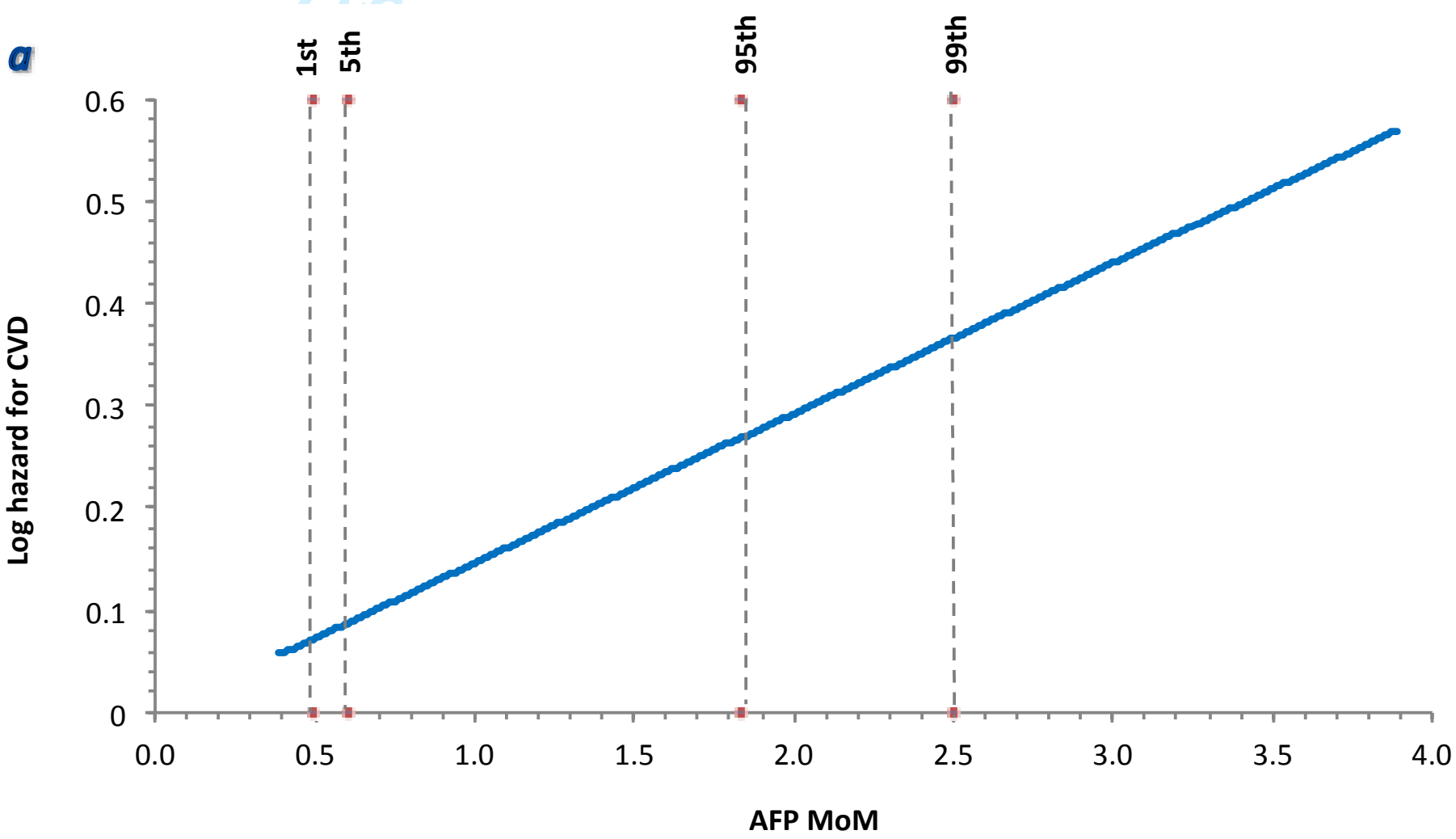
[b]Each is a condition used to broadly define a Maternal Placental Syndrome (MPS).

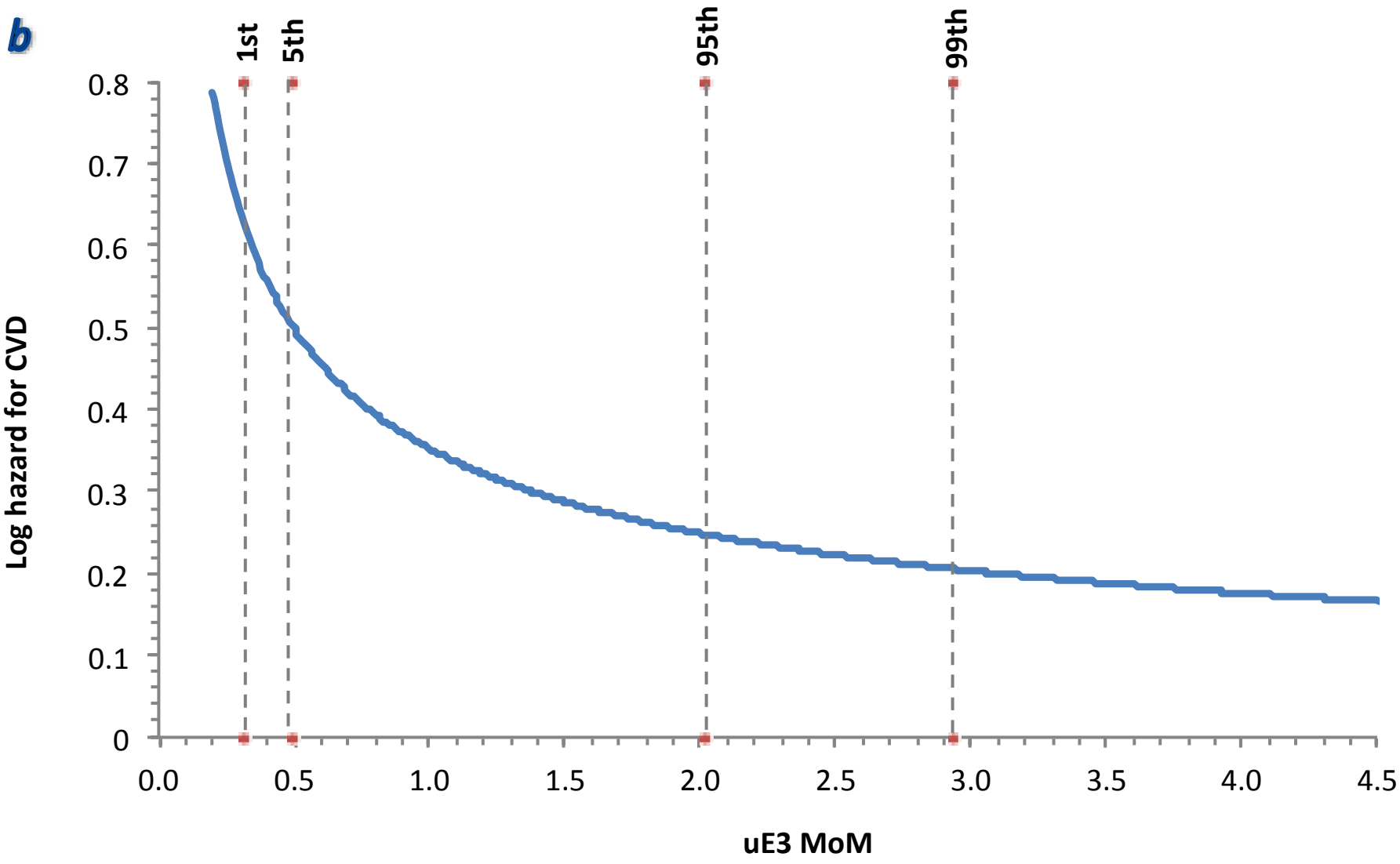
CIHI: Canadian Institute for Health Information; DAD: Discharge Abstract Database; ICD-9: International Classification of Diseases, 9th Revision; ICD-10-CA: International Classification of Diseases, 10th Revision, Canada; NACRS: National Ambulatory Care Reporting System; OHIP: Ontario Health Insurance Plan; SDS: Same Day Surgery Database

Supplementary file 3. Flow chart of inclusion and exclusions

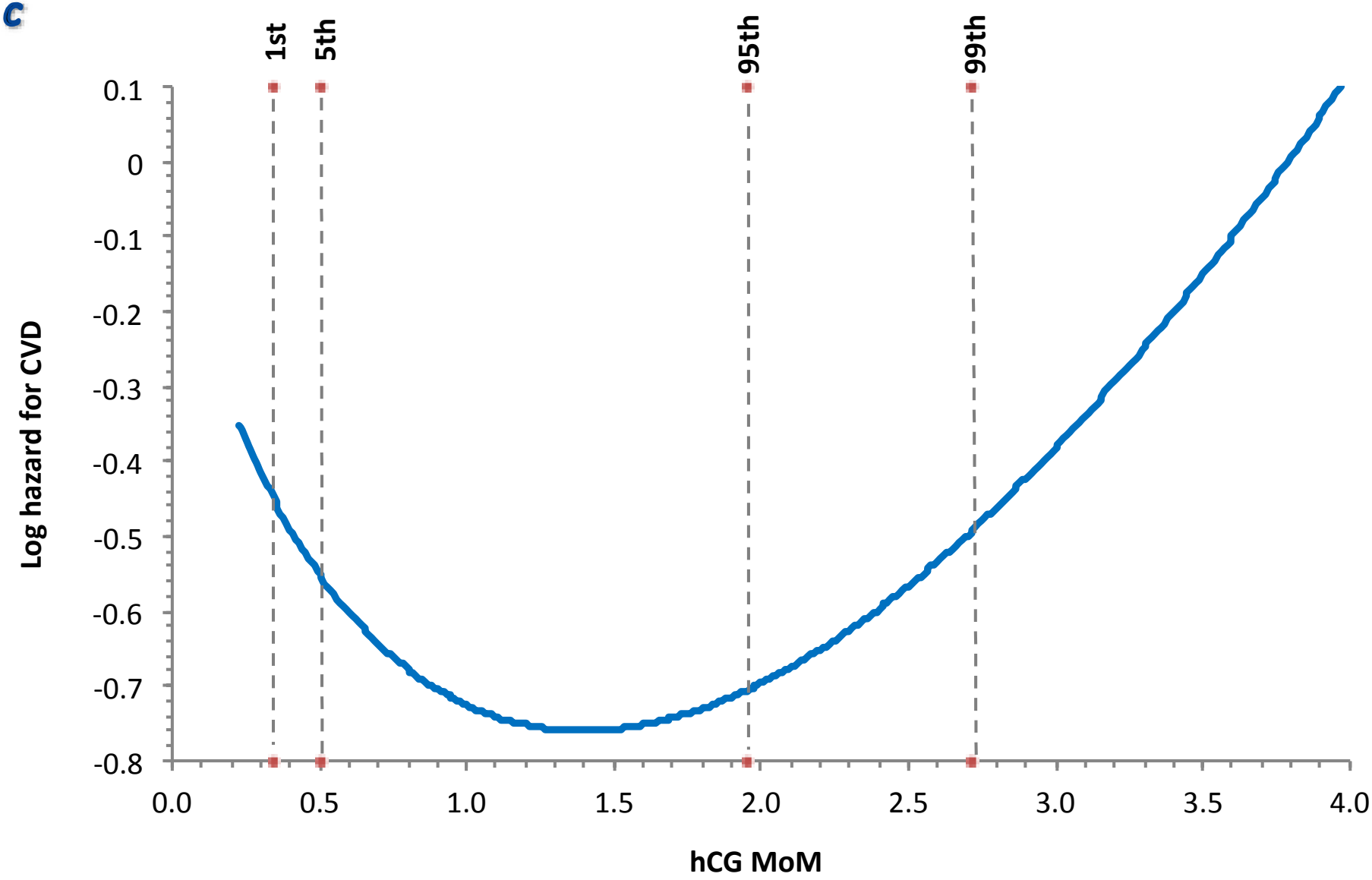


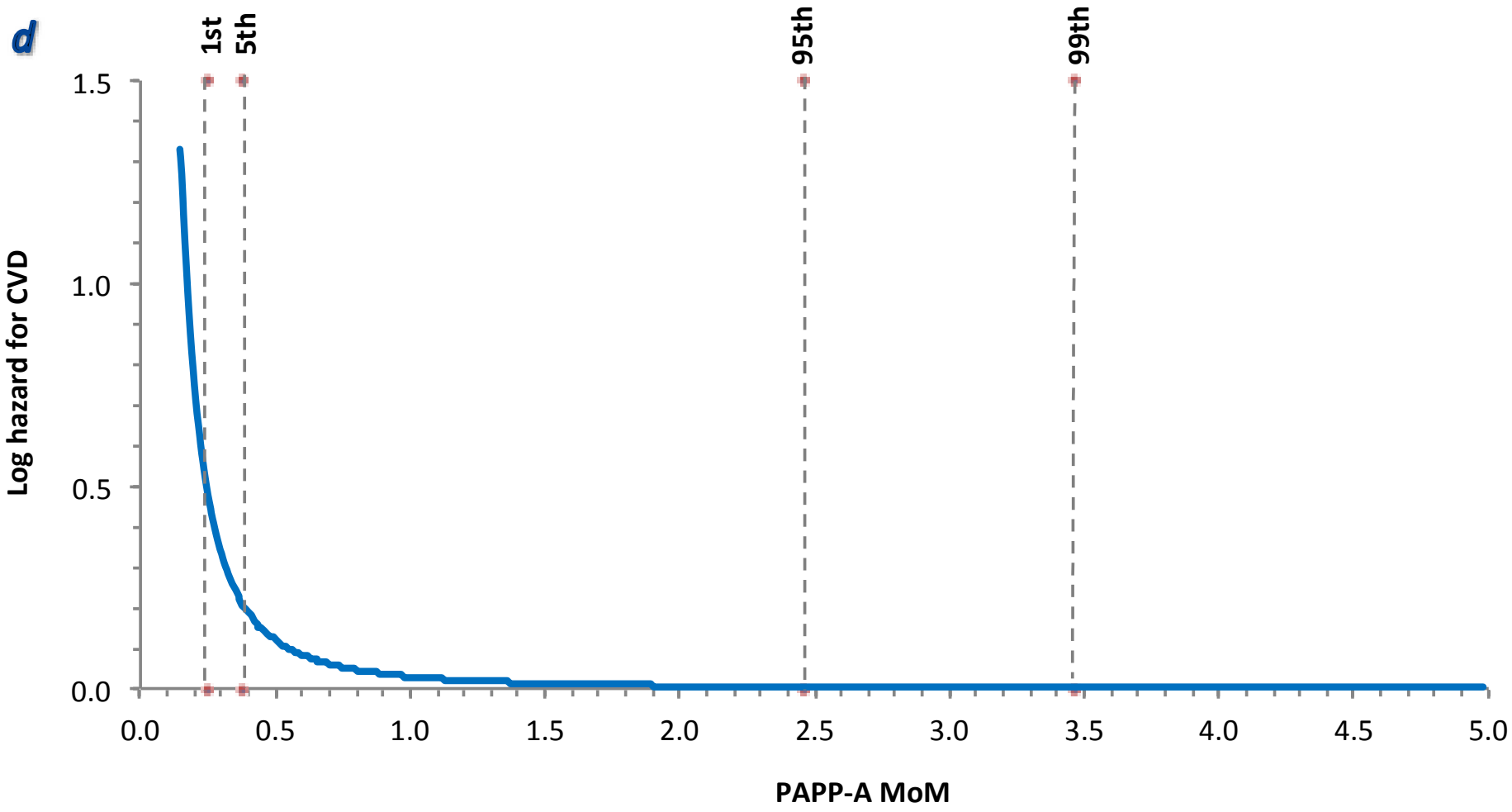
Supplementary file 4. Fractional polynomial derived best fitting plots of the continuous relation between the multiple of the median (MoM) for serum alphafetoprotein (AFP) (*a*), unconjugated estriol (uE3) (*b*), total human chorionic gonadotropin (hCG) (*c*), pregnancy-associated plasma protein A (PAPP-A) (*d*) and dimeric inhibin-A (DIA) (*e*), and the respective hazard for the cardiovascular disease (CVD) composite outcome of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia. The 1st, 5th, 95th and 99th percentile cut-points for each analyte are shown by vertical dashed lines.

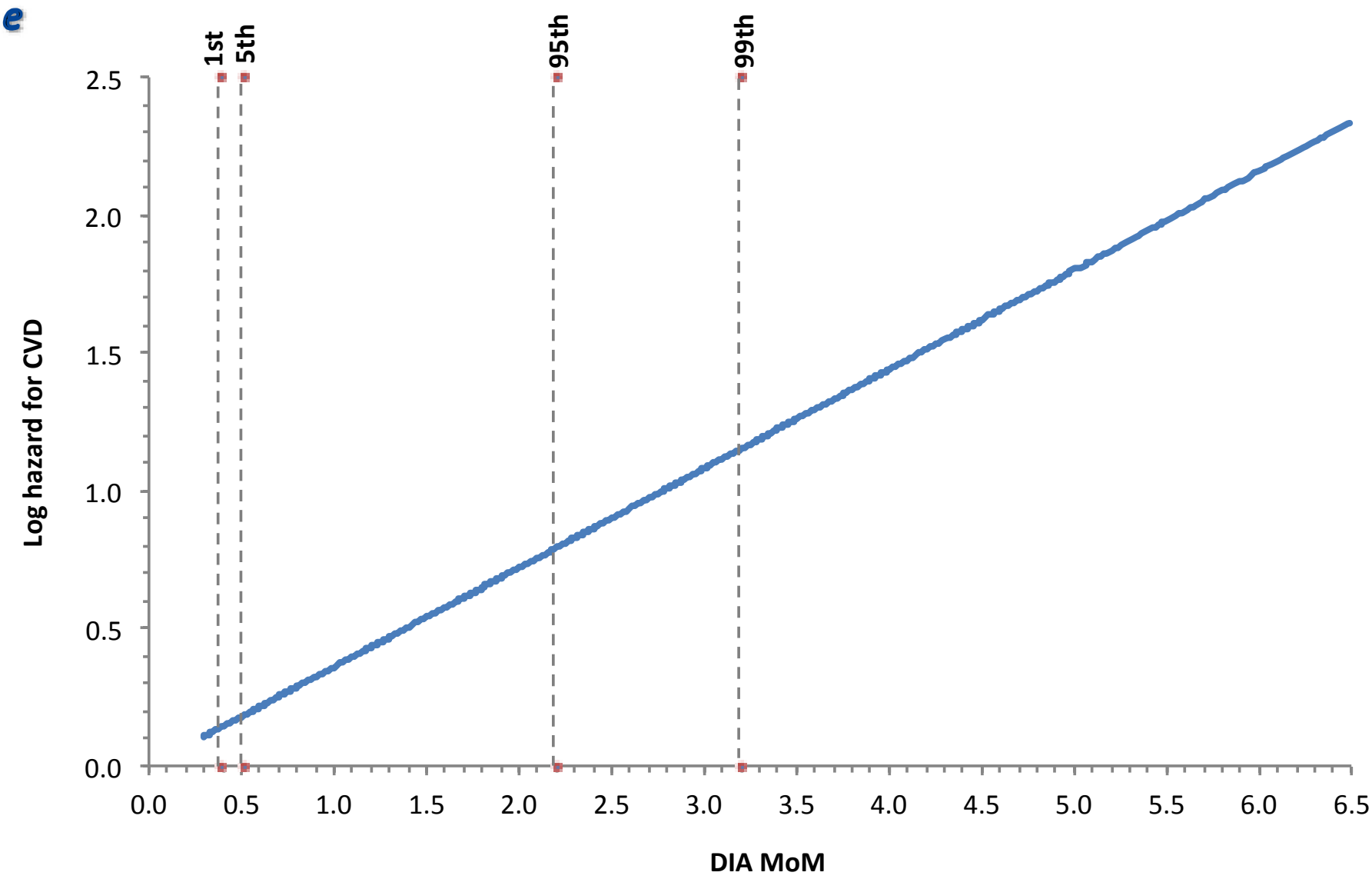



b
1st
5th
95th
99th
Log hazard for CVD
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
uE3 MoM

c


1st
5th
95th
99th
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
Log hazard for CVD
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
hCG MoM


d
1st
5th
95th
99th
1.5
1.0
0.5
0.0
Log hazard for CVD
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
PAPP-A MoM


1st
5th
95th
99th
Log hazard for CVD
DIA MoM
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5

Supplementary file 5 (Additional analysis 1). Risk of the cardiovascular disease composite outcome of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy, in association with an abnormal cut-point of the 5th or 95th percentile of the multiples of the median (MoM) for a given serum analyte. This analysis further adjusts for **maternal weight** at the time of prenatal biochemical screening in a sub-set of all the pregnancies.

		Cardiovascular disease composite outcome		
Abnormal serum analyte	**Cut-points used to define normal and abnormal**	**No. (incidence rate per 10000 person years)**	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)***
High alphafetoprotein	**Normal**: ≤ 95th percentile MoM (n = 688,426)	5,281 (5.9)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 39,317)	404 (7.6)	1.2 (1.1 to 1.3)	1.1 (1.0 to 1.3)
Low beta human chorionic gonadotropin	**Normal**: ≥ 5th percentile MoM (n = 673,615)	5,408 (6.0)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 36,205)	197 (5.8)	1.3 (1.2 to 1.4)	1.2 (1.0 to 1.4)
Low unconjugated estriol	**Normal**: ≥ 5th percentile MoM (n = 683,733)	5,055 (5.8)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 40,674)	553 (8.3)	1.3 (1.1 to 1.5)	1.3 (1.2 to 1.4)
High dimeric inhibin-A	**Normal**: ≤ 95th percentile MoM (n = 75,316)	217 (3.7)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 95th percentile MoM (n = 4147)	29 (8.9)	2.2 (1.5 to 3.2)	2.3 (1.5 to 3.4)
Low pregnancy-associated plasma protein A	**Normal**: ≥ 5th percentile MoM (n = 309,693)	861 (3.6)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 5th percentile MoM (n = 17,760)	67 (4.9)	1.4 (1.1 to 1.8)	1.3 (1.0 to 1.6)

*Adjusted for maternal weight (continuous), age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (category) and gestational age – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored on death or end of study (March 31, 2016).

Supplementary file 6 (Additional analysis 3). Incidence rates and hazard ratios for the cardiovascular disease composite outcome of any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy, in association with an abnormal cut-point of the 1st or 99th percentile of the multiple of the median (MoM) for a given serum analyte.

		Cardiovascular disease composite outcome		
Abnormal serum analyte	**Cut-points used to define normal and abnormal**	**No. (incidence rate per 10,000 person years)**	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)[a]**
High alphafetoprotein	**Normal**: ≤ 99th percentile MoM (n = 797,022)	5922 (6.0)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 99th percentile MoM (n = 10,270)	98 (7.3)	1.2 (1.0 to 1.4)	1.1 (0.9 to 1.3)
Low total human chorionic gonadotropin	**Normal**: ≥ 1st percentile MoM (n = 774,392)	5846 (6.0)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 1st percentile MoM (n = 10,235)	48 (5.4)	1.2 (0.9 to 1.6)	1.1 (0.8 to 1.5)
Low unconjugated estriol	**Normal**: ≥ 1st percentile MoM (n = 789,151)	5762 (5.9)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 1st percentile MoM (n = 10,212)	145 (8.8)	1.3 (1.1 to 1.5)	1.3 (1.1 to 1.5)
High dimeric inhibin-A	**Normal**: ≤ 99th percentile MoM (n = 90,761)	272 (4.0)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: > 99th percentile MoM (n = 1065)	9 (11.0)	2.7 (1.4 to 5.3)	2.4 (1.2 to 4.7)
Low pregnancy-associated plasma protein A	**Normal**: ≥ 1st percentile MoM (n = 387,651)	1045 (3.6)	1.0 (Ref.)	1.0 (Ref.)
	Abnormal: < 1st percentile MoM (n = 5748)	29 (6.8)	1.9 (1.3 to 2.7)	1.7 (1.2 to 2.5)

[a]Adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing), ethnicity (Asian, Black Caucasian, Hispanic, Oriental, other, missing), and gestational age (continuous) – each at the time of prenatal biochemical screening – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored on death or arrival at the end of study date of March 31, 2016.

Supplementary file 7 (Additional analysis 5). Comparison of 750,742 pregnancies with prenatal biochemical screening (screened cohort) and 750,742 pregnancies without biochemical screening (non-screened cohort), matched on year of delivery. One pregnancy was possible per woman. All data are presented as number (%) unless otherwise indicated.

Characteristic	Screened cohort (N = 750,742)	Non-screened cohort (N = 750,742)	Standardized difference
In the index pregnancy			
Mean maternal (SD) age, years	29.9 (5.3)	28.9 (5.9)	0.18
Income quintile (Q)			
Q1 (lowest)	170,988 (22.8)	184,721 (24.6)	-0.04
Q2	153,854 (20.5)	155,601 (20.7)	-0.01
Q3	150,552 (20.1)	148,776 (19.8)	0.01
Q4	149,474 (19.9)	142,983 (19.0)	0.02
Q5 (highest)	123,027 (16.4)	114,227 (15.2)	0.03
Unknown	2,847 (0.4)	4,434 (0.6)	-0.03
Residence			
Urban	694,690 (92.5)	644,616 (85.9)	0.22
Rural	55,078 (7.3)	105,757 (14.1)	-0.22
Unknown	974 (0.1)	369 (0.0)	0.03
Gravidity			
1	316,927 (42.2)	417,780 (55.6)	-0.27
≥ 2	423,400 (56.4)	332,962 (44.4)	0.24
Unknown	10,415 (1.4)	0 (0.0)	0.17
Pregnancy outcome			
Livebirth at ≥ 20 weeks' gestation	723,180 (96.3)	727,748 (96.9)	-0.03
Stillbirth at ≥ 20 weeks' gestation	3,684 (0.5)	4,476 (0.6)	-0.01
Miscarriage at < 20 weeks' gestation or ectopic pregnancy	3,146 (0.4)	22 (0.0)	0.09
Induced abortion at < 20 weeks' gestation	2,678 (0.4)	18,496 (2.5)	0.00
Unknown	18,054 (2.4)	0 (0.0)	0.22
Multifetal pregnancy	8,465 (1.1)	11,691 (1.6)	-0.04

Characteristic	Screened cohort (N = 750,742)	Non-screened cohort (N = 750,742)	Standardized difference
Year of screening/pregnancy outcome			
1993-2002	362,699 (48.3)	362,699 (48.3)	0.00
2003-2012	388,043 (51.7)	388,043 (51.7)	0.00
Conditions ≤ 365 days before, or up to 365 days after, the start of pregnancy			
Diabetes mellitus	41,185 (5.5)	42,796 (5.7)	-0.01
Chronic hypertension	24,896 (3.3)	43,584 (5.8)	-0.12
Dyslipidemia	8,216 (1.1)	11,259 (1.5)	-0.04
Renal disease	1,934 (0.3)	2,151 (0.3)	-0.01
Drug/alcohol/tobacco abuse	9,957 (1.3)	20,588 (2.7)	-0.10
Conditions at the time of a livebirth or stillbirth delivery			
Congenital or chromosomal anomaly	28,370 (3.9)	27,187 (3.7)	0.01
Preeclampsia/eclampsia	15,415 (2.1)	16,227 (2.2)	0.00
Gestational hypertension	22,832 (3.1)	23,173 (3.2)	0.00
Placental abruption	6,805 (0.9)	7,513 (1.0)	-0.01
Placental infarction	4,626 (0.6)	4,968 (0.7)	0.00
Conditions at the time of a livebirth delivery			
Preterm birth < 37 weeks' gestation	50,087 (6.9)	54,077 (7.4)	-0.02
Experienced the composite CVD outcome[a]			
No. events (incidence rate per 10,000 person-years)	6,504 (6.1)	5,901 (6.7)	--
Crude HR (95% CI)	1.0 (referent)	1.1 (1.1 to 1.1)	--
Adjusted HR (95% CI)[b]	1.0 (referent)	1.1 (1.0 to 1.1)	--

[a]Any hospitalization or revascularization for coronary artery, cerebrovascular or peripheral arterial disease, heart failure or dysrhythmia, arising ≥ 365 days after the start of the index pregnancy.

[b]Adjusted for maternal age (continuous), gravidity (1, 2+, missing), neighbourhood income quintile (1, 2, 3, 4, 5, missing), rural residence (rural, urban, missing) – each at the time of prenatal biochemical screening (screened cohort) or at the end of pregnancy (non-screened cohort) – as well as maternal diabetes mellitus, chronic hypertension, renal disease, tobacco/drug use and dyslipidemia within 365 days preceding the start

of the index pregnancy, up to and including 365 days after the start of the index pregnancy (i.e. time zero). Censored on arrival at the end of study date of March 31, 2016.