Difference in Serum Human Chorionic Gonadotrophin Levels Measured Using the World Health Organization 3rd versus 5th International Standard: A Correlation Study with Reference to Management of Pregnancy of Unknown Location

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Objective: To evaluate the difference in serum human chorionic gonadotrophin (hCG) level in pregnant women when using assays calibrated against the World Health Organization (WHO) 3rd versus 5th International Standard (IS), and to determine the implications for management of pregnancy of unknown location (PUL).
Methods: 105 samples of serum hCG obtained from pregnant women were tested using assays calibrated against the WHO 3rd IS versus 5th IS. The clinical course, ultrasound findings, final diagnosis, and clinical outcome were evaluated. The optimal cut-off value of 'discriminatory zone' for management of PUL was determined using receiver operating characteristic curve analysis.
Results: Both WHO 3rd IS and 5th IS were highly correlated (Pearson's r=0.996, r^2=0.992) but not equivalent. The mean percentage difference was 12.9%. 34 paired samples were included in a diagnostic-validation study, and the cut-off value of 'discriminatory zone' was 1500 IU/L for the 3rd IS (sensitivity=50.0%, specificity=87.5%, area under curve=77.9%) and 1745 IU/L for the 5th IS (sensitivity=60.0%, specificity=87.5%, area under curve=79.2%).
Conclusion: Calibration of serum hCG using the WHO 3rd IS and 5th IS was highly correlated but not equivalent. A larger prospective study is required before recommendations can be made with regard to the cut-off value of a new 'discriminatory zone'.

Introduction

Serum human chorionic gonadotrophin (hCG) level and transvaginal ultrasonography are important diagnostic tools for ectopic pregnancy. Pregnancy of unknown location (PUL) is diagnosed when neither an intra-uterine pregnancy nor an extra-uterine pregnancy can be visualized on ultrasonography. Using transvaginal ultrasonography, an ectopic pregnancy is suspected if an intra-uterine pregnancy cannot be visualised when the hCG level is between 1500 and 2000 IU/L, which is defined as the 'discriminatory zone'[1,2]. In these studies, the hCG assays were calibrated against the World Health Organization (WHO) 3rd International Standard (IS).

In 1986, the 3rd IS for hCG (coded as 75/537) was established by the WHO Expert Committee on Biological Standardization[3]. In 1999, the 4th IS for chorionic gonadotrophin (coded as 75/589), calibrated by the same procedure, was established[4]. Both standards were purified from urine but contained small amounts of the nicked and ß-subunit forms of hCG. In 2009, WHO introduced the 5th IS for hCG (coded 07/364)[5]. This new preparation has been highly purified from urine to remove contaminating forms of hCG, particularly the nicked and free ß-subunit that was present in the old assays. This study aimed to evaluate the correlation between the 3rd IS and the 5th IS, and determine the implications for management of PUL.

Methods

This was a diagnostic correlation and validation

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study carried out at Princess Margaret Hospital and Kwong Wah Hospital in Hong Kong. All urgent blood samples for serum hCG were sent to the Clinical Pathology Laboratory of Princess Margaret Hospital for analysis. All pregnant patients with serum hCG taken between 5 October 2015 and 17 October 2015 were included. They were identified through the Princess Margaret Hospital Chemical Pathology Laboratory Database. Ethics approval was obtained from the Kowloon West Cluster Research Ethics Committee of the Hospital Authority of Hong Kong in April 2016.

Each blood sample was analysed using both the WHO 3rd IS (Beckman Coulter access total βHCG) and the 5th IS (Beckman Coulter access total βhCG 5th IS assay). The individual clinical records were reviewed. The clinical course, ultrasonographic findings, final diagnosis, and clinical outcome were evaluated.

Samples unrelated to management of PUL were excluded: (1) serum hCG level of <5 IU/L (indicating no pregnancy), (2) serum hCG level of >10,000 IU/L in either assay (the management of PUL was unlikely to be altered even when there was a discrepancy between the two assays), and (3) serum hCG taken for other purposes, for example, as a tumour marker in gestational trophoblastic neoplasm.

Samples taken at the time the diagnosis of PUL was made were included in the diagnosis-validation study to evaluate the impact of any change to the 'discriminatory zone'. The clinical course, serial level of serum hCG, and ultrasonographic findings were reviewed until a final diagnosis was established: ectopic pregnancy, intra-uterine pregnancy, or miscarriage.

Statistical Analysis

Pearson's correlation between WHO 3rd IS and 5th IS of hCG was calculated. The Bland-Altman plot was used to evaluate the agreement and interchangeability between the two International Standards. The Passing-Bablok regression was used to estimate the analytical agreement and observe any systematic or proportional difference between the two assays. The confidence intervals (CI) were calculated with the bootstrap (quantile) method.

Receiver operating characteristic curve analysis was performed to define the optimal value of the new 'discriminatory zone' by maximising the weighted Youden's index with cost of 1 and sample prevalence[6]. The area under the curve (AUC), sensitivity, specificity, accuracy, positive and negative predictive values were evaluated. All statistical analysis was performed using Microsoft Excel and R version 3.1.2 with 'mcr' (method comparison regression)[7], 'pROC'[8], and 'epiR' packages[9].

Results

Among 132 paired samples retrieved, 105 were included in the correlation study. 23 pairs of samples were excluded as the serum hCG was normal (<5 IU/L), and three pairs were excluded as the serum hCG exceeded 10,000 IU/L. One pair of sample was used as a tumour marker and thus excluded (Figure 1).

The correlation between the 3rd IS and the 5th IS in the calibration of serum hCG was high (Pearson's r=0.996, r²=0.992). In the Bland-Altman plot (Figure 2), serum hCG values calibrated by the 5th IS were on average 12.9% higher (95% CI=10.6-15.2%) than those calibrated
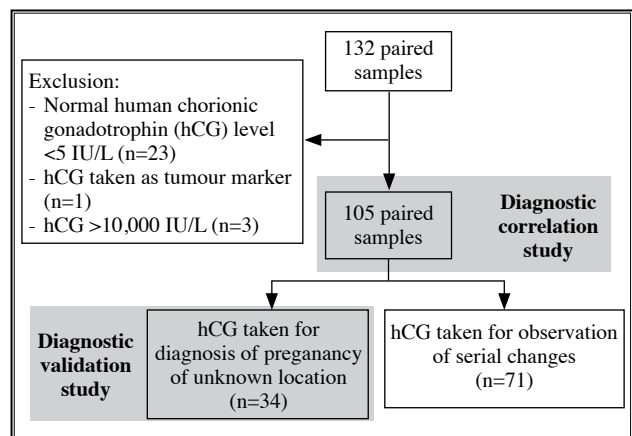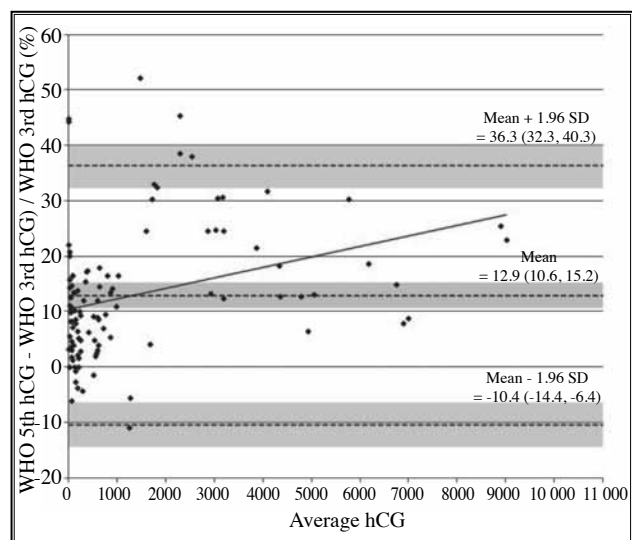

Figure 1. Study flowchart

Figure 2. Bland-Altman plot

by the 3rd IS, with 93.3% of the sample differences lying between the limits of agreement (± 1.96). The lower limit of agreement was -10.4% (95% CI= -14.4 to -6.4%), and the upper limit of agreement was 36.3% (95% CI=32.3-40.3%).
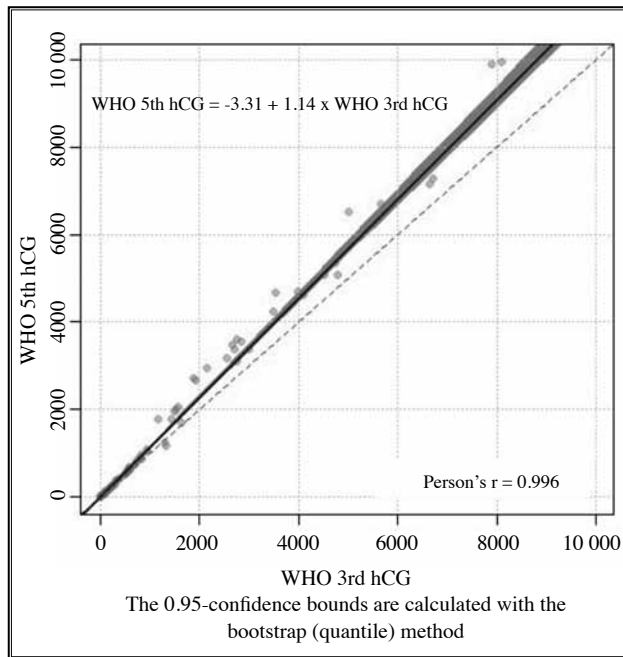

Figure 3. Passing-Bablok regression

In the Passing-Bablok regression (Figure 3), the slope was 1.14 (95% CI=1.12-1.18), and the intercept was -3.31 (95% CI= -8.44 to -0.87). There was a systematic difference and proportional difference between the two groups; the 3rd IS and 5th IS were not equivalent.

Defining the New 'Discriminatory Zone'

Among all the paired samples, 34 paired samples of serum hCG were taken when the diagnosis of PUL was made. Of these, the final diagnosis was ectopic pregnancy in 10, intra-uterine pregnancy in 9, and miscarriage in 15.

The cut-off values of the 'discriminatory zone' based on WHO 3rd and 5th IS assays were 1500 IU/L (sensitivity=50.0%, specificity=87.5%, AUC=77.9%) and 1745 IU/L (sensitivity=60.0%, specificity=87.5%, AUC=79.2%), respectively (Figure 4 and Table 1). Nonetheless, the number of samples was too small to make any recommendation for a change in the cut-off value of 'discriminatory zone'.

Discussion

In our study, serum hCG calibrated using the WHO 3rd IS and 5th IS were highly correlated (Pearson's r=0.996) but not equivalent. The mean percentage difference was 12.9% (95% CI=10.6-15.2%). The cut-off values of the 'discriminatory zone' were 1500 and 1745 IU/L for WHO 3rd and 5th IS, respectively.
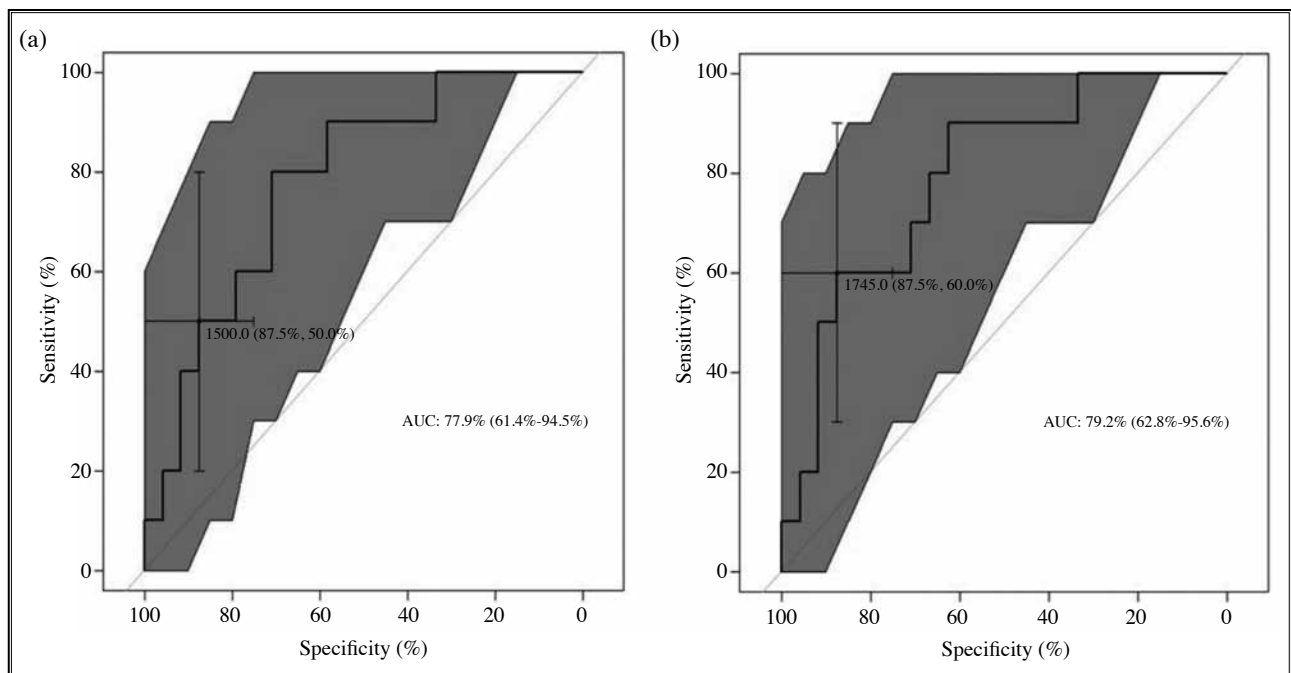

Figure 4. Receiver operating characteristic curve analysis for 'discriminatory zone' using the World Health Organization (a) 3rd and (b) 5th International Standard

Table 1. Receiver operating characteristic curve analysis

Characteristic	World Health Organization International Standard for serum human chorionic gonadotrophin	
	3rd	**5th**
Cut-off (IU/L)	1500	1745
Area under the curve (%)	77.92 (61.36-94.48)	79.17 (62.77-95.56)
Sensitivity (%)	50.00 (18.71-81.29)	60.00 (26.24-87.84)
Specificity (%)	87.50 (67.64-97.34)	87.50 (67.64-97.34)
Accuracy (%)	76.47 (58.83-89.25)	79.41 (62.10-91.30)
Positive predictive value (%)	62.50 (24.49-91.48)	66.67 (29.93-92.51)
Negative predictive value (%)	80.77 (60.65-93.45)	84.00 (63.92-95.46)

Table 2. Different analytical platforms used by different hospitals in the Hospital Authority

Hospital	Analytical platform	Calibration traceability
Kwong Wah Hospital	Beckman-Coulter	WHO 5th IS
Pamela Youde Nethersole Eastern Hospital	Abbott	WHO 4th IS
Prince of Wales Hospital	Roche	WHO 4th IS
Princess Margaret Hospital	Beckman-Coulter	WHO 5th IS
Queen Elizabeth Hospital	Abbott	WHO 4th IS
Queen Mary Hospital	Siemens	WHO 3th IS
Tseung Kwan O Hospital	Beckman-Coulter	WHO 5th IS
Tuen Mun Hospital	Abbott	WHO 4th IS
United Christian Hospital	Roche	WHO 4th IS

Abbreviations: IS = International Standard; WHO = World Health Organization

In Hong Kong, different hospitals use different assays and different analytical platforms for calibration of serum hCG (Table 2). Most laboratories will have to change to the new WHO 5th IS. Our study is the first in Hong Kong to evaluate the difference between the old and new assays.

Before the WHO 3rd IS was exhausted in October 2015, at Princess Margaret Hospital, the Department of Pathology and the Department of Obstetrics and Gynaecology collaborated to perform a parallel run of blood samples using the old and new assay. The transition period was short due to the short notice from the vendor, but the small number of paired samples were invaluable to compare the difference between the two assays.

To study the change of the 'discriminatory zone' for PUL, weighted Youden's index[6] was used to determine the appropriate cut-off for which sensitivity and specificity were maximised, taking cost and prevalence into account. If we aimed at a specificity of 0.875, precision of 0.1, and the confidence level at 95% (i.e. $\alpha=0.05$), then 60 samples were required to identify significant difference. Nonetheless, only 34 paired samples were included. The sample size was too small to make any recommendation for a new cut-off value of 'discriminatory zone' or change in clinical management. Nevertheless, our findings confirmed the differences between different assay standards in clinical use. To study the clinical correlation of the new WHO 5th IS assay, a larger prospective study is required.

The difference between new and old assays may potentially have different implications in different clinical scenarios. In a study to determine the suitability of the WHO 5th IS in Down's syndrome screening, a proportional increase of 33% in serum hCG levels was reported using the new assay, compared with the old assay[10]. There was no difference in the overall detection rate of Down's

syndrome screening, because the risk calculation was by multiples of the median of serum hCG. Nonetheless, in the management of PUL, the absolute value of serum hCG is used, and clinicians should be aware of the difference.

Our study focused on the management of PUL, and only samples with serum hCG between 5 and 10,000 IU/L were included. Ideally in a correlation study, the two extremities of serum hCG should also be evaluated. There was a possibility that serum hCG may have a larger bias if the level is higher. We suggest that the new assay should be further evaluated in other clinical conditions, for instance in gestational trophoblastic neoplasm where the serum hCG can be up to 10,000 or 100,000.

Clinical users should be aware of the different analytical platforms used by different hospitals, and the results of serum hCG should not be directly compared among different hospitals.

Conclusion

Serum hCG using WHO 3rd IS and 5th IS was highly correlated, but not equivalent. Based on our limited paired samples, the cut-off values of 'discriminatory zone' for management of PUL using 3rd IS and 5th IS were 1500 and 1745 IU/L, respectively. Further prospective studies are required to determine the appropriate 'discriminatory zone' when using the new WHO 5th IS.

Acknowledgement

Declaration

The authors have declared no conflicts of interest in this study.

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