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*Original Article*

# The value of ratio of hCG, progesterone in local blood of pregnancy location versus venous blood in the diagnosis of ectopic pregnancy

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**Abstract:** Objective: The aim of this study is to combine the ratios of venous serum/colporrhagia and hemoperitoneum/venous serum of human chorionic gonadotropin (hCG) and Progesterone (P) to generate and evaluate a new method to improve the prognosis of Ectopic pregnancy (EP). Methods: For patients with curettage procedure, curettage material and venous blood were obtained at the same time. For patients receiving culdocentesis and laparoscopic exploration, abdominal fluid and venous blood samples were obtained synchronously during surgery. Results: The sensitivity and specificity of Rp/v-hCG>1.0 and Rp/v-P>1.0 for diagnosis of EP was 88.2% and 80.71%, 93.8% and 87.53%, respectively. The sensitivity of parallel test (Rp/v-hCG>1.0 or Rp/v-P>1.0) was 92.23%. The specificity of serial test (Rp/v-hCG>1.0 and Rp/v-P>1.0) was 100%. For the area under the ROC curve of Rp/v-hCG and Rp/v-P, the parallel test and serial test were 0.91 and 0.82,0.90 and 0.87, respectively. At the determining threshold point of 1.0, the sensitivity of Rv/c-hCG and Rv/c-P for the diagnosis of EP was 56.73% and 60.01%. The specificity was 100% and 100%, respectively. The sensitivity of parallel test (Rv/c-hCG>1.0 or Rv/c-P>1.0) was 73.33%. For the area under the ROC curve of Rv/c-hCG, Rv/c-P and the parallel test was 0.78,0.80 and 0.87, respectively. Conclusions: It is proposed that EP can more rapidly and accurately be diagnosed by multiple biomarkers' test of Rp/v-hCG>1.0 and/or Rp/v-P>1.0, as well as Rv/c-hCG>1.0 and/or Rv/c-P>1.0 via culdocentesis or curettage.

**Keywords:** Ectopic pregnancy, human chorionic gonadotropin, progesterone

## Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and is responsible for pregnancy-related deaths in the first trimester [1], which is defined the implantation of a gestational sac outside the uterine corpus [2]. Approaching 1% to 2% of recognized pregnancies will be ectopic pregnancies [3]. And recent epidemiologic studies suggest that 6% of maternal deaths in the United States are attributable to EP [4]. The death rate from ectopic pregnancy has been declined in recent 30 years, which seems to be the result of earlier detection and treatment, but not the improvements in operative technique, anesthesia, or blood banking [5]. However, diagnostic problems still exist in these established methods and in 40-50% of cases the diagnoses made at presentation are incorrect [6]. So diagnosing ectopic pregnancy early and accurately is a great clinical concern for practitioner, and serum biomarkers are currently being investigated as a solution to need for a rapid and accurate test for ectopic pregnancy [7].

Earlier detection of ectopic pregnancy has been made possible due to more sensitive and specific multi-biomarkers' levels, high-resolution transvaginal ultrasound, culdocentesis, dilatation and curettage, as well as the widespread availability of laparoscopy [5]. Although the sensitivity and specificity of the available diagnostic tests have increased, there is no definitive non-surgical diagnostic test, and diagnosis often requires following patients over multiple visits for several days to weeks, which increases the risk of tubal rupture, the cause of most

EP-related deaths. Currently the most commonly used clinical method for the diagnosis of EP is the combination of transvaginal ultrasound and serial serum hCG levels, when the location of the pregnancy is unknown on initial presentation [2]. However, in many of the emergency rooms in developing countries, transvaginal ultrasound examination is absent, which limits the prompt diagnosis of EP. So a rapid and accurate biomarker test to detect the presence of an EP would permit early treatments to prevent mortality and morbidity of this condition with preservation of fallopian tube function and fertility [2, 8].

Currently, the only biomarker, human chorionic gonadotropin (hCG), is used routinely in clinical practice [9]. However, following serial hCG levels has limitations that the expected minimum rate of rise in 48 hours in a viable pregnancy varies in reports from 35% to 66% [10]. A single value of hCG is clinically useful in determining whether a gestational sac should reliably be visible on ultrasound (the "discriminatory zone") in an intrauterine pregnancy. Therefore, hCG is not diagnostic and can only assist diagnosis in combination with ultrasound use [11].

Progesterone (P), which is a critical hormone for the establishment of normal pregnancy, secreted by the corpus luteum has been studied extensively and used as an assist diagnosis to EP [12]. A systematic review of progesterone found that 3% of patients with a viable intrauterine pregnancy had serum progesterone value less than 5 ng/ml and 2.6% of patients with a serum progesterone level greater than 20 ng/ml had an EP. However, a low or high value of progesterone could not distinguish an intrauterine pregnancy form an EP [13]. However a low serum progesterone level can aid in identifying patients at higher risk for an EP who need to be followed vigilantly. Our group's previous work has indicated that the local hCG level of hemoperitoneum is much higher than that of venous serum for EP [14-16]. The reasons for this finding may be that (1) blood filling the posterior pouch of Douglas or Morisson's space is from the implantation site of gestational sac, into which the hCG secreted by cytotrophablasts directly flows (hCG secreted into venous serum is relatively low); (2) the metabolism of hCG in the hemoperitoneum is slower than that in venous serum [15].

Curettage is used for assisting diagnosis of ectopic pregnancy in many hospitals [17]. Ratio of blood hCG/curettage material hCG could be used as a reliable and fast diagnosis method for differentiating miscarriage and EP in early state. Accuracy rate of this method is 91.7% [18]. As none of the currently discovered biomarkers has consistently differentiated ectopic pregnancies, several researchers have attempted to combine several markers into one test with better diagnostics than individual markers [19]. The aim of this study is to combine ratio of blood/curettage material and hemoperitoneum/venous serum of hCG and P to generate and evaluate a new method to improve the prognosis of EP. In other words, the new method may be helpful for instantly discriminating early EP from IUP without any unnecessary surgical intervention.

## Materials and methods

### *Clinical samples*

From March 2013 to Feb 2014, this study recruited 167 subjects with suspected EP at the outpatient department or emergency center, which was conducted by the International Peace Maternal and Child Health Hospital, Shanghai Jiaotong University, Shanghai, China. Suspected EP was defined as the patient presenting with a positive pregnancy test, abdominal pain, history of amenorrhea, transvaginal sonography revealing no intrauterine gestational sac, and vaginal bleeding or hemoperitoneum found by culdocentesis. The subjects with serious medical complications, severe diseases, and presence of a gynecological tumor were excluded. The subjects without the appearance of vaginal bleeding or peritoneal fluid were excluded as well. Informed consent was obtained from all subjects before the study, which had been approved by Institutional Review Board.

Clinical information of all patients were collected for the following: age, gravidity, gestational age, with or without abdominal pain, the existence of vaginal bleeding, gynecological ultrasound, ectopic position of sac, the type of fallopian pregnancy, the appearance and volume of culdocentesisand peritoneal fluid or vaginal bleeding.

For patients with curettage procedure (62 cases), curettage material and venous blood (10 mL) were obtained at the same time. For patients receiving culdocentesis (19 cases) and laparoscopic exploration (116 cases), ab-

**Table 1.** Characteristics of patients in two groups

| Indices | EP group (n=167) | No-EP group (n=48) | P |
|---|---|---|---|
| Age | 25.22±5.03 | 25.50±5.42 | 0.760 |
| Gravidity | 1.69±0.559 | 1.73±0.594 | 0.792 |
| Stomach ache | 43/104 | 7/16 | 0.613 |
| Vaginal bleeding | 96/104 | 13/16 | 0.766 |
| Gestational age (days) | 43.68±7.02 | 43.81±7.12 | 0.913 |

**Table 2.** Compare celiac blood and blood indexes ratio between groups

| | | EP (n=167) | hIUP (n=16) | P |
|---|---|---|---|---|
| Rp/v-hCG | P25 | 2.29 | 0.47 | |
| | P50 | 5.74 | 0.57 | <0.001 |
| | P75 | 26.66 | 0.77 | |
| Rp/v-P | P25 | 1.13 | 0.40 | |
| | P50 | 2.40 | 0.74 | <0.001 |
| | P75 | 4.32 | 0.94 | |
| Rp/v-CK | P25 | 0.40 | 0.69 | |
| | P50 | 0.87 | 0.92 | 0.923 |
| | P75 | 1.36 | 1.06 | |

dominal fluid (5 mL) and venous blood (5 mL) samples were obtained synchronously during surgery. After centrifuged with 2100 rpm, all blood samples were sent immediately to measure the concentrations (U/l) of hCG and P.

*Marker measurements*

Bayer's ADVIA Centaur automated chemiluminescence immunoassay system and the test kits for total hCG and P were used to measure hCG and P concentration.

*The criteria of EP*

Subjects were separated into two groups (the EP and non-EP group) according to clinical gold standards as the following: Intrauterine gestational sacs were finally confirmed by dilation and curettage in the presence of chorionic villi or falling serum hCG levels (<5 U/l), or subsequent sonography. Abdominal gestational sacs were confirmed either by histological diagnosis or by exclusion of an intrauterine gestational sac [14].

*Statistical analysis*

Date analysis was performed using the Statistical Package for the Social Sciences (SPSS 17.0). Calculate the ratios of peritoneal/venous serum or venous serum/curettage material of HCG and P. Independent sample test and Mann Whitney U tests were used to compare the differences between the groups with values expressed as mean ± standard deviation or median, 25th and 75th percentiles. Receiver operating characteristic (ROC) curve was drawn for determining threshold of identifying EP and miscarriage (intrauterine pregnancy with hemoperitoneum or vaginal bleeding) and evaluating the sensitivity, specificity and accuracy in diagnosis. For all tests, the probability level of $P<0.05$ was considered statistically significant.

## Results

*Clinical characteristics of two groups*

Among total 167 subjects of suspected EP, 119 patients were diagnosed with EP (EP group) and 48 patients were diagnosed with intrauterine pregnancy (non-EP group) according to clinical gold standards. Among 119 EP, 30 of them had performed curettage procedure before surgery, 114 patients underwent laparoscopy and 5 patients received laparotomy by evaluating the clinic symptoms. In non-EP group, 32 patients performed curettage procedure, 12 patients received conservative treatment, and 4 patients underwent laparoscopy based on the appearance of culdocentesis fluid (deep red). The demographic data and clinic characteristics between the two groups are summarized in **Table 1**. There was no significant difference between age, the percentage of abdominal pain, vaginal bleeding and gestational age in two groups.

*Analysis of peritoneal/venous serum index*

In non-EP group, only 16 cases obtained abdominal fluid sample, which meant hemoperitoneum associated with either an intrauterineoraborted pregnancy (hIUP). The data between two groups is shown in **Table 2**. The median of Rp/v-hCG and Rp/v-P were both significantly greater in EP group than in hIUP group ($P<0.001$). ROC analysis shows that Rp/v-hCG and Rp/v-P could be used for the differential diagnosis of EP for hIUP. The threshold of Rp/v-hCG and Rp/v-P for the diagnosis of EP is 1.0

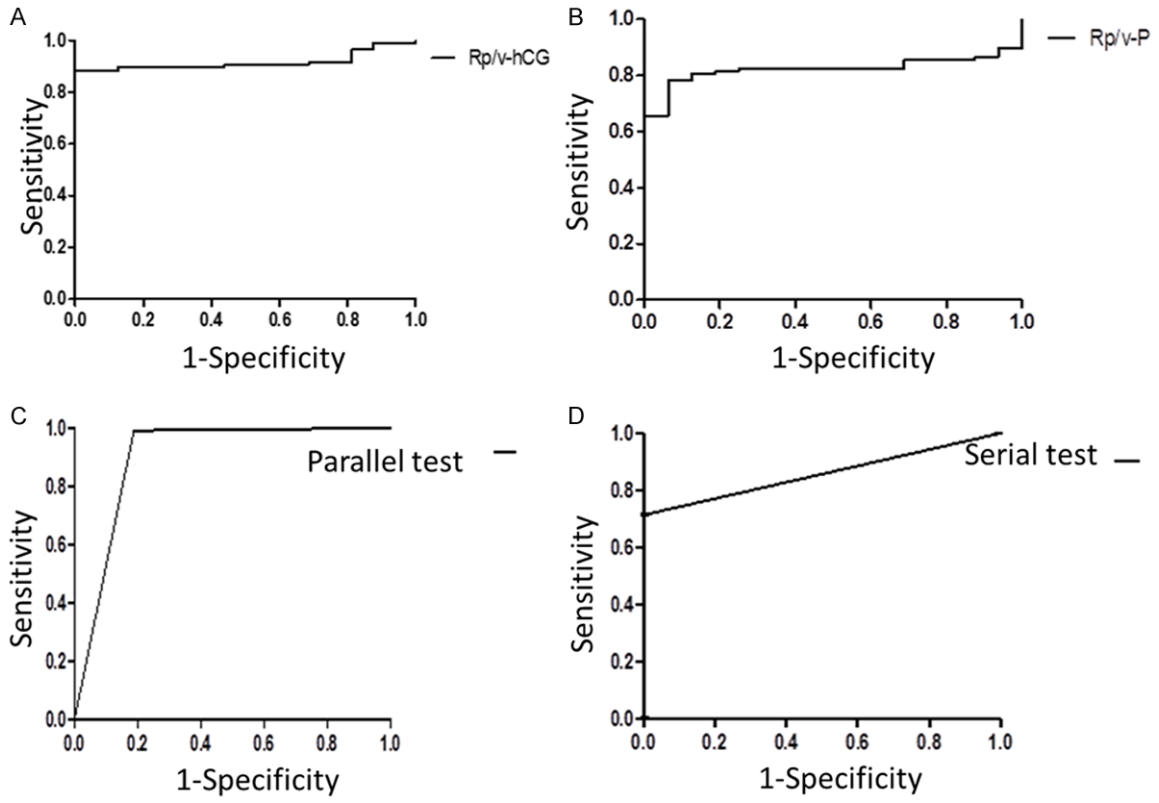


**Figure 1.** The area under the ROC curve of Rp/v-Hcg (A), Rp/v-P (B), serial test (C) and parallel test (D).

**Table 3.** Comparison of venous blood vaginal ratio of each index between groups

| | | EP (n=30) | IUP (n=32) | P |
|---|---|---|---|---|
| Rv/c-hCG | P25 | 0.29 | 0.20 | |
| | P50 | 2.22 | 0.31 | 0.001 |
| | P75 | 11.58 | 0.43 | |
| Rv/c-P | P25 | 0.67 | 0.40 | |
| | P50 | 1.77 | 0.50 | <0.001 |
| | P75 | 4.20 | 0.64 | |
| Rv/c-CK | P25 | 0.83 | 0.73 | |
| | P50 | 1.00 | 1.05 | 0.611 |
| | P75 | 1.34 | 1.27 | |

(at this point sensitivity = 88.2% and 80.71%; specificity = 93.8% and 87.53%, respectively). The sensitivity of parallel test (the criterion of Rp/v-hCG>1.0 or Rp/v-P>1.0 as the diagnostic threshold) was 92.23%. The specificity of serial test (the criterion of Rp/v-hCG>1.0 and Rp/v-P>1.0 in the meantime as the diagnostic threshold) was 100%. For the area under the ROC curve of Rp/v-hCG, Rp/v-P, parallel test and serial test were 0.91, 0.82, 0.90 and 0.87, respectively (**Figure 1**).

*Analysis of venous serum/colporrhagia index*

30 cases in EP group and 32 cases in non-EP group obtained curettage material sample. The median of Rv/c-hCG and Rv/c-P were both found to be significantly greater for EP compared to intrauterine pregnancy (IUP) (P=0.001 and P<0.001, respectively), shown in **Table 3**. Rv/c-hCG andRv/c-P could be used for identifying EP and IUP by ROC analysis. At the determining threshold point of 1.0, the sensitivity of Rv/c-hCG and Rv/c-P for the diagnosis of EP was 56.73% and 60.01%, the specificity were 100% and 100%, respectively. The sensitivity of parallel test (the criterion of Rv/c-hCG>1.0 or Rv/c-P>1.0 as the diagnostic threshold) was 73.33. For the area under the ROC curve of Rv/c-hCG, Rv/c-P and parallel test were 0.78, 0.80, and 0.87, respectively (**Figure 2**).

## Discussion

*Rp/v-hCG, Rp/v-P and Diagnosis of EP*

Our group's previous study demonstrates that Rp/v-hCG>1.0 can be used for diagnosis of EP,

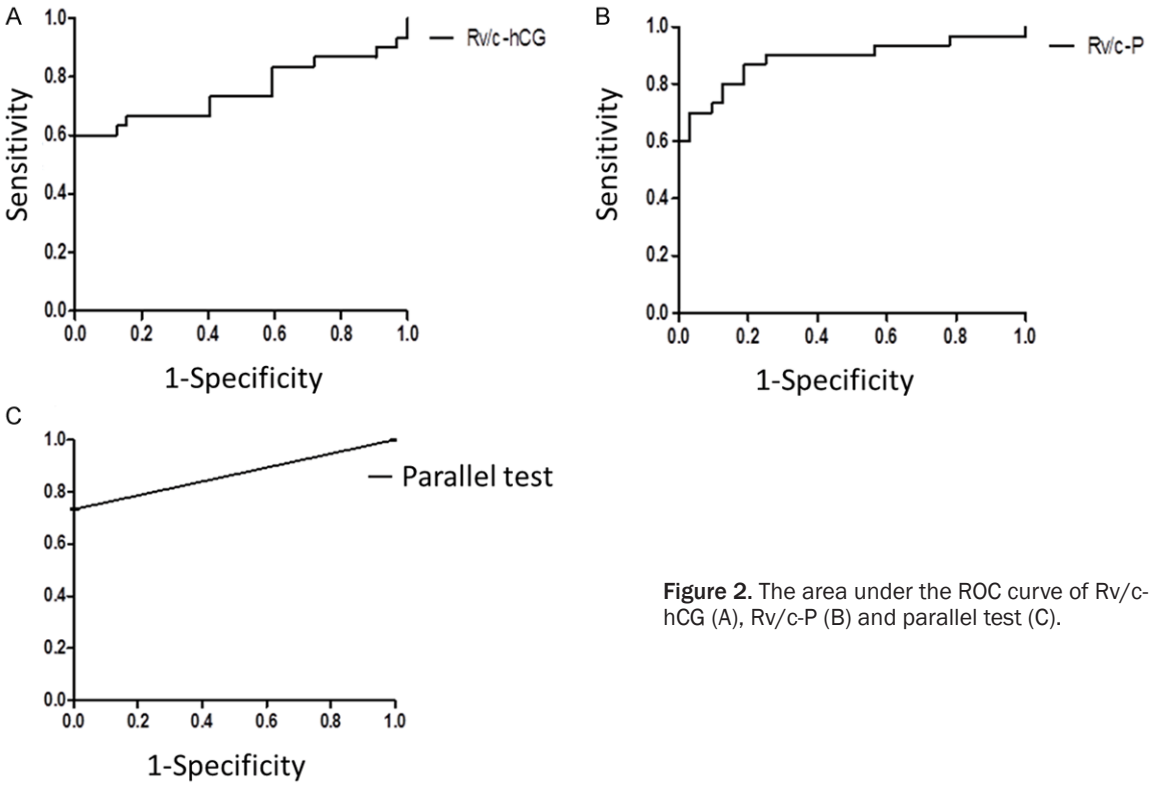


**Figure 2.** The area under the ROC curve of Rv/c-hCG (A), Rv/c-P (B) and parallel test (C).

which is an accurate, rapid, and convenient method for diagnosis of EP, has high sensitivity and specificity [14-16]. With the gestational sac of an EP invasion of the oviduct wall, amounts of hCG in local blood will flow directly into the pelvic (relatively less HCG secreted into venous serum), and hCG metabolism in the hemoperitoneum is slower than that in circulation. So hCG level in pelvic is much higher than that of venous serum for EP. We expect to find a biochemical indicator combined with hCG to improve the diagnostic sensitivity and specificity. Then we hypothesized that if that local progesterone level would also be higher than that of venous serum for EP. The study shows that diagnosis of EP by single test of Rp/v-P>1.0 also has high sensitivity and specificity but not as good as diagnosis by single test of Rp/v-hCG>1.0. The sensitivity of parallel test which combines Rp/v-hCG>1.0 with Rp/v-P>1.0 is higher than single test. It is useful for preoperative and intra-operative screening and reducing the rate of missed diagnosis. Serial tests raise the specificity of diagnosis and reduce the rate of misdiagnosis when compared with single test too.

Formerly culdocentesis alone only can verify hemoperitoneum, but not differentiate EP from hIUP. According to our result, culdocentesis may be routinely performed to women diagnosed in PUL with hemoperitoneum, which can differential diagnostic algorithm for patients with a positive urine pregnancy test and hemoperitoneum.

*Rv/c-hCG, Rv/c-P and diagnosis of EP*

Patients with suspected EP may occur colporrhagia and/or hemoperitoneum. These patients can be diagnosed for EP by the ratio of abdominal fluid and venous blood. However, some patients only experience vaginal bleeding, or the volume of hemoperitoneum is too petty for culdocentesis. Under that condition, we hypothesized that if the ratio of venous blood and vaginal blood could assist diagnosis of EP. The previous study shows that the local concentration of hCG and P of abdominal fluid is greater than that of venous blood for the patients with EP. If the vaginal blood flows out of the uterine cavity for abnormal intrauterine pregnancy, it is local blood in pregnancy location and the concentration of hCG and P should be greater than that of venous blood. Vice versa, the concentration of hCG and P of venous blood should be greater than that of vaginal blood in EP. There was a study shown that ratio of blood hCG/

curettage material hCG could be used as a diagnosis method for differentiating miscarriage and EP in early state. Accuracy rate of this method is 91.7% [18]. The result of our study also verifies the hypothesis. But in our study, the sensitivity of a single test of Rv/c-hCG or Rv/c-P>1.0 is low for diagnosis of EP. The sensitivity of tests combined Rv/c-hCG and P is higher when compared with single test which can help reducing missed diagnosis. If the sensitivity of Rv/c-hCG>1.0 and/or Rv/c-P>1.0 is low, it won't help the clinicians to conduct preoperative screening for EP patients. We suspect that is because part of the blood flows to the pelvic cavity and part of it flows to uterine cavity, therefore the concentration of hCG and P of the vaginal blood of some EP patients is greater than that of venous blood. Meanwhile, the specificity of Rv/c-hCG or Rv/c-P>1.0 for diagnosis of EP reaches 100%, so almost there won't be a misdiagnosis. Patients with Rv/c-hCG or Rv/c-P>1.0 can be diagnosed as EP. Determination of EP by the ratio of venous blood and vaginal blood is a new diagnostic method for suspected patients without hemoperitoneum. Uterine curettage was performed as an assist diagnosis of ectopic pregnancy in many hospitals [20]. Currently there is a change in clinical practice that curettage should not be used in the routine for women wanted pregnancies with a PUL to avoid termination of pregnancy [21].

It is proposed that EP can rapidly and accurately be diagnosed by single or multiple biomarkers test of Rp/v-hCG>1.0 and Rp/v-P>1.0, as well as Rv/c-hCG>1.0 and Rv/c-P>1.0 via culdocentesis or curettage. The multiple markers tests taken advantage of different biologic mechanisms can be more efficient. The clinical applicability of the diagnostic algorithm is yet to be further evaluated in larger samples of retrospective and prospective studies. In addition, combining multi-biomarkers, such as vascular endothelial growth factor and markers of abnormal trophoblast, would be a promising trial for predicting the outcome of EP and differentiating EP from hIUP or abnormal IUP. Extensive sample research will be needed for future work and new simple biochemical indicators should be found to improve the diagnosis value.

In conclusion,it is proposed that EP can more rapidly and accurately be diagnosed by multiple biomarkers' test of Rp/v-hCG>1.0 and/or Rp/v-P>1.0, as well as Rv/c-hCG>1.0 and/or Rv/c-P>1.0 via culdocentesis or curettage.

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## Disclosure of conflict of interest

None.

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