OBSTETRICS

Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes

Francisco J. Schneuer, MBA; Christine L. Roberts, DrPH; Anthony W. Ashton, PhD; Cyrille Guilbert, MSc; Vitomir Tasevski, PhD; Jonathan M. Morris, PhD; Natasha Nassar, PhD

OBJECTIVE: To assess angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and the Ang-1/Ang-2 ratio levels in the first trimester of pregnancy, their association with adverse pregnancy outcomes, and their predictive accuracy.

STUDY DESIGN: This cohort study measured serum Ang-1 and Ang-2 levels in 4785 women with singleton pregnancies attending first trimester screening in New South Wales, Australia. Multivariate logistic regression models were used to assess the association and predictive accuracy of serum biomarkers with subsequent adverse pregnancy outcomes (small for gestational age, preterm birth, preeclampsia, miscarriage >10 weeks, and stillbirth).

RESULTS: Median (interquartile range) levels for Ang-1, Ang-2, and the Ang-1/Ang-2 ratio for the total population were 19.6 ng/mL (13.6—26.4), 15.5 ng/mL (10.3—22.7), and 1.21 (0.83—1.73), respectively. Maternal age, weight, country of birth, and socioeconomic status significantly affected Ang-1, Ang-2, and the Ang-1/Ang-2 ratio levels. After adjusting for maternal and clinical risk factors, women with low Ang-2 levels (<10th percentile) and high Ang-1/Ang-2 ratio (>90th percentile) had increased risk of developing most adverse pregnancy outcomes. Compared with the Ang-1/Ang-2 ratio alone, maternal and clinical risk factors had better predictive accuracy for most adverse pregnancy outcomes. The exception was miscarriage (Ang-1/Ang-2 ratio area under receiver operating characteristic curve = 0.70; maternal risk factors = 0.58). Overall, adding the Ang-1/Ang-2 ratio to maternal risk factors did not improve the ability of the models to predict adverse pregnancy outcomes.

CONCLUSION: Our findings suggest that the Ang-1/Ang-2 ratio in first trimester is associated with most adverse pregnancy outcomes, but do not predict outcomes any better than clinical and maternal risk factor information.

Key words: adverse pregnancy outcomes, angiopoietins, Ang-1, Ang-2, first trimester, serum levels

Cite this article as: Schneuer FJ, Roberts CL, Ashton AW, et al. Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes. Am J Obstet Gynecol 2014;210:345.e1-9.

Neovascularisation, or new vessel formation, is essential for placental growth throughout gestation and is driven by changes in the balance between pro- and antiangiogenic factors present in the extracellular milieu.[1] Angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2) are angiogenic factors that play a critical role in the development of the placental vascular system. Although Ang-1 helps capillary maturation and maintains vessel integrity, Ang-2 antagonises Ang-1 and destabilizes vessels. In the presence of proangiogenic factors, such as vascular endothelial growth factor (VEGF) or placental growth factor, this destabilization results in vessel sprouting and enhanced angiogenesis.[2] More than just vessel growth, signalling from angiopoietins is a significant stimulus for trophoblast growth and remodelling during placentation. Thus, the interplay between Ang-1, Ang-2, and other angiogenic factors (such as VEGF) controls placental growth and tissue neovascularization during pregnancy.[3] It is not therefore surprising that the amount of circulating Ang-1 and Ang-2 shifts from a dominance of Ang-1 to Ang-2 during gestation reflecting the requirement for new vessel formation.[4]

Impaired placental vascular development related to imbalances in angiogenic factors are implicated in pathologic pregnancies. As such, Ang-1 and Ang-2 are potential biomarkers for adverse pregnancy outcomes as they indicate the progression of placental growth and maternal vascular health during

From Clinical and Population Perinatal Health Research, Kolling Institute of Medical Research (Mr Schneuer, Drs Roberts, Ashton, Morris, and Nassar, and Mr Guilbert), University of Sydney, Sydney, and Fetal Maternal Medicine (PaLMs), Royal North Shore Hospital (Dr Tasevski), St Leonards, NSW, Australia.

Received July 26, 2013; revised Oct. 21, 2013; accepted Nov. 4, 2013.

The authors report no conflict of interest.

This work was funded by Australian National Health and Medical Research Council (NHMRC) Project Grant number 632653 and Centre for Research Excellence Grant number 1001066. NN is supported by an NHMRC Career Development Fellowship (#632955) and CLR by a NHMRC Senior Research Fellowship (#1021025). NHMRC had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Presented as a poster at the 33rd annual meeting of the Society for Maternal-Fetal Medicine, San Francisco, CA, Feb. 11-16, 2013.

Reprints: Francisco J. Schneuer, Building 52, Royal North Shore Hospital, St Leonards NSW 2065, Australia. francisco.schneuer@sydney.edu.au.

0002-9378/$36.00 • • http://dx.doi.org/10.1016/j.ajog.2013.11.012

gestation.[4] Circulating levels of Ang-1 and Ang-2 have previously been associated with poor pregnancy outcome. We have reported that women whose fetuses develop intrauterine growth restriction (IUGR) have lower serum levels of Ang-2 in first trimester, suggesting impaired placental angiogenesis may be pathogenic in the disease.[5] Others have reported lower serum Ang-1 and Ang-2 in women that had an abortion or an ectopic pregnancy, with promising predictive results.[6] Preeclampsia has been associated with both low second trimester levels of Ang-2[7] and low ratio of Ang-1/Ang-2.[8] However, these studies included a small number of cases and did not report results for other adverse pregnancy outcomes. There are no large population-based studies assessing the association of Ang-1 and Ang-2 in early pregnancy and risk of subsequent pregnancy outcomes. It is also unknown whether these angiogenic biomarkers provide any additional value to usual maternal and clinical information identifying pregnancies at risk. If women at risk of developing adverse pregnancy outcomes can be identified early in pregnancy this would allow ample time for monitoring and implementing potential preventive strategies.

The aims of this study were 3-fold; (1) to evaluate serum levels of serum Ang-1, Ang-2 concentrations and Ang-1/Ang-2 ratio in first trimester of pregnancy. (2) To assess the association between maternal serum Ang-1, Ang-2 concentrations and the Ang-1/Ang-2 ratio and risk of adverse pregnancy outcomes; and (3) to determine their accuracy in predicting adverse pregnancy outcomes.

Materials and Methods

Study population and sample testing

This cohort study was conducted on women attending first trimester Down syndrome screening between July 2006 and June 2007 in New South Wales (NSW), Australia. Serum samples were collected by the Pacific Laboratory Medicine Services, and then archived and stored at −80°C. During this period, this was the state's only public screening service and received samples from throughout NSW.

Serum samples for this study were thawed and serum levels of Ang-1 and Ang-2 were measured by a semi-automated enyzimed linked sorbent assay immunoassay (R & D Systems, Minneapolis, MN). Intraassay and interassay coefficient of variation were <9.5% and the reported analytic sensitivity of the immunoassay was 0.06−84.3 ng/mL for Ang-1 and 0.05−108.9 ng/mL for Ang-2.

Data sources

Maternal information for archived serum samples was derived from the laboratory database and corresponding pregnancy and birth outcomes were ascertained via record linkage to the Perinatal Data Collection (PDC) and Admitted Patient Data Collection (APDC). The PDC is a statutory surveillance system of all births in NSW of at least 400 g birthweight, or at least 20 weeks' gestation and includes demographic, medical, and obstetric information on the mother, labor, delivery, and birth outcome. The APDC is a census of all patient hospital admissions from NSW public and private hospitals, with records for both mothers and liveborn infants. It includes demographic, clinical, and health services information for each admission and relevant diagnoses and procedures are recorded for each hospital admission. These are coded according to the *International Classification of Diseases* version 10−Australian Modification (ICD10-AM) and Australian Classification of Healthcare Interventions, respectively. Validation studies of the PDC and the APDC show excellent level of agreement with the medical record and low rates of missing data.[9,10] Reporting in both datasets have high specificity (>99%) indicating few false positive reports. Only factors and outcomes accurately reported in birth or hospital data were included in analyses.[11] The NSW Centre for Health Record Linkage conducted the record linkage and identifying information was removed before the release of data for analysis. The CHeReL assesses the linkage quality for each study, and for this study reported <5/1000 missed links and <2/1000 false positive links. The study was approved by the NSW Population and Health Services Research Ethics Committee.

Study outcomes and explanatory factors assessed included: small for gestational age (SGA), preterm birth, preeclampsia, gestational diabetes, miscarriage, and stillbirth. SGA was defined as birthweight <10th percentile and <3rd percentile (severe SGA) of the distribution for gestational age and infant sex.[12] Gestational age is reported in the birth data in completed weeks of gestation and determined by the best clinical estimate including early ultrasound (>97%) and last menstrual period. Preterm birth was defined as delivery at less than 37 weeks and very preterm birth less than 34 weeks' gestation. Information on preeclampsia was obtained from both the APDC and PDC data, to maximize ascertainment.[13,14] Preeclampsia (regardless of severity) was determined either by the box being checked in the PDC record, or if any APDC record had a diagnosis in any of the 55 fields of gestational hypertension (ICD10-AM: O13 and O16), preeclampsia (O11 and O14), or eclampsia (O15).[14,15] Early onset preeclampsia was defined as women with preeclampsia requiring delivery at ≤34 weeks' gestation. Miscarriage was defined as a spontaneous pregnancy loss between 10-20 weeks' gestation and identified from APDC data, whereas stillbirth was defined as a spontaneous pregnancy loss after 20 weeks' gestation and was identified from PDC data. To replicate an earlier study of ours, we defined a proxy measure of IUGR using combined criteria of SGA <10th percentile and preterm birth <37 weeks.

The key explanatory variables were Ang-1, Ang-2, and the Ang-1/Ang-2 ratio levels and covariates used in this analysis included maternal age and weight (kilograms) ascertained at the time of first trimester screening, parity (nulliparous/multiparous), smoking during pregnancy, previous diagnosed hypertension, previous miscarriage, country of birth, and socioeconomic disadvantage quintile. Socioeconomic disadvantage was defined according to the Socioeconomic Indexes for Areas relative disadvantage scores developed by the Australian Bureau of Statistics.[16] Maternal weight was missing in 831 (16.3%) of the records. Multiple imputation was used to account