

THE USE OF MEASUREMENTS OF hCG AND OTHER PREGNANCY-ASSOCIATED PROTEINS AND STEROIDS IN THE DIAGNOSIS OF ECTOPIC PREGNANCY

STABILE.I., LRCP, MRCS. MRCOG, PhD

GRUZINSKAS J.G. MD, FRCOG

ACADEMIC UNIT OF OBSTETRICS AND GYNAECOLOGY,
THE LONDON HOSPITAL MEDICAL COLLEGE WHITECHAPEL, LONDON UK.

ABSTRACT

The measurement of serum of urinary hCG is commonly used to make or exclude the diagnosis of ectopic pregnancy in women presenting with symptoms suspicious of ectopic pregnancy. Detection of depressed hCG levels is indicative of early pregnancy failure. Depressed serum levels of proteins of hormone of trophoblastic, ovarian or endometrial origin make the diagnosis of ectopic gestation more likely, whilst high values are likely to exclude the possibility of this condition.

INTRODUCTION

Unlike lower animals, implantation in the human species can occur in a variety of locations. The incidence of ectopic pregnancy has more than doubled in the United States from 4.5 to 9.4 per 1000 reported pregnancies from 1970 to 1978 (1). Apart from the fetal wastage involved, ectopic pregnancy remains the major cause of maternal mortality in the first trimester of pregnancy in the western world (2). A recent study has shown an increased risk of ectopic pregnancy mortality among black as compared with white women, and among young as compared with old women (3). The poor fertility prognosis for women who have had an ectopic pregnancy should be emphasised. Less than half will conceive again, and only one-third will bear a live child (4, 5). The risk of a second ectopic pregnancy is approximately 8 per cent. Curran (6) has predicted that by the year 2000, 10 per cent of all women of reproductive age will be sterile because of pelvic inflammatory disease (PID), and approximately 3 per cent will have had an ectopic pregnancy. These dismal statistics form the basis of the rationale for earlier diagnosis and treatment of this serious disease.

DIAGNOSIS OF ECTOPIC PREGNANCY

In the acute situation, where there is clinical evidence of haemoperitoneum, the only diagnostic test required is a

laparoscopy or occasionally emergency laparotomy. In the clinically stable woman with a positive pregnancy test complaining of abdominal pain, the diagnosis of ectopic pregnancy is aided by a higher index of suspicion and a few well chosen diagnostic tests. It is noteworthy that the early stages of ectopic pregnancy may mimic the symptoms of other gynaecological conditions such as PID, ruptured corpus luteum, dysfunctional uterine bleeding and threatened or incomplete abortion (7). Subfertile patients who have a past history of ectopic gestation, have undergone tubal surgery or attend an in-vitro fertilisation (IVF) or Gamete Intra Fallopian Transfer (GIFT) programme are considered at high risk for ectopic pregnancy. In having the advantage of intensive clinical, biochemical and sonographic monitoring, the diagnosis is often made earlier in this group. At present there is no effective biochemical measurement which discriminates between failed intrauterine pregnancy and ectopic gestation. This review will summarise current opinion on the measurement of trophoblastic proteins (human chorionic gonadotrophin [hCG], Schwangerschaftsprotein 1 [SP1], human placental lactogen [hPL], and pregnancy-associated plasma protein A [PAPP-A]), endometrial (progesterone dependent endometrial protein [PEP]/placental protein 14 [PP14]) and decidual proteins (insulin-like growth factor binding protein [IGF-BP]/placental protein 12 [PP12]), fetal proteins (alphafetoprotein [AFP]), and ovarian steroids (oestradiol and progesterone), in the management of suspected ectopic pregnancy.

HUMAN CHORIONIC GONADOTROPHIN (hCG)

hCG estimations remain the mainstay of the differential diagnosis of abdominal pain in women of reproductive age (8). Although early and fast detection of low levels of hCG is important, the efficiency of any given pregnancy test based on hCG depends on its sensitivity and specificity, which are in turn, a reflection of the sample fluid tested and technique used. Urinary latex agglutination inhibition tests have a sensitivity of 700-1000 IU/l and are positive in up to 50 per cent of patients with

ectopic gestation (9). Simple dipstick techniques for the measurement of hCG based on enzyme labelled monoclonal antibodies (10) or immunofluorometric assays (11) have been introduced into clinical practice. The high sensitivity (IU/I) and speed (10 minutes incubation time) of these new generation assay techniques have contributed greatly to the increased clinical acceptability of this bedside test. However, it should be noted that very low levels of hCG which may result from human pituitary hCG (12) have also been recorded in the serum of non-pregnant and postmenopausal women.

The false negative rate of hCG estimations in patients with ectopic pregnancy clearly depends on the cut-off level used. Poulsen and colleagues (13) have reported 8 cases of ectopic pregnancy with undetectable or very low levels of circulating hCG (<25 IU/I) emphasising that a high index of suspicion should be maintained if a diagnosis of ectopic pregnancy is suspected, even if serum hCG is undetectable in the peripheral circulation. Olson (14) and Romero and colleagues (15) report false negative rates for ectopic pregnancy of 0.5 per cent at a detection limit of 10 IU/I (IRP) [= 5 IU/I 2nd IS] and 0 per cent at <3 IU/I.

Ectopic pregnancies generally produce hCG at a slower rate than normal pregnancies (16). Normal hCG values are found more often in early and in unruptured ectopic pregnancies (17). This has led to the theory that the more viable the ectopic pregnancy is, the more likely it is to rupture. In addition, negative (<25 IU/I 2nd IS) hCG estimations are associated with marked necrosis of chorionic tissue (13), supporting the concept that the reduction in functional trophoblast mass is due to poor vascularisation and cell necrosis. It has also been suggested that the lower hCG levels in ectopic pregnancy may be related to the high incidence of blighted ova (18) in this group of pregnancy failure. Indeed, normal hCG levels are recorded when normally developed embryos are removed from the fallopian tube (19). Individual variations in levels of serum hCG are large, such that there is a great deal of overlap between normal and pathological pregnancies at any given gestational age. This suggests that unless the time of conception is known with some accuracy, hCG estimations alone are of limited value in the diagnosis of ectopic pregnancy (20).

To further improve the diagnostic value of hCG estimations, some workers have analysed the rate of hCG production, correlating it with the sonographic detection of a gestational sac. Kadar and colleagues (21) have established a discriminatory hCG zone (6000 to 6500 IU/I), i.e. the levels of hCG at which a gestational sac first becomes visible using abdominal ultrasound. By calculating the doubling rates of hCG they have devised a simple algorithm for the management of suspected ectopic pregnancy based on serial quantitative hCG estimations. hCG measurements should be performed at 48 hour intervals, as after one day there is only a 30 per cent increase in the hCG titre, a value which may overlap with the level of an intrauterine pregnancy. After spontaneous

abortion, the half-life of hCG is reported to be between 3 and 7 days (22). Thus the information obtained from a single hCG estimation may not reflect the actual state of the trophoblast. The shorter half-life of the alpha subunit of hCG may make it a better diagnostic test. L'Hermite-Baleriaux and colleagues (23) have shown that if hCG levels are depressed, beta-hCG is less affected than the alpha subunit, suggesting that the alpha subunit of hCG reflects an early alteration of the trophoblast at a time when hCG secretion is still preserved. The pathophysiology of these observations is as yet unknown, but warrants further investigation.

SCHWANGERSCHAFTSPROTEIN 1 (SP 1)

SP1 is a glycoprotein with a molecular weight of 90KD. It is secreted by the syncytiotrophoblast and appears in the maternal circulation shortly after implantation (24). SP1 measurements have also been evaluated in the diagnosis of ectopic pregnancy. Despite encouraging initial results (25), the high false-positive rates diminish its value in excluding ectopic gestation (26).

Our own observations in a group of 294 women with a subacute presentation of suspected ectopic pregnancy, revealed that all but 25 of the 105 women with confirmed ectopic pregnancy had normal circulating levels of SP1. This would suggest that the synthesis of this protein is independent of the site of implantation, thus limiting its usefulness in the diagnosis of ectopic pregnancy. Nevertheless, SP1 can be used as a pregnancy test in women who have received hCG for ovulation induction and hence it may retain a limited role in women undergoing assisted conception techniques who are at risk of this condition (27).

PREGNANCY-ASSOCIATED PLASMA PROTEIN A (PAPP-A)

PAPP-A estimations appears to be of greater value than other pregnancy proteins in the diagnosis of ectopic pregnancy (13, 28-36). Contrary to the findings of the first published report (37), several studies have documented undetectable or depressed PAPP-A levels in 50 per cent of women with histologically proven ectopic pregnancy. By contrast, hCG and SP1 were detected in almost all patients studied (30). In our own study of 294 women with suspected ectopic pregnancy, the condition was confirmed in 105. Depressed levels of hCG were found in all but 15 women, 9 of whom had a live extrauterine pregnancy confirmed at ultrasound examination. By contrast, all but 25 patients had normal circulating levels of SP1. Circulating PAPP-A was detected in only 34 women with ectopic pregnancy, of whom 24 had levels within the normal range. In the remaining women, PAPP-A was undetectable by our in-house radioimmunoassay (RIA) with a detection limit of 0.02 IU/I. It should be noted, however, that this protein cannot be detected at less than 7 weeks gestation, such that a negative PAPP-A result alone prior to this date cannot exclude an ectopic pregnancy.

Immunohistochemical techniques have demonstrated the relative paucity of PAPP-A in tubal trophoblastic tissue (38), which may be a reflection of either poor vascularisation at the ectopic implantation site or a selective compromise of PAPP-A synthesis in this condition. The apparently normal distribution of other pregnancy proteins and hormones (hCG, hPL, SP1) in ectopic pregnancy as determined by immunohistochemical localisation studies, would suggest that the controlling mechanism for the synthesis of these proteins differs from that of PAPP-A. In vitro explant studies are in progress to investigate the possible mechanisms of reduced circulating PAPP-A levels in ectopic gestation.

HUMAN PLACENTAL LACTOGEN (hPL)

This placental hormone with a molecular weight of 25KD is detectable in the maternal circulation by radioimmunoassay (RIA) from 6 weeks gestation. There is a correlation between hCG and hPL levels in ectopic pregnancy, which is not surprising as this hormone is merely one of a number of substances produced by the fetoplacental unit. The predictive value of hPL in suspected ectopic pregnancy is limited as hPL values are more frequently normal than the levels of hCG or SP1 (26).

ALPHAFETOPROTEIN (AFP)

Maternal serum alphafetoprotein (AFP) levels have been measured in 44 women with histologically proven ectopic pregnancy (39). As expected, highest levels were observed in five women in whom a live fetus was seen ultrasonically pre-operatively, supporting the view that the fetus is the primary source of maternal AFP. The overlap in levels between failed intrauterine pregnancy and ectopic pregnancy renders these observations of little clinical value.

OVARIAN STEROIDS

The activity of the corpus luteum as reflected in progesterone levels is receiving greater attention in the diagnosis of ectopic pregnancy. Early studies showed depressed progesterone levels in up to two-thirds of patients with ectopic pregnancy (40-42). Matthews and colleagues (43) have reported the use of the rapid determination of progesterone levels (within 2 to 4 hours) as an adjunct to clinical diagnosis. They have suggested that in the patient with supporting history and physical signs of ectopic pregnancy, a depressed progesterone level (<15 ng/ml) makes the diagnosis of an accident of pregnancy more likely. The predictive value positive of a progesterone level >15 ng/l at less than 8 weeks gestation for a normal outcome of that pregnancy reaches 95 per cent. The clinical value of these observations remains uncertain, and the results of prospective studies of the specificity of depressed progesterone levels for ectopic pregnancy are awaited. By comparing progesterone levels in normal and ectopic pregnancies with similar hCG levels, Hubinont and colleagues (44) have shown that the luteal

insufficiency observed in ectopic pregnancy cannot be explained solely by the decrease of hCG. Furthermore, Lower and colleagues (45) have confirmed these findings, notably in women who have ovulated spontaneously in the asymptomatic phase, at the time when pregnancy was first diagnosed and selecting women only if hCG levels were rising. Nevertheless, low progesterone levels are known to occur in patients with early spontaneous abortion in whom the discrepancy between hCG and ovarian steroids also occurs.

The value of oestradiol estimations in ectopic pregnancy has been addressed by a few studies. Depressed levels of oestradiol have been reported in ectopic pregnancy (19, 44). Eighty percent of women with ectopic pregnancy had serum oestradiol levels below the 10th centile of the normal range in these studies, which we were not able to confirm in the women described above (45). The overlap between values in intra- and extrauterine pregnancy remains the major limitation of the use of this pregnancy steroid in the management of suspected ectopic gestation.

PREGNANCY PROTEIN 12 (IGF-BINDING PROTEIN)

Placental protein 12 (PP12), a glycoprotein identical to the small molecular weight binding protein for insulin-like growth factor (IGF-BP), is synthesised principally by decidual cells in the decidua compacta (46). The rate of synthesis of IGF-bp increased dramatically between 6 and 12 weeks of pregnancy, reflecting decidualisation of the endometrium during this period. Preliminary observations from our group (47) have suggested that regardless of the implantation site outside the uterus, ectopic pregnancy is associated with normal IGF-bp levels. Thus, the decidual synthesis of this protein is probably not dependent on the intimate relationship between decidua and trophoblast.

PREGNANCY PROTEIN 14 (PP14)

This endometrial protein has a molecular weight of 42 to 56 KD and is synthesised and secreted by the glandular epithelium (48) [synonym: progesterone-dependent endometrial protein (PEP)] (49). Trends in blood levels throughout pregnancy have shown a remarkable similarity with hCG (49), the highest levels being found in amniotic fluid at the end of the first trimester (50). Preliminary observations from Denmark and London (30, 31) have indicated that high circulating PP14 levels may be used to distinguish between women with normal intrauterine and extrauterine pregnancy. These findings have been confirmed by Pedersen et al (51), but further studies are necessary to examine this phenomenon in more detail.

CONCLUSIONS

Protein and hormone synthesis by the trophoblast (hCG, SP1, PAPP-A), endometrium (PP14), and ovary (pro-

gesterone and oestradiol) is depressed in ectopic gestation. hCG remains the primary biochemical diagnostic test used in the clinically stable woman suspected of harbouring an ectopic pregnancy. Nevertheless, the clinician should be armed with detailed knowledge of the limitations of the assay techniques available. In particular, negative serum hCG levels should not be equated

with the absence of ectopic pregnancy, unless the detection limit of the assay used is below 3 IU/l. The presence of hCG and depressed levels of one other parameter, e.g. PAPP-A, progesterone or PEP increased the likelihood of ectopic gestation. Finally, the interpretation of the test result should take into account the method of conception and the mode of clinical presentation.

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