Peer Review File

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Reviewer A

The study is a good one, congratulations. I add some tips in order to improve the quality of the article.
From my point of view, the title should mention someway the rest of the tumour markers, otherwise it seems as if only the homocysteine has been tested.
Reply: Thank you for your helpful suggestion to include the HE4 marker in the title. It has been added.

Abstract: the "Methods" should describes what has been done it the study, not only the statistical description. Apart from this, it is very well structured.
Reply: Thank you for your suggestion. We have already included all the necessary information in the methods section. If there is something specific you would like us to address, please provide more clarification so that we can make appropriate modifications.

Introduction: well done. The goals and hypothesis are clear and concise.
Method: there is a good statistical design and description, furthermore, I would find interesting to know why there are two parts in the research. Is it a test-retest research? What for?
Reply: Thank you for your suggestion. The experiment consists of two parts: the pre-experiment and the validation experiment. In the pre-experiment, we established a diagnostic equation (H-H). In the validation experiments, we estimated the sample size based on the pre-experimental results and checked the applicability of the diagnostic equation (H-H). The consistency between the two parts of the experiment confirms the validity and reliability of the experimental conclusion

Results are accurate and well summarized.
Discussion: the first paragraphs are similar to the introduction, moreover, there are not description of other similar studies such as results of sensitivity and specificity of HE4 in endometrial cancer. Very good mention about economic aspect as it is realistic and practical.
The conclusion is good.
Reply: Thank you for your useful suggestion. We have made modifications based on it, as shown in the second paragraph of the discussion.

There are currently no blood biomarkers in routine clinical use in EC. HE4 is a glycoprotein that is overexpressed in the serum of patients with EC, making it a good candidate for use as a diagnostic biomarker (1, 2) Some studies have shown that when

HE4 was used for the diagnosis of EC, AUC of it was fluctuate between 0.76 to 0.97,meaning it a promising non-invasive biomarker. However, HE4 levels rise with age and renal dysfunction, which may affect the interpretation of results (3, 4). Therefore, the combined use of multiple markers can further enhance the application value.

Reference, figures and tables are well. Table 1 should calculate the BMI instead of the height and weight separately.

Reply: Thank you for your valuable suggestion. We have made modifications based on it, as indicated in table 1.

Reviewer B

1. Please specify the **name** of the hospital in below sentences.

115 Patients

116 A total of 143 patients hospitalized for abnormal vaginal bleeding or discharge and

117 diagnosed with EC at our Hospital from January 2016 to May 2019 were included in the pre-

125 group of patients with benign gynecological diseases who visited our hospital during the same

126 period was included in this study. Clinical data were collected retrospectively. A verification

127 experiment was performed between June 2019 and February 2020, and 118 patients were

128 recruited. None of the patients recruited in this study had any other physical disorders (for

129 example, other malignant tumors, thrombotic diseases, poor blood glucose or blood pressure

130 control, endocrinological diseases, coagulation dysfunction, or liver and kidney dysfunction).

131 This study was approved by the Medical Ethics Committee of our Hospital (No. NFKSL-003)

Reply: Thank you for your helpful suggestion. The hospital name has been added to the corresponding location.

2. Ethical statement is incomplete. Please confirm and indicate in your manuscript (in both Methods section and Ethical statement in Footnote) that the study conformed to the provisions of the **Declaration of Helsinki (as revised in 2013)**, available at: https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf.

***- Suggested wording**: "The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013)."*

Reply: Thank you for your helpful suggestion. Complete ethical statement has been added to the corresponding location.

3. Please kindly reword this sentence to avoid absolute expression.

223 This is the first study to investigate the role of Hcy in EC diagnosis. The area under ROC

224 was greater than 0.5, indicating that Hcy could be used for the diagnosis of EC. Moreover,

Reply: Thank you for your helpful suggestion. We have made changes. Hcy is an important serum tumor marker for qualitative diagnosis of disease. However, there is limited research on its role in the diagnosis of EC. Therefore, we conducted research on this topic.

4. You've mentioned "studies", while only one reference was cited in this sentence. Please check. (You could either choose to revise it to singular form or to give **more than one reference** in this sentence. In the latter case, please keep the citations consecutively in text.)

239 results confirmed its application in predicting EC, which is consistent with previous studies

240 (37)

Reply: Thank you for your helpful suggestion. We have made changes.

5. Table 1

a. Please check if table header is missing here.

	Pre-experiment			Verification experiment		
	EG	CG	p	EG	CG	p
Number	143	143	-	59	59	-
Year	53.518 (8.319)	48.238 (9.214)	0.000	56.051 (8.827)	46.373 (8.428)	0.000

Reply: Thank you for your helpful suggestion. This line represents grouping information and We have added in table 1.

b. Please define these two kinds of data either inside the table or in table footnote.

Table 1 Clinical characteristics of included Patients.

	Pre-experiment			Verification experiment		
	EG	CG	p	EG	CG	p
Number	143	143	-	59	59	-
Year	53.518 (8.319)	48.238 (9.214)	0.000	56.051 (8.827)	46.373 (8.428)	0.000
BMI	24.088 (3.262)	25.148 (3.922)	0.014	25.897 (16.650)	25.433 (3.664)	0.209
Fib	2.5 (2.205-2.894)	2.296 (2.00-2.627)	0.000	2.852 (2.409-3.279)	2.41 (2.10-2.69)	0.000
D-D	0.25 (0.18-0.40)	0.20 (0.14-0.32)	0.001	0.33 (0.19-0.49)	0.205 (0.19-0.39)	0.038
Hcy	8.80 (7.01-11.30)	7.2 (6.20-8.50)	0.000	7.80 (7.10-8.58)	6.83 (6.10-8.00)	0.001
HE4	68.35 (51.84-102.90)	52.96 (44.44-61.44)	0.000	61.05 (46.16-80.80)	42.39 (38.20-51.19)	0.000

Reply: Thank you for your helpful suggestion. We have included an explanation in the table footnote.

6. Table 2:

a. Please check if table header is missing here.

	Univariate			Multivariate		
	Relative risk	95% CI	P-value	Relative risk	95% CI	P-value
BMI	1.087	1.016-1.163	.015	1.109	1.022-1.203	.013

Reply: Thank you for your helpful suggestion. Table header is not missing here. It is simply an empty space with no additional significance.

b. Please supplement those data.

	Univariate			Multivariate		
	Relative risk	95% CI	P-value	Relative risk	95% CI	P-value
BMI	1.087	1.016-1.163	.015	1.109	1.022-1.203	.013
Hcy	1.34	1.202-1.494	.000	1.279	1.131-1.445	.000
Fibrinogen	2.538	1.562-4.124	.000	1.988	1.135-3.481	.016
D-dimer	2.376	0.977-5.776	.056	-		
CA125	1.001	0.997-1.007	.566	-		
HE4	1.047	1.030-1.064	.000	1.039	1.022-1.056	.000
CA199	1.038	1.017-1.059	.000	1.029	1.006-1.052	.012

Reply: Thank you for your helpful suggestion. We have added the relevant data.

7. Table 3: Please check if table header is missing here.

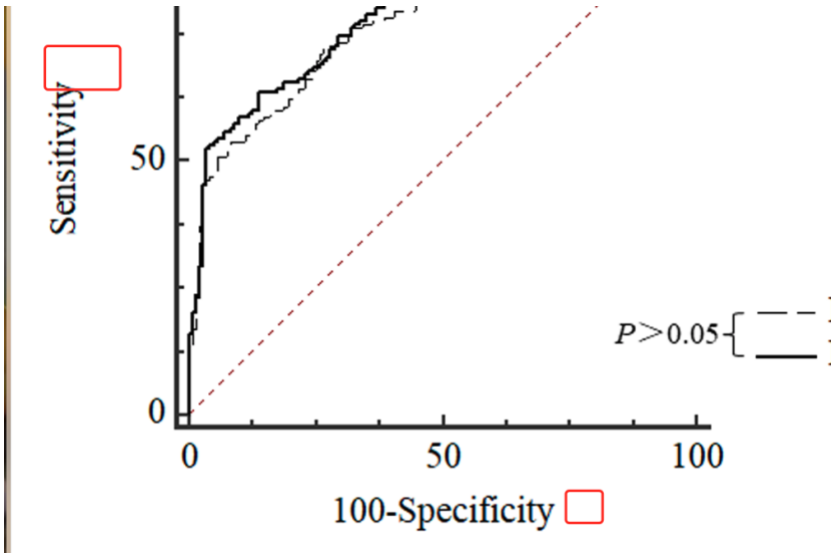
	ROC-AUC	95% CI	p*
HE4	0.747	0.693	0.796

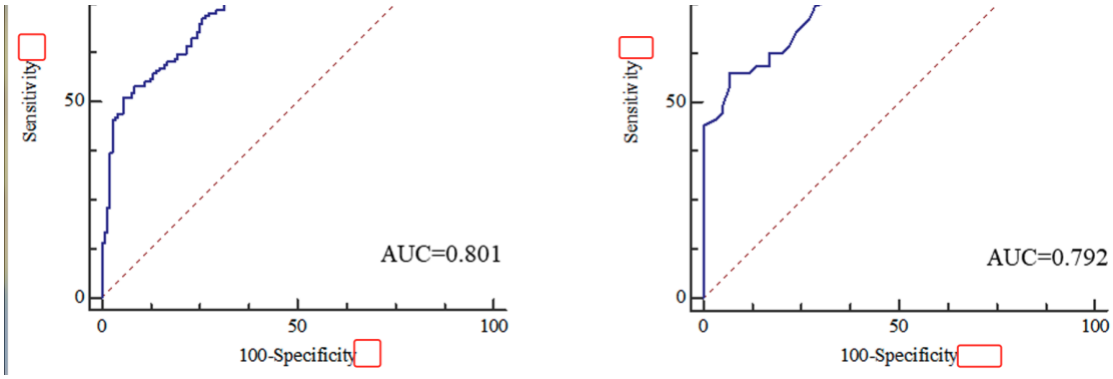
Reply: Thank you for your helpful suggestion. We have added table header in the space

8. Please define **ALL abbreviations** in Table 1-4 footnotes **respectively**.
Reply: Thank you for your helpful suggestion. All abbreviations in Table 1-4 have been added in footnotes.

9. Please provide **editable** version of Figure 1 in Word/PPT format since it's a flow diagram.
Reply: Thank you for your helpful suggestion. Figure 1 has been converted to PPT format

10. Please supplement units for X- and Y-axis and resend us updated Figure 2-3.
Reply: Thank you for your helpful suggestion. We made the necessary changes in Figure 2-3.





12. Please define **ALL abbreviations** shown in Figure 2-4 in their **figure legends** respectively.

Reply: Thank you for your helpful suggestion. ALL abbreviations in Figure 2-4 have been added in their figure legends respectively.

13. Originality checking of your manuscript shows that this study shares the **same approval number and registration ID** with your another study published on Technol Cancer Res Treat (https://pubmed.ncbi.nlm.nih.gov/32167027/). **Please explain**. Attached is a report for your reference.

Reply: Thank you for your helpful suggestion. I appreciate your serious and cautious approach. Both experiments are focused on diagnosing endometrial cancer but are at different stages. In our clinical experience, we have observed elevated levels of HCY in patients with endometrial cancer, prompting this study. Prior to conducting the validation experiment, we sought approval from the Ethics Office, who confirmed that both experiments can be shared, along with the necessary clinical registrations and ethical consent forms.

References

1. Barr, C.E., K. Njoku, E.R. Jones, et al., Serum CA125 and HE4 as Biomarkers for the Detection of Endometrial Cancer and Associated High-Risk Features. Diagnostics (Basel), 2022. 12(11).
2. Behrouzi, R., C.E. Barr, and E.J. Crosbie, HE4 as a Biomarker for Endometrial Cancer. Cancers (Basel), 2021. 13(19).
3. Omer, B., S. Genc, O. Takmaz, et al., The diagnostic role of human epididymis protein 4 and serum amyloid-A in early-stage endometrial cancer patients. Tumour Biol, 2013. 34(5): p. 2645-50.
4. Hertlein, L., P. Stieber, A. Kirschenhofer, et al., Human epididymis protein 4 (HE4) in benign and malignant diseases. Clin Chem Lab Med, 2012. 50(12): p. 2181-8.