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The use of serum inhibin A and activin A levels in predicting the outcome of 'pregnancies of unknown location'

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Abstract

Introduction: The aim was to evaluate the role of serum inhibin A and activin A in the prediction of the outcome of women with 'pregnancies of unknown location' (PULs).

Materials and Methods: Serum hCG, progesterone, inhibin A and activin A levels were measured at 0 and 48 hours. Differences in the mean levels and the change in levels over 48 hours expressed as a ratio (48 hours/0 hours) were examined between the three outcome groups - failing PUL, intra-uterine pregnancy (IUP) or ectopic pregnancy. Variables were incorporated into logistic regression models to predict the pregnancy outcomes, which were evaluated using receiver operator characteristic (ROC) curves.

Results: 141 PULs: 67 failing PULs (47.5%), 58 IUPs (41.1%) and 16 ectopic pregnancies (11.4%). Activin A levels were not significantly different between the three outcome groups. Inhibin A levels were significantly lower in failing PULs. The logistic regression model based on serum inhibin levels gave an area under the curve (AUC) of 0.88 for failing PUL, 0.87 for IUP and 0.60 for ectopic pregnancy. The model based on serum activin levels gave an AUC of 0.61 for failing PUL, 0.64 for IUP and 0.51 for ectopic pregnancy and the model based on serum hCG levels gave an AUC of 0.95 for failing PUL, 0.97 for IUP and 0.67 for ectopic pregnancy

Conclusion: Serum activin A levels at 0 and 48 hours are not helpful in predicting the outcome of PULs. Although serum inhibin A levels may be of use in the prediction of failing PULs and IUPs in the PUL populations, they do not perform as well as serum hCG levels

Introduction

A 'pregnancy of unknown location' (PUL) is defined by the finding of an empty uterus with no signs of an intra-uterine or extra-uterine pregnancy on a transvaginal ultrasound scan (TVS) in a woman with a positive pregnancy test. There are three possible final clinical outcomes – a failing PUL (serum human chorionic gonadotrophin (hCG) levels spontaneously decrease to < 5 IU/L), an intra-uterine pregnancy (IUP) or an ectopic pregnancy. At present there is no universally accepted method for predicting the final pregnancy outcome. Previous studies have shown that PULs occur in about 10% of women attending an early pregnancy unit (EPU). Due to the uncertainty of the outcomes and the risk of an underlying ectopic pregnancy these women require serial TVS or serum tests in order establish a diagnosis; they therefore represent a disproportionate amount of workload for EPUs, and women with this diagnosis are at risk of unnecessary diagnostic laparoscopy.

Measurements of serum progesterone and serum hCG have been used to predict pregnancy outcome. A serum progesterone of < 20 nmol/L has been shown to be predictive of failing pregnancy (Banerjee et al., 2001). Single serum hCG levels have been used to describe a discriminatory zone – a level of serum hCG above which it is thought, that, if present, an IUP should be visualised on TVS (Romero et al., 1985). Serial measurements of serum hCG are also often used to help predict the final pregnancy outcome. In 1981 Kadar et al. first described the minimal rate of increase in serum hCG for an IUP to be 66% in two

days (Kadar et al., 1981). Since then various cut-offs have been suggested to predict the different pregnancy outcomes (Barnhart et al., 2004a, Barnhart et al., 2004b, Seeber et al., 2006, Silva et al., 2006). The change in serum hCG over 48 hours has also been expressed as the hCG ratio (hCG 48 hours / hCG 0 hours) and used to predict pregnancy outcome, both by itself and when incorporated into mathematical models (Condous et al., 2004, Condous et al., 2006, Kirk et al., 2006, Condous et al., 2007a, Condous et al., 2007b).

Whilst much of the work on the prediction of outcome of PULs has centered on the use of serum hCG and progesterone levels, some other markers have been studied. These include creatine kinase and cancer antigen 125 (CA125). Whilst CA 125 levels and creatine kinase levels alone were not able to predict pregnancy outcome, the CA 125 ratio was found to be able to help distinguishing failing PULs from IUPs, but was not able to detect those at risk of ectopic pregnancy (Condous et al., 2005).

Inhibin A is a glycoprotein secreted by the feto-placental unit. It has been suggested that it may be a specific marker of early pregnancy loss (Muttukrishna et al., 2002). Both single and serial measurements have been used to predict subsequent pregnancy loss in women with recurrent miscarriage (Muttukrishna et al., 2002, Al-Azemi et al., 2003, Prakash et al., 2005). Inhibin A has also been used to predict pregnancy outcome in women undergoing in vitro fertilization where it has been shown to be more accurate than hCG in predicting preclinical

pregnancy loss (Hauzman et al., 2004). In addition, serum inhibin levels have been shown to be lower in spontaneously conceived ectopic pregnancies compared to intrauterine pregnancies (Seifer et al., 1996).

Activin A is another glycoprotein secreted by the feto-maternal unit and it is thought to be a marker of trophoblastic invasion with the potential to identify patients at risk of miscarriage and ectopic pregnancy. Both single and serial measurements of activin A levels have been used to predict miscarriage in women with recurrent miscarriage (Prakash et al., 2005). Levels were significantly lower at 7-8 weeks of gestation in those who subsequently miscarried compared to those with ongoing pregnancies. Serum activin A levels have been used to predict ectopic pregnancy in women with PULs (Florio et al., 2007). Serum levels were found to be significantly lower in women with ectopic pregnancies compared to those with IUPs or failing PULs. Using a cut-off value of 0.37 ng/ml gave a sensitivity and specificity of 100% and 99.6%, respectively for the prediction of ectopic pregnancy (Florio et al., 2007).

The aim of this study was therefore to evaluate the role of single and serial measurements of inhibin A and activin A in the prediction of the outcome of pregnancies of unknown location (PULs).

Methods

This was a prospective observational study on women attending the Early Pregnancy Unit (EPU) of an inner London teaching hospital. Women attended the unit for a number of reasons including: pain, vaginal bleeding, pain with vaginal bleeding, unsure menstrual dates, history of a previous pregnancy complication and maternal anxiety. Women could self refer or were referred by their general practitioner, midwife, the accident and emergency department or another hospital department.

At the time of presentation all women attending the unit underwent a TVS examination using a 5MHz transducer for B mode imaging (Aloka SSD 900, 2000 or 4000, Aloka Co. Japan). They were diagnosed with an IUP (viable or non-viable) or an ectopic pregnancy on the basis of this TVS examination, using the sonographic criteria in Table 1. If no pregnancy could be visualised on TVS, they were classified as a PUL. All haemodynamically stable, pain-free women classified as having a PUL were invited to participate in this study, which was granted research ethics approval.

Women were given an information sheet about the study and were asked for written consent. Blood samples were taken at the time of the TVS examination (time 0 hours) and 48 hours later to measure serum hCG (World Health Organisation, Third International Reference 75/537) and progesterone (Roche Elecsys 2010 Progesterone II test) levels. **Serum was aliquoted and stored at −20ºC and** levels of serum inhibin A and activin A (Serotec, Kidlington, UK) were

also measured at 0 and 48 hours. All samples were analysed in duplicate over 8 batches. The detection limit for activin was 0.05. The average coefficient of variation (CV) of the duplicates was 3.48% (range 2.92-4.43). For inhibin the detection limit was 1.00. The average CV of the duplicates was 4.47% (range 3.54-5.64).

The women were followed up until the final pregnancy outcome was known – failing PUL, IUP or ectopic pregnancy, according to the unit protocol. In general, if the serum hCG level increased by > 66% in 48 hours (Kadar et al., 1981, Hahlin et al., 1995), women were classified as an IUP and rescanned in 7-14 days to confirm the diagnosis. If the initial serum progesterone level was < 20 nmol/L, the women were classified as a failing PUL (Banerjee et al., 2001). They were also classified as a failing PUL if the serum hCG decreased by more than 20% in 48 hours. Serum hCG levels were repeated at 7-14 days to confirm the diagnosis. Women who did not fall into either of these categories were thought to be at risk of ectopic pregnancy and were reviewed every 48-72 hours, until a pregnancy (IUP or ectopic) could be visualised on TVS (using the criteria in Table 1) or had a spontaneous decrease in serum hCG levels to < 5 IU/L, confirming a failed PUL.

Statistical analysis

Missing values were observed in 1% of the patients for serum hCG levels at 0 hours, and in 6% of the patients for levels at 48 hours. For progesterone, inhibin,

and activin, missing values were observed in 6% and 25%, 9% and 24%, and 9% and 25%, respectively. Using multiple imputation (Schafer, 1999; Little & Rubin, 2002), missing values were imputed five times, resulting in five completed data sets. To impute missing values of one marker (e.g. inhibin at 48 hours), information of the other hormonal markers as well as information of so-called auxiliary variables was used. The auxiliary variables were age, gestational age, endometrial thickness, level of vaginal bleeding, nulliparity, history of miscarriages, history of termination of pregnancies, history of ectopic pregnancies, and final pregnancy outcome. Each completed data set was analyzed using standard statistical techniques, and the results were combined to obtain the final results.

The change in serum hCG, inhibin A and activin A levels over 48 hours were expressed as a ratio (level at 48 hours / level at 0 hours). Univariate multicategory logistic regression was used to detect differences in hormonal variables between the three outcome groups – failing PUL, IUP and ectopic pregnancy. Additionally, we investigated how well different hormonal indices were able to detect the different pregnancy outcomes by computing the area under the receiver operating characteristic (ROC) curve (AUC) for each outcome separately.

To evaluate the value of inhibin and activin levels for predicting the three pregnancy outcome groups, multivariate multicategory logistic regression was

used. Log transformations were performed where the variables were highly skewed. The reference model contained hCG information: log of the hCG ratio and log of the average hCG. The AUC for each pregnancy outcome was computed to determine the effectiveness of the models to predict pregnancy outcome. The models' AUC performances were estimated using bootstrapping (Efron & Tibshirani, 1993; Steyerberg et al, 2001). This method starts with computing the data set AUCs, which are too optimistic because the model is fitted and evaluated on the same data. Then, using 200 bootstrap samples, an estimate of each AUC's optimism is derived and subtracted from the AUCs to obtain corrected AUCs. Statistical analyses were conducted with SAS version 9 (SAS Institute Inc., Cary, USA).

Results

Serum hCG, progesterone, inhibin A and activin A levels were available from 141 women with a classification of PUL. **The samples were collected between the 1st July 2004 and the 30th November 2004.** The final pregnancy outcomes were: 67 failing PULs (47.5%), 58 IUPs (41.1%) and 16 ectopic pregnancies (11.4%).

The median hCG, progesterone, inhibin A and activin A levels with ranges are presented in Table 2. The hCG ratio and average progesterone levels were significantly different between the three pregnancy outcome groups. The activin A level was different between the outcome groups at 0 hours but not at 48 hours

(Table 2). However there seemed to be differences for both inhibin A levels, with failing PULs having the lowest levels and IUPs the highest. Changes in activin A or inhibin A levels over time (the ratios) did not differ across pregnancy groups.

Table 3 shows the separate AUCs for each pregnancy outcome to assess the effectiveness of individual hormonal variables in predicting a particular pregnancy outcome. The progesterone levels at 0 and 48 hours were good predictors of a failing PUL or an IUP, but were poor at predicting ectopic pregnancy, Table 3. A decrease in inhibin A levels over 48 hours appears to be the 'best' predictor of ectopic pregnancy (AUC = 0.61). Ectopic pregnancies had the highest median activin level at presentation. Using a cut-off of 0.37 ng/ml on the serum activin A level at presentation to predict ectopic pregnancy (Florio et al., 2007) gave an average sensitivity over the completed data sets of 93% (14.8/16) and an average specificity of 13% (16.4/125).

The multicategory logistic regression model based on serum activin A levels, using the log of the average level and the log of the ratio as predictors, was not helpful in predicting pregnancy outcome (see Table 4 for the corrected AUCs for each pregnancy outcome). Although a model using serum inhibin A levels (using the ratio and the log of the average level as predictors) was of use in the prediction of failing PULs and IUPs in the PUL population, it did not perform as well as the model using serum hCG levels (Table 4). The corrected AUCs for failing PUL, IUP and ectopic pregnancy using the hCG model were 0.95, 0.97

and 0.67 respectively. Addition of both inhibin variables to the hCG model did not improve its performance. The corrected AUCs for failing PUL, IUP and ectopic pregnancy using a model with hCG and inhibin information (log of the average hCG, hCG ratio, hCG ratio square, log of the average inhibin, inhibin ratio) were 0.96, 0.97 and 0.69 respectively.

Discussion

In this study we have shown that in women with PUL maternal serum inhibin A levels can help predict pregnancy loss, while levels of activin A correlate weakly with pregnancy outcome.

Inhibin A

To the best of our knowledge this is the first study to examine the use of inhibin A in the prediction of PUL outcome. Previous studies have shown that inhibin A can be used to predict pregnancy loss in women with recurrent miscarriages (Muttukrishna et al., 2002, Al-Azemi et al., 2003, Prakash et al., 2005), those with threatened miscarriages (Johns et al., 2007) and those who have undergone in vitro fertilization (Treetampinich et al., 2000, Hauzman et al., 2004). Serum inhibin A has also been found to be a predictor of complete miscarriage in women with miscarriage managed expectantly (Elson et al., 2005). We have found that inhibin A levels are different in the three pregnancy outcome groups. There was however no significant difference in the change in serum inhibin A levels over time between the different outcome groups. A low inhibin A level at 0

hours was found to be a good predictor of pregnancy loss (failing PUL, AUC = 0.88, Table 3). Conversely we found high levels of inhibin A at 0 hours to be a good predictor of an IUP (AUC = 0.87, Table 3). However, inhibin levels were no better at predicting PUL outcome than progesterone, Table 3.

A previous study found that inhibin A levels were lower in spontaneously conceived ectopic pregnancies compared to IUPs (Seifer et al., 1996). However this was a case-control study measuring inhibin A levels in women with ectopic pregnancies undergoing surgery using gestation matched controls with IUPs. No comparison was made to women with failed pregnancies. Whilst we found that inhibin A levels were significantly different amongst the three possible PUL outcome groups, with levels lower in ectopic pregnancies compared to IUPs, levels were still higher in ectopic pregnancies than in failing PULs (Table 2). We therefore found that a single inhibin A measurement was not a good predictor of ectopic pregnancy (inhibin A 0 hour AUC = 0.55, Table 3). Using serial inhibin A levels, the prediction of ectopic pregnancy was not much better (AUC = 0.60, Table 4), and did not perform as well as the model based on just hCG measurements (AUC = 0.67, Table 4).

As it is secreted by trophoblast serum inhibin A has been evaluated as a possible surveillance marker after conservative surgical management of ectopic pregnancy as its half-life is significantly shorter than that of hCG (D'Antona et al., 1998). However interpretation of results is complicated as inhibin A is also

secreted by the ovary and increases in levels may also occur with the return of normal ovarian function. Inhibin A was therefore concluded not to be a useful marker for persistent trophoblast after conservative surgical treatment for ectopic pregnancy. However the study did suggest that pre-treatment inhibin A levels may provide an accurate assessment of the viability of trophoblastic tissue within ectopic pregnancies as some ectopic pregnancies with very high hCG levels had very low inhibin A levels. They therefore proposed that inhibin A levels may be useful in helping assess whether ectopic pregnancy management should be expectant, medical or surgical. If true, this may explain why the women with ectopic pregnancies in our study had varying serum inhibin A levels (3.6 – 305 pg/ml at presentation). Some ectopic pregnancies will have active trophoblast and behave like IUPs and others will be failing and behave like failing PULs. A previous study examining serial hCG levels in women with ectopic pregnancies initially classified as PULs found that the changes in serum hCG mimicked an IUP in 20% and a failing pregnancy in 8% of the cases in their series (Silva et al., 2006).

Activin A

Activin A levels have been found to be significantly lower in women with a threatened miscarriage who subsequently miscarried compared to those who had live births (Johns et al., 2007). It has also been suggested that activin A may be used as a marker to predict pregnancies that are likely to miscarry in women with recurrent miscarriage (Prakash et al., 2005). However other studies have

shown that activin A levels are similar in women with miscarriages and controls (Muttukrishna et al., 2002, Wallace et al., 2004).

We found activin A levels are not a good predictor of PUL outcome (Table 3). In contrast to previous studies suggesting lower levels in women who subsequently suffer a miscarriage, in our study activin A levels were higher in failing PULs compared to IUPs (Table 2). Using the activin A level at presentation gave an AUC of 0.62 to predict failing PUL, 0.66 to predict IUP, and 0.58 to predict ectopic pregnancy (Table 3). This is in contrast to those obtained in a recent study evaluating the use of a single measurement of activin A in the diagnosis of ectopic pregnancy in women classified with a PUL (Florio et al., 2007), who found that activin A levels were significantly lower in women with ectopic pregnancies compared to IUPs and failing PULs. In the study by Florio, ROC analysis suggested that activin A, at a cut-off of 0.37 ng/ml, gave a sensitivity and specificity of 100% and 99.6% respectively for the prediction of ectopic pregnancy. When we evaluated this cut-off on our data we found the sensitivity to be 93%, but with a very poor specificity of 13%. Activin A levels in the women with ectopic pregnancies in our study ranged from 0.35 – 0.85 ng/ml at presentation compared to 0.27 ± 0.06 ng/ml in the study by Florio et al. (2007). They suggested that the lower levels of activin A in ectopic pregnancies compared to those with failing PULs may be due to the difficulty of the ectopic trophoblast to correctly implant. Our results do not agree with their findings, possibly because our ectopic pregnancies may have been more of a

heterogeneous group. Certainly in our study there were ectopic pregnancies that had low progesterone levels or low hCG ratios as often seen in failing PULs whilst others had high progesterone levels or high hCG ratios as seen in IUPs. The median progesterone level in the ectopic pregnancies was 24.5 nmol/L (range 2 – 121 nmol/L) and the median hCG ratio 1.09 (range 0.16 – 2.73), (Table 2). Florio et al. did not examine changes in serum hCG over time in their PULs but progesterone levels in their ectopic pregnancies were significantly lower (14.7 ± 0.9 ng/ml) compared to their IUPs (31.8 ± 0.4 ng/ml) suggesting many of them were failing in any event.

In this study we have therefore shown that neither single nor serial measurements of inhibin A or activin A can be used to reliably predict all three possible final outcomes of PULs. The challenge still remains to identify a universal marker of PUL outcome, which combines accuracy, reproducibility and simplicity. However, it may be that a combination of markers may be needed in order to maximize sensitivity and specificity for the various outcomes. For the moment our data suggests that a model based on serum hCG levels at 0 and 48 hours is the optimal approach to the prediction of PUL outcome until such a marker can be identified.

References

Al-Azemi M, Ledger WL, Diejomaoh M, Mousa M, Makhseed M, Omu A (2003). Measurement of inhibin A and inhibin pro-alphaC in early human pregnancy and their role in the prediction of pregnancy outcome in patients with recurrent pregnancy loss. Fertil Steril. 80:1473-9.

Banerjee S, Aslam N, Woelfer B, Lawrence A, Elson J, Jurkovic D (2001). Expectant management of early pregnancies of unknown location: a prospective evaluation of methods to predict spontaneous resolution of pregnancy. BJOG 108:158-163.

Barnhart KT, Sammel MD, Rinaudo RF, Zhou L, Hummel AC, Guo W (2004a). Symptomatic patients with an early viable intra-uterine pregnancy: hCG curves redefined. Obstet Gynecol. 104: 50-5.

Barnhart KT, Sammel MD, Chung K, Zhou L, Hummel AC, Guo W (2004b). Decline of serum human chorionic gonadotropin and spontaneous complete abortion: defining the normal curve. Obstet Gynecol. 104:975-81.

Condous G, Okaro E, Khalid A, Timmerman D, Lu C, Zhou Y, Van Huffel S, Bourne T (2004). The use of a new logistic regression model for predicting the outcome of pregnancies of unknown location. Hum Reprod 19(8): 1900-10.

Condous G, Kirk E, Syed A, Van Calster B, Van Huffel S, Timmerman D, Bourne T (2005). Do levels of serum cancer antigen 125 and creatine kinase predict the outcome in pregnancies of unknown location? Hum Reprod. 20: 3348-3354.

Condous G, Kirk E, Van Calster B, Van Huffel S, Timmerman D, Bourne T (2006). Failing pregnancies of unknown location: a prospective evaluation of the human chorionic gonadotrophin ratio. BJOG 113: 521-527.

Condous G, Van Calster B, Kirk E, Haider Z, Timmerman D, Van Huffel S, Bourne T (2007a). Prediction of ectopic pregnancy in women with a pregnancy of unknown location. Ultrasound Obstet Gynecol. 29:680-7.

Condous G, Van Calster B, Kirk E, Timmerman D, Van Huffel S, Bourne T (2007b). Prospective cross-validation of three methods of predicting failing pregnancies of unknown location. Hum Reprod. 22:1156-60.

D'Antona D, Mamers PM, Lowe PJ, Balazs N, Groome NP, Wallace EM (1998). Evaluation of serum inhibin A as a surveillance marker after conservative management of tubal pregnancy. Hum Reprod. 13: 2305-7.

Efron B, Tibshirani RJ (1993). An introduction to the bootstrap. New York: Chapman & Hall.

Elson J, Tailor A, Salim R, Hillaby K, Dew T, Jurkovic D (2005). Expectant management of miscarriage-prediction of outcome using ultrasound and novel biochemical markers. Hum Reprod. 20:2330-3.

Florio P, Severi FM, Bocchi C, Luisi S, Mazzini M, Danero S, Torricelli M, Petraglia F (2007). Single serum activin A testing to predict ectopic pregnancy. J Clin Endocrinol Metab 92: 1748-53.

Hauzman E, Fedorcsák P, Klinga K, Papp Z, Rabe T, Strowitzki T, Urbancsek J (2004). Use of serum inhibin A and human chorionic gonadotropin measurements to predict the outcome of in vitro fertilization pregnancies. Fertil Steril. 81: 66-72.

Johns J, Muttukrishna S, Lygnos M, Groome N, Jauniaux E (2007). Maternal serum hormone concentrations for prediction of adverse outcome in threatened miscarriage. Reprod Biomed Online. 15:413-21.

Kadar N, Caldwell BV, Romero R (1981). A method of screening for ectopic pregnancy and its indications. Obstet Gynecol. 58: 162-6.

Kirk E, Condous G, Haider Z, Lu C, Van Huffel S, Timmerman D, Bourne T (2006). The practical application of a mathematical model to predict the outcome of pregnancies of unknown location (PULs). Ultrasound Obstet Gynecol. 27: 311-315.

Little RJA, Rubin DB (2002). Statistical analysis with missing data (2nd edition). New York: Wiley.

Muttukrishna S, Jauniaux E, Greenwold N, McGarrigle H, Jivraj S, Carter S, Elgaddal S, Groome N, Regan L (2002). Circulating levels of inhibin A, activin A and follistatin in missed and recurrent miscarriages. Hum Reprod. 17: 3072-8.

Prakash A, Laird S, Tuckerman E, Li TC, Ledger WL (2005). Inhibin A and activin A may be used to predict pregnancy outcome in women with recurrent miscarriage. Fertil Steril. 836:1758-63.

Romero R, Kadar N, Jeanty P, Copel JA, Chervenak FA, DeCherney A, Hobbins JC (1985). Diagnosis of ectopic pregnancy: value of the discriminatory human chorionic gonadotropin zone. Obstet Gynecol. 66:357-60.

Schafer JL (1999). Multiple imputation: a primer. Stat Methods Med Res. 8:3-15.

Seeber BE, Sammel MD, Guo W, Zhou L, Hummel A, Barnhart KT (2006). Application of redefined human chorionic gonadotrophin curves for the diagnosis of women at risk for ectopic pregnancy. Fertil Steril. 86: 454-9.

Seifer DB, Lambert-Messerlian GM, Canick JA, Frishman GN, Schneyer AL (1996). Serum inhibin levels are lower in ectopic than intrauterine spontaneously conceived pregnancies. Fertil Steril. 65:667-9.

Silva C, Sammel MD, Zhou L, Gracia C, Hummel AC, Barnhart KT (2006). Human chorionic gonadotrophin profile for women with ectopic pregnancy. Obstet Gynecol 107: 605-10.

Steyerberg EW, Harrel FE Jr, Borsboom GJJM, Eijkemans MJC, Vergouwe Y, Habbema JDF. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol. 54:774-781.

Treetampinich C, O'Connor AE, MacLachlan V, Groome NP, de Kretser DM (2000). Maternal serum inhibin A concentrations in early pregnancy after IVF and embryo transfer reflect the corpus luteum contribution and pregnancy outcome. Hum Reprod. 15:2028-32.

Wallace EM, Marjono B, Tyzack K, Tong S (2004). First trimester levels of inhibins and activin A in normal and failing pregnancies. Clin Endocrinol (Oxf). 60:484-90.

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Introduction: The aim was to evaluate the role of serum inhibin A and activin A in the prediction of the outcome of women with 'pregnancies of unknown location' (PULs).
Materials and Methods: Serum hCG, progesterone, inhibin A and activin A levels were measured at 0 and 48 hours. Differences in the mean levels and the change in levels over 48 hours expressed as a ratio (48 hours/0 hours) were examined between the three outcome groups - failing PUL, intra-uterine pregnancy (IUP) or ectopic pregnancy. Variables were incorporated into logistic regression models to predict the pregnancy outcomes, which were evaluated using receiver operator characteristic (ROC) curves.
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Whilst much of the work on the prediction of outcome of PULs has centered on the use of serum hCG and progesterone levels, some other markers have been studied. These include creatine kinase and cancer antigen 125 (CA125). Whilst CA 125 levels and creatine kinase levels alone were not able to predict pregnancy outcome, the CA 125 ratio was found to be able to help distinguishing failing PULs from IUPs, but was not able to detect those at risk of ectopic pregnancy (Condous et al., 2005).

Inhibin A is a glycoprotein secreted by the feto-placental unit. It has been suggested that it may be a specific marker of early pregnancy loss (Muttukrishna et al., 2002). Both single and serial measurements have been used to predict subsequent pregnancy loss in women with recurrent miscarriage (Muttukrishna et al., 2002, Al-Azemi et al., 2003, Prakash et al., 2005). Inhibin A has also been used to predict pregnancy outcome in women undergoing in vitro fertilization where it has been shown to be more accurate than hCG in predicting preclinical

pregnancy loss (Hauzman et al., 2004). In addition, serum inhibin levels have been shown to be lower in spontaneously conceived ectopic pregnancies compared to intrauterine pregnancies (Seifer et al., 1996).

Activin A is another glycoprotein secreted by the feto-maternal unit and it is thought to be a marker of trophoblastic invasion with the potential to identify patients at risk of miscarriage and ectopic pregnancy. Both single and serial measurements of activin A levels have been used to predict miscarriage in women with recurrent miscarriage (Prakash et al., 2005). Levels were significantly lower at 7-8 weeks of gestation in those who subsequently miscarried compared to those with ongoing pregnancies. Serum activin A levels have been used to predict ectopic pregnancy in women with PULs (Florio et al., 2007). Serum levels were found to be significantly lower in women with ectopic pregnancies compared to those with IUPs or failing PULs. Using a cut-off value of 0.37 ng/ml gave a sensitivity and specificity of 100% and 99.6%, respectively for the prediction of ectopic pregnancy (Florio et al., 2007).

The aim of this study was therefore to evaluate the role of single and serial measurements of inhibin A and activin A in the prediction of the outcome of pregnancies of unknown location (PULs).

Methods

This was a prospective observational study on women attending the Early Pregnancy Unit (EPU) of an inner London teaching hospital. Women attended the unit for a number of reasons including: pain, vaginal bleeding, pain with vaginal bleeding, unsure menstrual dates, history of a previous pregnancy complication and maternal anxiety. Women could self refer or were referred by their general practitioner, midwife, the accident and emergency department or another hospital department.

At the time of presentation all women attending the unit underwent a TVS examination using a 5MHz transducer for B mode imaging (Aloka SSD 900, 2000 or 4000, Aloka Co. Japan). They were diagnosed with an IUP (viable or non-viable) or an ectopic pregnancy on the basis of this TVS examination, using the sonographic criteria in Table 1. If no pregnancy could be visualised on TVS, they were classified as a PUL. All haemodynamically stable, pain-free women classified as having a PUL were invited to participate in this study, which was granted research ethics approval.

Women were given an information sheet about the study and were asked for written consent. Blood samples were taken at the time of the TVS examination (time 0 hours) and 48 hours later to measure serum hCG (World Health Organisation, Third International Reference 75/537) and progesterone (Roche Elecsys 2010 Progesterone II test) levels. **Serum was aliquoted and stored at –20ºC and** levels of serum inhibin A and activin A (Serotec, Kidlington, UK) were

also measured at 0 and 48 hours. All samples were analysed in duplicate over 8 batches. The detection limit for activin was 0.05. The average coefficient of variation (CV) of the duplicates was 3.48% (range 2.92-4.43). For inhibin the detection limit was 1.00. The average CV of the duplicates was 4.47% (range 3.54-5.64).

The women were followed up until the final pregnancy outcome was known – failing PUL, IUP or ectopic pregnancy, according to the unit protocol. In general, if the serum hCG level increased by > 66% in 48 hours (Kadar et al., 1981, Hahlin et al., 1995), women were classified as an IUP and rescanned in 7-14 days to confirm the diagnosis. If the initial serum progesterone level was < 20 nmol/L, the women were classified as a failing PUL (Banerjee et al., 2001). They were also classified as a failing PUL if the serum hCG decreased by more than 20% in 48 hours. Serum hCG levels were repeated at 7-14 days to confirm the diagnosis. Women who did not fall into either of these categories were thought to be at risk of ectopic pregnancy and were reviewed every 48-72 hours, until a pregnancy (IUP or ectopic) could be visualised on TVS (using the criteria in Table 1) or had a spontaneous decrease in serum hCG levels to < 5 IU/L, confirming a failed PUL.

Statistical analysis

Missing values were observed in 1% of the patients for serum hCG levels at 0 hours, and in 6% of the patients for levels at 48 hours. For progesterone, inhibin,

and activin, missing values were observed in 6% and 25%, 9% and 24%, and 9% and 25%, respectively. Using multiple imputation (Schafer, 1999; Little & Rubin, 2002), missing values were imputed five times, resulting in five completed data sets. To impute missing values of one marker (e.g. inhibin at 48 hours), information of the other hormonal markers as well as information of so-called auxiliary variables was used. The auxiliary variables were age, gestational age, endometrial thickness, level of vaginal bleeding, nulliparity, history of miscarriages, history of termination of pregnancies, history of ectopic pregnancies, and final pregnancy outcome. Each completed data set was analyzed using standard statistical techniques, and the results were combined to obtain the final results.

The change in serum hCG, inhibin A and activin A levels over 48 hours were expressed as a ratio (level at 48 hours / level at 0 hours). Univariate multicategory logistic regression was used to detect differences in hormonal variables between the three outcome groups – failing PUL, IUP and ectopic pregnancy. Additionally, we investigated how well different hormonal indices were able to detect the different pregnancy outcomes by computing the area under the receiver operating characteristic (ROC) curve (AUC) for each outcome separately.

To evaluate the value of inhibin and activin levels for predicting the three pregnancy outcome groups, multivariate multicategory logistic regression was

used. Log transformations were performed where the variables were highly skewed. The reference model contained hCG information: log of the hCG ratio and log of the average hCG. The AUC for each pregnancy outcome was computed to determine the effectiveness of the models to predict pregnancy outcome. The models' AUC performances were estimated using bootstrapping (Efron & Tibshirani, 1993; Steyerberg et al, 2001). This method starts with computing the data set AUCs, which are too optimistic because the model is fitted and evaluated on the same data. Then, using 200 bootstrap samples, an estimate of each AUC's optimism is derived and subtracted from the AUCs to obtain corrected AUCs. Statistical analyses were conducted with SAS version 9 (SAS Institute Inc., Cary, USA).

Results

Serum hCG, progesterone, inhibin A and activin A levels were available from 141 women with a classification of PUL. **The samples were collected between the 1st July 2004 and the 30th November 2004.** The final pregnancy outcomes were: 67 failing PULs (47.5%), 58 IUPs (41.1%) and 16 ectopic pregnancies (11.4%).

The median hCG, progesterone, inhibin A and activin A levels with ranges are presented in Table 2. The hCG ratio and average progesterone levels were significantly different between the three pregnancy outcome groups. The activin A level was different between the outcome groups at 0 hours but not at 48 hours

(Table 2). However there seemed to be differences for both inhibin A levels, with failing PULs having the lowest levels and IUPs the highest. Changes in activin A or inhibin A levels over time (the ratios) did not differ across pregnancy groups.

Table 3 shows the separate AUCs for each pregnancy outcome to assess the effectiveness of individual hormonal variables in predicting a particular pregnancy outcome. The progesterone levels at 0 and 48 hours were good predictors of a failing PUL or an IUP, but were poor at predicting ectopic pregnancy, Table 3. A decrease in inhibin A levels over 48 hours appears to be the 'best' predictor of ectopic pregnancy (AUC = 0.61). Ectopic pregnancies had the highest median activin level at presentation. Using a cut-off of 0.37 ng/ml on the serum activin A level at presentation to predict ectopic pregnancy (Florio et al., 2007) gave an average sensitivity over the completed data sets of 93% (14.8/16) and an average specificity of 13% (16.4/125).

The multicategory logistic regression model based on serum activin A levels, using the log of the average level and the log of the ratio as predictors, was not helpful in predicting pregnancy outcome (see Table 4 for the corrected AUCs for each pregnancy outcome). Although a model using serum inhibin A levels (using the ratio and the log of the average level as predictors) was of use in the prediction of failing PULs and IUPs in the PUL population, it did not perform as well as the model using serum hCG levels (Table 4). The corrected AUCs for failing PUL, IUP and ectopic pregnancy using the hCG model were 0.95, 0.97

and 0.67 respectively. Addition of both inhibin variables to the hCG model did not improve its performance. The corrected AUCs for failing PUL, IUP and ectopic pregnancy using a model with hCG and inhibin information (log of the average hCG, hCG ratio, hCG ratio square, log of the average inhibin, inhibin ratio) were 0.96, 0.97 and 0.69 respectively.

Discussion

In this study we have shown that in women with PUL maternal serum inhibin A levels can help predict pregnancy loss, while levels of activin A correlate weakly with pregnancy outcome.

Inhibin A

To the best of our knowledge this is the first study to examine the use of inhibin A in the prediction of PUL outcome. Previous studies have shown that inhibin A can be used to predict pregnancy loss in women with recurrent miscarriages (Muttukrishna et al., 2002, Al-Azemi et al., 2003, Prakash et al., 2005), those with threatened miscarriages (Johns et al., 2007) and those who have undergone in vitro fertilization (Treetampinich et al., 2000, Hauzman et al., 2004). Serum inhibin A has also been found to be a predictor of complete miscarriage in women with miscarriage managed expectantly (Elson et al., 2005). We have found that inhibin A levels are different in the three pregnancy outcome groups. There was however no significant difference in the change in serum inhibin A levels over time between the different outcome groups. A low inhibin A level at 0

hours was found to be a good predictor of pregnancy loss (failing PUL, AUC = 0.88, Table 3). Conversely we found high levels of inhibin A at 0 hours to be a good predictor of an IUP (AUC = 0.87, Table 3). However, inhibin levels were no better at predicting PUL outcome than progesterone, Table 3.

A previous study found that inhibin A levels were lower in spontaneously conceived ectopic pregnancies compared to IUPs (Seifer et al., 1996). However this was a case-control study measuring inhibin A levels in women with ectopic pregnancies undergoing surgery using gestation matched controls with IUPs. No comparison was made to women with failed pregnancies. Whilst we found that inhibin A levels were significantly different amongst the three possible PUL outcome groups, with levels lower in ectopic pregnancies compared to IUPs, levels were still higher in ectopic pregnancies than in failing PULs (Table 2). We therefore found that a single inhibin A measurement was not a good predictor of ectopic pregnancy (inhibin A 0 hour AUC = 0.55, Table 3). Using serial inhibin A levels, the prediction of ectopic pregnancy was not much better (AUC = 0.60, Table 4), and did not perform as well as the model based on just hCG measurements (AUC = 0.67, Table 4).

As it is secreted by trophoblast serum inhibin A has been evaluated as a possible surveillance marker after conservative surgical management of ectopic pregnancy as its half-life is significantly shorter than that of hCG (D'Antona et al., 1998). However interpretation of results is complicated as inhibin A is also

secreted by the ovary and increases in levels may also occur with the return of normal ovarian function. Inhibin A was therefore concluded not to be a useful marker for persistent trophoblast after conservative surgical treatment for ectopic pregnancy. However the study did suggest that pre-treatment inhibin A levels may provide an accurate assessment of the viability of trophoblastic tissue within ectopic pregnancies as some ectopic pregnancies with very high hCG levels had very low inhibin A levels. They therefore proposed that inhibin A levels may be useful in helping assess whether ectopic pregnancy management should be expectant, medical or surgical. If true, this may explain why the women with ectopic pregnancies in our study had varying serum inhibin A levels (3.6 – 305 pg/ml at presentation). Some ectopic pregnancies will have active trophoblast and behave like IUPs and others will be failing and behave like failing PULs. A previous study examining serial hCG levels in women with ectopic pregnancies initially classified as PULs found that the changes in serum hCG mimicked an IUP in 20% and a failing pregnancy in 8% of the cases in their series (Silva et al., 2006).

Activin A

Activin A levels have been found to be significantly lower in women with a threatened miscarriage who subsequently miscarried compared to those who had live births (Johns et al., 2007). It has also been suggested that activin A may be used as a marker to predict pregnancies that are likely to miscarry in women with recurrent miscarriage (Prakash et al., 2005). However other studies have

shown that activin A levels are similar in women with miscarriages and controls (Muttukrishna et al., 2002, Wallace et al., 2004).

We found activin A levels are not a good predictor of PUL outcome (Table 3). In contrast to previous studies suggesting lower levels in women who subsequently suffer a miscarriage, in our study activin A levels were higher in failing PULs compared to IUPs (Table 2). Using the activin A level at presentation gave an AUC of 0.62 to predict failing PUL, 0.66 to predict IUP, and 0.58 to predict ectopic pregnancy (Table 3). This is in contrast to those obtained in a recent study evaluating the use of a single measurement of activin A in the diagnosis of ectopic pregnancy in women classified with a PUL (Florio et al., 2007), who found that activin A levels were significantly lower in women with ectopic pregnancies compared to IUPs and failing PULs. In the study by Florio, ROC analysis suggested that activin A, at a cut-off of 0.37 ng/ml, gave a sensitivity and specificity of 100% and 99.6% respectively for the prediction of ectopic pregnancy. When we evaluated this cut-off on our data we found the sensitivity to be 93%, but with a very poor specificity of 13%. Activin A levels in the women with ectopic pregnancies in our study ranged from 0.35 – 0.85 ng/ml at presentation compared to 0.27 ± 0.06 ng/ml in the study by Florio et al. (2007). They suggested that the lower levels of activin A in ectopic pregnancies compared to those with failing PULs may be due to the difficulty of the ectopic trophoblast to correctly implant. Our results do not agree with their findings, possibly because our ectopic pregnancies may have been more of a

heterogeneous group. Certainly in our study there were ectopic pregnancies that had low progesterone levels or low hCG ratios as often seen in failing PULs whilst others had high progesterone levels or high hCG ratios as seen in IUPs. The median progesterone level in the ectopic pregnancies was 24.5 nmol/L (range 2 – 121 nmol/L) and the median hCG ratio 1.09 (range 0.16 – 2.73), (Table 2). Florio et al. did not examine changes in serum hCG over time in their PULs but progesterone levels in their ectopic pregnancies were significantly lower (14.7 ± 0.9 ng/ml) compared to their IUPs (31.8 ± 0.4 ng/ml) suggesting many of them were failing in any event.

In this study we have therefore shown that neither single nor serial measurements of inhibin A or activin A can be used to reliably predict all three possible final outcomes of PULs. The challenge still remains to identify a universal marker of PUL outcome, which combines accuracy, reproducibility and simplicity. However, it may be that a combination of markers may be needed in order to maximize sensitivity and specificity for the various outcomes. For the moment our data suggests that a model based on serum hCG levels at 0 and 48 hours is the optimal approach to the prediction of PUL outcome until such a marker can be identified.

References

Al-Azemi M, Ledger WL, Diejomaoh M, Mousa M, Makhseed M, Omu A (2003). Measurement of inhibin A and inhibin pro-alphaC in early human pregnancy and their role in the prediction of pregnancy outcome in patients with recurrent pregnancy loss. Fertil Steril. 80:1473-9.

Banerjee S, Aslam N, Woelfer B, Lawrence A, Elson J, Jurkovic D (2001). Expectant management of early pregnancies of unknown location: a prospective evaluation of methods to predict spontaneous resolution of pregnancy. BJOG 108:158-163.

Barnhart KT, Sammel MD, Rinaudo RF, Zhou L, Hummel AC, Guo W (2004a). Symptomatic patients with an early viable intra-uterine pregnancy: hCG curves redefined. Obstet Gynecol. 104: 50-5.

Barnhart KT, Sammel MD, Chung K, Zhou L, Hummel AC, Guo W (2004b). Decline of serum human chorionic gonadotropin and spontaneous complete abortion: defining the normal curve. Obstet Gynecol. 104:975-81.

Condous G, Okaro E, Khalid A, Timmerman D, Lu C, Zhou Y, Van Huffel S, Bourne T (2004). The use of a new logistic regression model for predicting the outcome of pregnancies of unknown location. Hum Reprod 19(8): 1900-10.

Condous G, Kirk E, Syed A, Van Calster B, Van Huffel S, Timmerman D, Bourne T (2005). Do levels of serum cancer antigen 125 and creatine kinase predict the outcome in pregnancies of unknown location? Hum Reprod. 20: 3348-3354.

Condous G, Kirk E, Van Calster B, Van Huffel S, Timmerman D, Bourne T (2006). Failing pregnancies of unknown location: a prospective evaluation of the human chorionic gonadotrophin ratio. BJOG 113: 521-527.

Condous G, Van Calster B, Kirk E, Haider Z, Timmerman D, Van Huffel S, Bourne T (2007a). Prediction of ectopic pregnancy in women with a pregnancy of unknown location. Ultrasound Obstet Gynecol. 29:680-7.

Condous G, Van Calster B, Kirk E, Timmerman D, Van Huffel S, Bourne T (2007b). Prospective cross-validation of three methods of predicting failing pregnancies of unknown location. Hum Reprod. 22:1156-60.

D'Antona D, Mamers PM, Lowe PJ, Balazs N, Groome NP, Wallace EM (1998). Evaluation of serum inhibin A as a surveillance marker after conservative management of tubal pregnancy. Hum Reprod. 13: 2305-7.

Efron B, Tibshirani RJ (1993). An introduction to the bootstrap. New York: Chapman & Hall.

Elson J, Tailor A, Salim R, Hillaby K, Dew T, Jurkovic D (2005). Expectant management of miscarriage-prediction of outcome using ultrasound and novel biochemical markers. Hum Reprod. 20:2330-3.

Florio P, Severi FM, Bocchi C, Luisi S, Mazzini M, Danero S, Torricelli M, Petraglia F (2007). Single serum activin A testing to predict ectopic pregnancy. J Clin Endocrinol Metab 92: 1748-53.

Hauzman E, Fedorcsák P, Klinga K, Papp Z, Rabe T, Strowitzki T, Urbancsek J (2004). Use of serum inhibin A and human chorionic gonadotropin measurements to predict the outcome of in vitro fertilization pregnancies. Fertil Steril. 81: 66-72.

Johns J, Muttukrishna S, Lygnos M, Groome N, Jauniaux E (2007). Maternal serum hormone concentrations for prediction of adverse outcome in threatened miscarriage. Reprod Biomed Online. 15:413-21.

Kadar N, Caldwell BV, Romero R (1981). A method of screening for ectopic pregnancy and its indications. Obstet Gynecol. 58: 162-6.

Kirk E, Condous G, Haider Z, Lu C, Van Huffel S, Timmerman D, Bourne T (2006). The practical application of a mathematical model to predict the outcome of pregnancies of unknown location (PULs). Ultrasound Obstet Gynecol. 27: 311-315.

Little RJA, Rubin DB (2002). Statistical analysis with missing data (2nd edition). New York: Wiley.

Muttukrishna S, Jauniaux E, Greenwold N, McGarrigle H, Jivraj S, Carter S, Elgaddal S, Groome N, Regan L (2002). Circulating levels of inhibin A, activin A and follistatin in missed and recurrent miscarriages. Hum Reprod. 17: 3072-8.

Prakash A, Laird S, Tuckerman E, Li TC, Ledger WL (2005). Inhibin A and activin A may be used to predict pregnancy outcome in women with recurrent miscarriage. Fertil Steril. 836:1758-63.

Romero R, Kadar N, Jeanty P, Copel JA, Chervenak FA, DeCherney A, Hobbins JC (1985). Diagnosis of ectopic pregnancy: value of the discriminatory human chorionic gonadotropin zone. Obstet Gynecol. 66:357-60.

Schafer JL (1999). Multiple imputation: a primer. Stat Methods Med Res. 8:3-15.

Seeber BE, Sammel MD, Guo W, Zhou L, Hummel A, Barnhart KT (2006). Application of redefined human chorionic gonadotrophin curves for the diagnosis of women at risk for ectopic pregnancy. Fertil Steril. 86: 454-9.

Seifer DB, Lambert-Messerlian GM, Canick JA, Frishman GN, Schneyer AL (1996). Serum inhibin levels are lower in ectopic than intrauterine spontaneously conceived pregnancies. Fertil Steril. 65:667-9.

Silva C, Sammel MD, Zhou L, Gracia C, Hummel AC, Barnhart KT (2006). Human chorionic gonadotrophin profile for women with ectopic pregnancy. Obstet Gynecol 107: 605-10.

Steyerberg EW, Harrel FE Jr, Borsboom GJJM, Eijkemans MJC, Vergouwe Y, Habbema JDF. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol. 54:774-781.

Treetampinich C, O'Connor AE, MacLachlan V, Groome NP, de Kretser DM (2000). Maternal serum inhibin A concentrations in early pregnancy after IVF and embryo transfer reflect the corpus luteum contribution and pregnancy outcome. Hum Reprod. 15:2028-32.

Wallace EM, Marjono B, Tyzack K, Tong S (2004). First trimester levels of inhibins and activin A in normal and failing pregnancies. Clin Endocrinol (Oxf). 60:484-90.