# Exploration of tumor-infiltrating immune cells on the prognosis of endometrial cancer

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**Abstract**

Setting:Immune cells played a vital role in the progression of endometrial cancer, whereas immunologic mechanism and clinical prognostic biomarkers remained to be identified. Objective: To explore the landscape of tumor-infiltrating immune cells in endometrial cancer and the correlation between TIICs and EC prognosis. Methods: Data of 530 patients was downloaded from TCGA. CIBERSORTx estimated the abundance of immune cells. Ranked data used Spearman coefficient to measure the relationship between immune cells and clinical characteristics. Univariate COX hazard analysis was performed to explore the relationship between immune cells and clinical outcomes. Lasso regression was used to screen variables. Multivariate regression was performed for stepwise regression variable screening. Univariate and multivariate Independent prognosis analysis was performed to validate the reliability of the multivariate risk model. A nomographic chart was used to grade and assess each patient's prognosis. Results: 11 type TIICs were increased in cancer tissues($P<0.05$). Univariable regression revealed that 5 types TIICs were significantly related with EC prognosis. TTMMD model was constructed via lasso regression and multivariable cox regression. Log-rank was used to perform survival analysis, and the result was presented by Kaplan-Meier curve(p=5.13e-03). The Roc curve was simultaneously fulfilled to validate the model's accuracy and predictability(AUC=0.676). The Kaplan-Meier curve of the training set was statistically significant(p=3.807e-02). ROC curve replied the feasibility of the model(AUC=0.678). The risk model was an independent factor for predicting EC prognosis($p<0.001$). Conclusion: 11 types TIICs were significantly associated with the prognosis of endometrial cancers. The immune-cells-based risk model was reliable for prognosis assessment.

## Introduction:

Endometrial cancers mainly affect women post-menopaused, around 60 years old. The incidence of this malignant disease ranks second among gynecological cancers, second only to cervical cancer[1], and third for mortality worldwide, nearly 382069 individuals were diagnosed with endometrial cancer in 2018 among 185 countries, and the number is rising, owing to obesity. According to histopathology, endometrial cancer can be classified to two types, differing in morbidity, sensitivity to estrogen, and outcome. Altogether stage, histology, comprising grade and histological subtype, older age and race are responsible for the prognosis of endometrial cancer patients. Early-stage diagnosed and type one has a favorable prognosis, and their 5-year survival rate is approximately 80% to 90%. In 2013, TCGA stratified endometrial cancer into four novel prognosis subtypes: comprising overexpression of p53 and p16, microsatellite instability, PTEN mutation and deficiency[2]. Furthermore, corresponding researches have been proposed to evaluate the efficiency of surgical and adjuvant treatment, several indexes as for biomarkers have been proposed to assess clinical outcomes, immune cells played a vital role in the progression of metastasis and invasion of endometrial

cancer, but a comprehensive cognition of immunologic mechanism is still perplexing, and clinical prognostic biomarkers remains to be identified[3].

**Methods:**

Data Acquisition

All the data was downloaded from TCGA database(*http://xena.ucsc.edu/* ), including gene expression data and phenotype data. After integrating the gene expression data and clinical characteristics, consisting of age, stage, grade, overall survival, 35 normal cases and 530 tumor cases were included. The gene expression was upload to website

(*https://cibersortx.stanford.edu/index.php* ). Cibersortx is an analytical tool, using cell specific expression of gene to assess the abundance of cell types in tissues. Impute Cell Fractions was selected for analysis module, 22 type immune cell was chosen for signature matrix file and 1000 permutations was set for significance analysis. $P<0.05$ was considered statistically significant.

Data analysis

All the data and results were visualized by R4.0.1. The correlation analysis between TIICs and clinical stage and pathology classification was measured by Spearman's rank correlation coefficient. We divided the data set into training set and validation set in a ratio of 2:1 randomly. Univariate regression was performed and five most prognosis-related immune cells were selected. Lasso regression was performed to further select the variates. Multivariate COX regression was performed to establish a prognostic risk model, the method of backward and forward gradual regression was chosen. the efficiency of the model was confirmed by validation set, via Kaplan-Meier, calculated-AUC and independent prognosis, showed by forest plot, and turn out to be statistically significant($p<0.05$) . Log-rank method was used to compare the difference between low-risk and high-risk patients.

**Results:**

Clinical characteristics of 530 patients, containing age, sex, stage, grade, was summarized in **Table1** .

**Landscape of 22 types of tumor-infiltrating immune cells**

The relative abundance of tumor-infiltrating immune cells was showed by heatmap. (Figure 1A) The proportion of 22 types immune cells in each samples summed up to 1. The distribution of immune cells differed in each sample. High-level infiltration was represented by red, on the contrary, green revealed low-level infiltration. (Figure 1B) The correlation matrix of immune cells (Figure 1C) showed relationship between each immune cell. The violin plot indicated distribution difference of TIICs in normal samples and EC samples(Figure 1D). In contrast to Plasma cells, T cells CD4 memory activated, T cells follicular helper, T cells regulatory(Tregs), Macrophages M0, Macrophage M1, Dendritic cells activated, Eosinophils and Neutrophils increased in endometrial cancer samples, T cells CD4 memory resting, T cells gamma delta, Monocytes, Dendritic cells resting and Mast cells resting are decreased in EC patients.

The result of correlation analysis of TIICs and clinical stage was shown by the bubble plot(Figure 2A). B cells memory, T cells CD4 memory resting, T cells CD4 memory activated, Monocytes, Macrophages M0, Mast cells resting, Mast cells activated and T cells gamma delta are significantly related to clinical stage. T cells CD8, T cells CD4 naive, T cells gamma delta, Macrophages M0 and Mast cells activated are closely related with pathology classification(Figure 2B). Five types prognosis-relative immune cells, containing T cells CD4 naive, T cells regulatory, Macrophage M1, Macrophage M2, Dendritic cells activated, were filtered up by univariate regression analysis with the standard of $p<0.05$(Table 2).

**TTMMD model was constructed via lasso regression and multivariable cox regression**

Lasso regression analysis was performed to further filter variables(Figure 3A&3B). The result showed that no cell was excluded. A multivariable COX model was constructed based on T cells CD4 naïve, T cells regulatory, Macrophage M1, Macrophage M2, and Dendritic cells activated. Patients in the model was divided

into high-risk and low-risk according to their individual immune risk score(Figure 3C). The distribution of survival status between high and low risk group(Figure 3D). Macrophage M2 and Dendritic cells activated were relatively more infiltrating in high risk patients compared to Macrophage M1 and T cells naïve. T cells regulatory were, on the contrary, more abundant in low risk patients, demonstrating it was a protective factor(Figure 3E). Comparison of the survival rate between high and low risk patients by time progressing was illustrated by Kaplan-Meier curve(Figure 4A). The ROC curve was performed and AUC was calculated(AUC=0.676) (Figure 4B).

**The validation of the immune risk model**

The survival condition of validation set patients was revealed by Kaplan-Meier (Figure 4C) to compare the survival difference among two-level patients. ROC curve showed the sensitivity and specificity of the cox risk model to predicting prognosis. (Figure 4D).The risk plot of low-risk and high-risk patients was showed in validation set(Figure 4E). The survival status of low-risk and high-risk patients in validation set(Figure 4F). The distribution of 5 types immune cells in patients of validation set(Figure 4G). Corresponding to training set, Macrophage M2 and Dendritic cells activated were more abundantly infiltrating, and macrophage M1 was relatively more abundant in high-risk patients, regarded as risk factors, compared to T cells regulatory as a suppressor. Moreover, both in training set and validating set, the infiltration level of T cells CD4 naive was awfully low.

**The risk model was an independent factor for predicting EC prognosis**

The risk model was otherwise inspected by univariate and multivariate independent prognostic analyzing(Figure 5A&5B). The forest plot confirmed that the model can be used as an independent factor to estimate clinical outcomes. Furthermore, validation set was performed meaningful results(Figure 5C&5D). Nomograph was performed to generate a prognostic score for each patient, based on their age, clinical stage, pathological grade and survival rate(Figure 6A&6B).

**Discussion:**

The inner immune system can identify and specifically eliminate mutated cells to maintain the homeostasis. The mechanism explaining Immune escaped can be concluded: 1. Down-regulated expression of tumor associated antigen. 2.Weak immunogenicity of tumor. 3. Exhaustion of the anti-tumor antibody, along with the disabled ADCC effection functioned by immune cells, combining with Fe receptors located on NK cells. 4. Deficiency of antigen presentation induced by MHC I molecules and the lack expression of MHC II molecules, failing effectively activating T helper cells. 5. Absence of costimulatory signal, including ICAM-I, IFA-3, VCAM-1, to activate T cells. 6. The apoptosis induced by Fas/FasL. Meanwhile, tumor-associated immuno-suppressive factors and tumor-associated immuno-cells, which participated in the formation of tumor microenvironment (TME), played an important role in tumor immunology[4].

The result showed that Plasma cells, T cells CD4 memory activated, T cells follicular helper, T cells regulatory(Tregs), Macrophages M0, Macrophage M1, Dendritic cells activated, Eosinophils, and Neutrophils increased in tumor issue compared with normal issue. ($P<0.05$) Other studies had revealed that macrophage cells was recruited induced by chemokines, such as CCL8[5], CCL-2, MCSF, VEGF[6].

Macrophage cells, Tregs, Dendritic cells were associated with the prognosis of endometrial patients. Macrophage cells and Tregs were known as tumor-associated immunosuppressive cells. Macrophage had two phenotypes, consisting of macrophage M1 and macrophage M2. They had multiple functions, containing antigen presenting, and phagocytosis. With the stimulation by IFN-$\gamma$ and MCSF, macrophage cells polarizated to M1 subtype , characterized with inducing inflammation , enhancing Th1 cells response and supporting anti-tumorigenic functions. Nevertheless, macrophage M2, characterized with pro-tumorigenic and tissue repairing, were stimulated by IL-4 and IL-13[7]. The increase of tumor-associated macrophage cells(TAM) was closely related to poor prognosis. Numerous studies indicated that TAMs was more likely to be M2 subtye[8-9], which affected the vascularization , muscular infiltration, extracellular matrix degradation and invasion[7][10]. Furthermore, TAMs induced the immunosuppression via supporting Tregs differentiation

and restraining the activation of T cells. Tregs were closely associated with tumor progression, affecting the tumor vascularization and damaging the CD8+T cells cytotoxicity[11-13]. Dendriyic cells were divided into three subtypes, according to different function and phenotype. Dendritic cells could stimulate T cells to be activated[14],promoting anti-tumor immunity. Different subtypes corresponded to different effect T cells, correlating with diverse prognosis in cancer[15-16].

**Conclusion and perspective:**

we comprehensively analyzed the distribution of tumor-infiltrating immune cells, the interaction between immune cells, the potential mechanism of cell polarization, and metabolism.The risk model performed as an independent prognosis factor. Immunotherapy will get better, benefiting from more accurate assessment tools and measures. The studies will be widen from epigenetics to genomics, more therapeutic targets will be find to benefit more people.

**Declarations:**

**Authors' contributions:**

Jiayi Zhou, Lin Han: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation. Chi Chi: Visualization, Validation. Yueming Zhang: Supervision, Project administration. Jing He: Software, Validation. Qiao Gu: Writing- Reviewing and Editing, Wenjie Hou: Conceptualization, Resources, Writing- Reviewing and Editing.

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**Ethical statement:**

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