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The morphological characteristics and arrangements of cells in the liquid-based cytology preparation of patients with endometrial lesions

Ming-Zhe Wu[1], Na-Jin Gu[2], Ming-Ming Xiao[3], Dong-Ge Liu[4], Mu-Lan Jin[5], Xu-Yan Liu[6], Jian Wang[7], Hong-Tao Xu[7], Yi Zhang[1*] and Guang-Ping Wu[7*]

Abstract

Background The accurate cytological diagnosis of endometrial carcinomas by minimally invasive method has a broad application. There are several articles described the morphological characteristics but not arrangements of endometrial lesion cells on LBC slides.

Methods A retrospective study was conducted using 175 endometrial samples obtained by direct negative pressure suction with disposable endometrial sampler. All lesions were diagnosed both cytologically and histologically, and the diagnostic results were compared and analyzed.

Results The cytological diagnoses of polyps, simple or complex hyperplasia, and atypical hyperplasia were highly consistent with the histological diagnosis. The cytological features of polyps and normal endometrium, as well as simple and complex hyperplasia, are the same. Among 82 cases of histologically confirmed adenocarcinoma, the cytological diagnosis were adenocarcinoma cells (46 cases, 56.10%), suspected for adenocarcinoma cells (22 cases, 26.83%), and false negative (14 cases,17.07%). Retrospective reviewing the slide suggest diagnostic parameters such as significantly enlarged nuclei, multistage papillary arrangements, large and numerous nucleoli, and large vacuoles containing neutrophils in the cytoplasm are reliable diagnostic criteria for endometrial carcinoma cells; on the other hand, ignorance of lobulated arrangements and escaped arrangements are the main reasons for missed diagnosis.

Conclusions The cytological diagnosis of endometrial lesions not only depends on the morphological characteristics of cells, but also need careful observations of the cellular arrangements.

Keywords Cytological diagnosis, Endometrial polyps, Endometrial hyperplasia, Endometrial atypical hyperplasia, Endometrial carcinoma

*Correspondence:
Yi Zhang
zhangyi@cmu1h.com
Guang-Ping Wu
wugp@cmu1h.com
Full list of author information is available at the end of the article

Introduction

Endometrial carcinoma (EC) is the most common gynecological malignant tumor, and its incidence rate has shown increased in recent years [1]. In considerable number of cases, once ECs were confirmed, the diagnosis are in the middle and late stages. This is mainly due to lack of early tumor diagnosis [2]. Most hospitals—both domestic and international—are still using traumatic dilatation and curettage as the only way to obtain endometrial specimens. This diagnostic method is time-consuming, and expensive [3]. Meanwhile, patients suffer a lot of pain [3] and with fewer cell components obtained which results in failing to meet the diagnostic needs [4]. In order to improve the prognosis of EC patients, a cytological screening of endometrial lesions has been developed and proven to be the effective method for detecting and clarifying the diagnosis of cancer and non-tumor endometrial lesions [5]. The advent of disposable endometrial sampler not only brings a new dawn to the early and accurate diagnosis for patients with endometrial lesions [6, 7], but also poses a new challenge to the cytological diagnosis of endometrial lesions. This method is a minimally invasive method, without need anesthesia or other special equipment. Furthermore, by this new method, we can obtain rich cellular components for cytological diagnosis [6]. It is suitable for almost all patients with endometrial lesions. Recently, many studies reports classify endometrial lesion using gene sequenceing, but the sensitivity and specificity of sequencing-based diagnosis varies a lot between reports [8–10]. This is due to multiple detection methods are applied and, the types of genes involved are increasing, which results in the final results are sometimes inconsistent [11, 12]. For example, PTEN mutation and PAX2 inactivation are both critical for endometrial atypical hyperplasia and endometrial carcinoma [13]. But these 2 molecular markers cannot distinguish endometrial atypical hyperplasia from endometrioid carcinoma; Another example is that TP53 gene mutations and p16 expression were reported in 96% and more than 90% of endometrial serous carcinomas, respectively [14, 15]. But neither genetic alteration is specific for serous carcinoma. In addition, the availability and cost of molecular tests (such as next gene sequencing) limit their widespread application. Therefore, it can only be used as an auxiliary detection method and cannot completely replace cytological diagnosis.

Liquid-based cytology (LBC) preparation has increased the sensitivity of endometrial pathology detection in postmenopausal subjects up to 100%, and at approximately 53% for premenopausal women [16]. The Yokohama System, a new reporting system, has been introduced as a standard reporting system for endometrial lesion, similar to The Bethesda System for the uterine cervical cytology. LBC has great advantages in cell preservation and clearance of background. Both of which are very important for accurate diagnoses [17, 18]. It has also been reported that the application of cell block assisted liquid-based cytology significantly improve the sensitivity and specificity of the diagnosis of endometrial lesions [19, 20]. However, what's the key point for the improved diagnostic accuracy is not clear. The main purpose of this study was to evaluate the morphological characteristics and arrangements of cells in the LBC preparation of patients with endometrial lesions. Especially evaluate the common arrangements of endometrial cancer cells and the arrangements those previous are misdiagnosis.

Materials and methods

This study was a retrospective analysis of cytological diagnoses of endometrial lesions that had been histologically confirmed. All procedures in this study were conducted in accordance with the First Hospital of China Medical University's (APPROVAL NUMBER/2023-189) approved protocols. Written informed consent was obtained from the patients for their anonymized information to be published in this article. A total of 82 patients with endometrial carcinomas and 18 patients with atypical hyperplasia confirmed by histology in the first hospital of China Medical University from March 2019 to August 2021 were included in the study. An additional 75 randomly selected patients with benign endometrial lesions were included in the study and used as a control group, including 34 cases of simple or complex hyperplasia and 41 cases of polyps. All patients ranged in age from 27 to 81 years and had cytological and histological (biopsy and/or postoperative biopsy) diagnosis. When there is a discrepancy between biopsy and postoperative histological diagnosis, postoperative histological diagnosis is used as the gold standard. The endometrial samplings were obtained by direct negative pressure suction using a disposable endometrial sampler (ES Sampler, C0121JN11, Junocare, Beijing, China) by two experienced gynecologists. Refer to the literature for specific operation procedures [6]. The samples obtained were divided into two parts. The part containing tissue fragments were put into the tissue collection bottles containing a fixed solution for histological diagnosis, and the remaining cells were immediately rinsed into the cell collection bottles containing a preservative-fixative solution for cytological diagnosis.

Histological and cytological samples were processed separately by different laboratories in the department of pathology, formalin-fixed paraffin-embedded technology is used for histological preparation, and liquid-based cytology technology was used for cytological preparation. Histological slides were stained with hematoxylin

and eosin, while cytological samples were automatically prepared by SurePath (BD Tripath, Burlington, NC, USA) and stained with Papanicolaou. Satisfactory criteria for the number of cells collected in LBC samples: Thirty or more endometrial epithelial cells forming a cluster are defined as "a cell- cluster," and, if the number of "cell-clusters" exceeds 10 or 5 (60 years old or older), the sample is defined as "satisfactory" [21]. However, if clear pathological cells are found, even if the number of cells does not meet the above criteria, it is considered a satisfactory specimen. The Yokohama System for Reporting Endometrial Cytology was used for cytology diagnosis [21].

Based on the histological diagnoses and the cytological diagnoses, the diagnosis results were recorded into histological and cytological groups, respectively. Statistical analysis was performed using SPSS 16.0 software package (SPSS, Inc. Chicago, IL, USA). The chi-square test or Fisher's exact test was used for the comparison of positive cases between different groups. A *P*-value of less than 0.05 was considered statistically significant.

Results

First, we found that most common benign endometrial lesions were polyps (Fig. 1A) and simple or complex endometrial hyperplasia. The cellular arrangements and morphological characteristics of them are shown in Table 1.

Second, we need to interpret the endometrial atypical hyperplasia cells that are easy to be confused with endometrial carcinoma cells (Fig. 1B). The atypical hyperplasia cells of endometrium are closer to cancer cells in both morphological characteristics and arrangements of cells. The morphological characteristics and arrangements of them are also shown in Table 1.

In this study, 82 cases of adenocarcinomas were confirmed by histology, and the cytological diagnosis results showed 46 cases of adenocarcinoma cells, 22 cases of suspected for cancer cells, 10 cases of atypical hyperplasia cells, and 4 cases of glandular epithelial cells; 18 cases of atypical hyperplasia confirmed by histology, of which 6 cases were under-diagnosed by cytology as glandular epithelial cells; 34 cases of simple hyperplasia or complex hyperplasia confirmed by histology, of which 3 cases were over-diagnosed by cytology as atypical hyperplasia; Except for 2 cases over-diagnosed as atypical hyperplasia, the cytological findings of the other polyps were similar to those of normal glandular epithelial cells. The results are shown in Table 2.

In order to find the root causes of missed diagnosis for endometrial carcinoma cells, the original cytology slides were reviewed again. Based on the interpretation criteria of The Yokohama System for Reporting Endometrial Cytology [19] and our diagnostic experience, the morphological characteristics of this group of lesions were further classified. The results showed that the significantly enlarged nuclei, (Fig. 1C), multistage papillary arrangements (Fig. 1D), large and numerous nucleoli (Fig. 1E), and large vacuoles containing neutrophils in the cytoplasm (Fig. 1F). All of these findings were consistent with the histological diagnosis. The significantly enlarged nuclei means that nuclei were significantly enlarged to more than five times compared with that of normal endometrial nuclei. On the contrary, the 6 cases of lobulated arrangements (Fig. 1G) and all 8 cases of escaped arrangements (Fig. 1H) were the main causes for missed diagnosis of endometrial carcinoma cells. The lobulated arrangements usually show a tendency for a cell cluster to differentiate into multiple small cell clusters, while there are still some connections between each small cell clusters. The characteristic of escaped arrangement is that several cancer cells located at the edge of the cancer cell cluster have a tendency to escape from the original cell cluster. The nuclei of the escaping cancer cells are significantly elongated and perpendicular to the cell cluster. The results are shown in Table 3.

Discussion

The minimally invasive method has a broad application in accurate cytological diagnosis of endometrial carcinomas [22]. Although new auxiliary screening

(See figure on next page.)

Fig. 1 The morphological characteristics and arrangements of cells in the LBC preparations of patients with endometrial lesions (Papanicolaou stain, 400). **A** Endometrial polyps. The glandular epithelial cells were evenly distributed and arranged in flat sheets. The cells were round and oval, with the same size and equal cell spacing. **B** Atypical hyperplasia cells. The glandular epithelial cells were crowded and overlapped, and the two-dimensional flat sheet distribution had disappeared. The nuclear enlargement was within three times compared with that of normal endometrial nuclei. **C** Endometrial carcinoma cells. One cancer cell nucleus at the left edge of the cell cluster was significantly enlarged, which was more than five times compared with that of other nuclei. **D** Endometrial carcinoma cells. The clusters of cancer cells were arranged in multistage papillary arrangements. **E** Endometrial carcinoma cells. Most of the nuclei in the cancer cell cluster showed large and numerous nucleoli. **F** Endometrial carcinoma cells. Some cancer cells in the cancer cell cluster exhibit large vacuoles containing neutrophils in the cytoplasm. **G** Endometrial carcinoma cells. Lobulated arrangements: a cluster of cancer cells is about to divide into six small clusters of cells, and there are still connections between the small clusters. **H** Endometrial carcinoma cells. On the surface of the cell cluster, there were many escape cancer cells with elongated nuclei

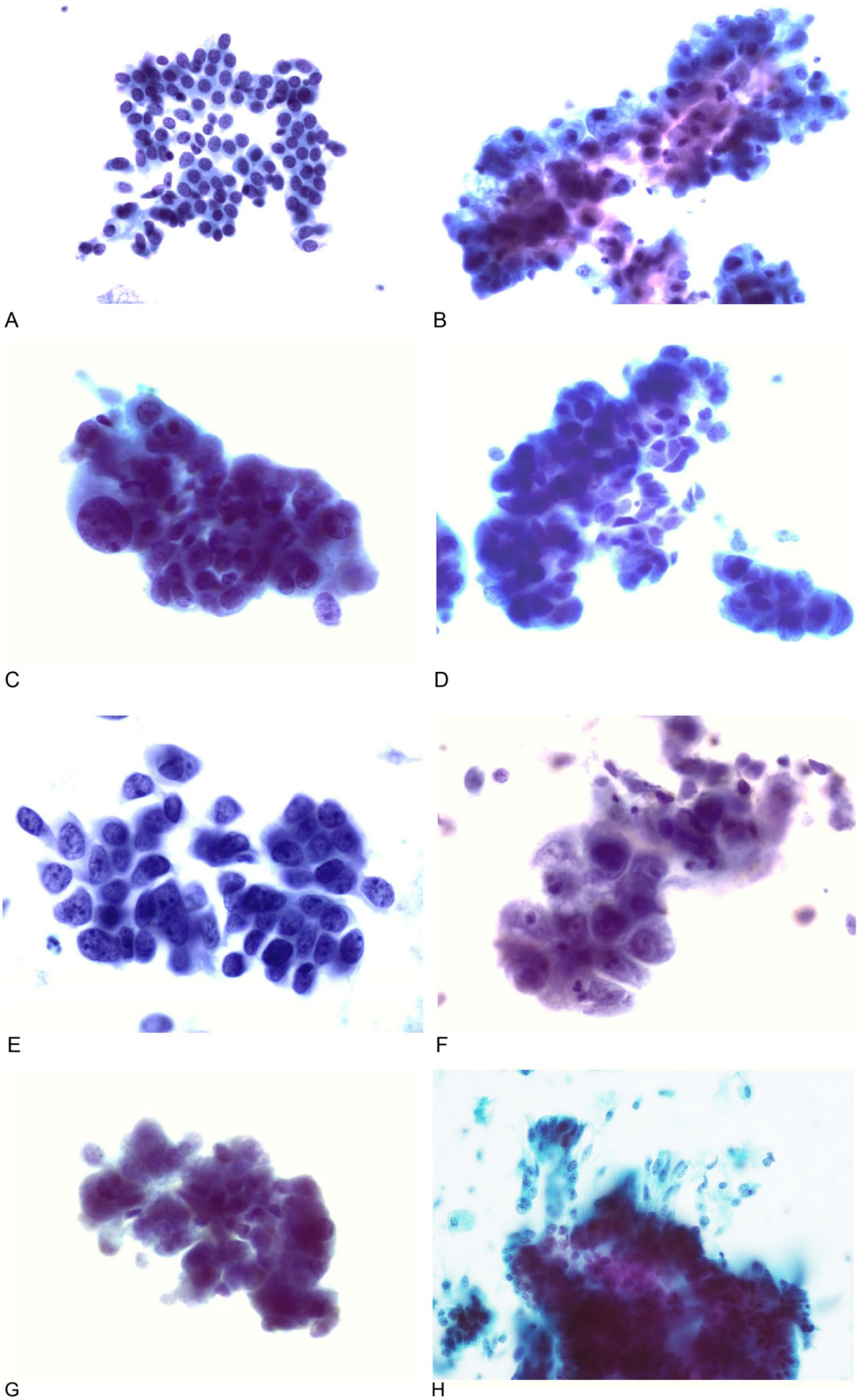

Fig. 1 (See legend on previous page.)

Table 1 The arrangements and morphological characteristics of common benign and malignant cells in endometrium

lesion types	cell arrangements	morphological characteristics
endometrial polyps	evenly distributed and arranged in flakes	The cells are round and oval, with the same size and equal cell spacing
S or C hyperplasia	evenly distributed and closely arranged	The cells are round and oval, similar in size, increased in number and decreased in cell spacing
atypical hyperplasia	crowded, overlapping, and two-dimensional flat sheet distribution has disappeared	Enlarged nuclei are within three times compared with that of normal endometrial nuclei
adenocarcinoma cells	atypical adenoid arrangements with obvious three-dimensional sense	Significantly enlarged nuclei are more than three times compared with that of normal endometrial cell nuclei, and shows some unique features

S or C Hyperplasia: simple hyperplasia or complex hyperplasia

Table 2 Comparison of histological diagnosis with cytological diagnosis

Histological Diagnosis	n	Cytological		Diagnosis	
		AC	SAC	AH	GE
Adenocarcinoma	82	46(56.10)	22(26.83)	10(12.19)	4(4.88)
Atypical Hyperplasia	18	0	0	12(66.67)	6(33.33)
S or C Hyperplasia	34	0	0	3(8.82)	31(91.18)
Polyps	41	0	0	2(4.88)	39(95.12)
Total	175	46	22	27	80

S or C Hyperplasia: simple hyperplasia or complex hyperplasia

AC Adenocarcinoma cells, *SAC* Suspected for adenocarcimoma cells, *AH* Atypical hyperplasia cells, *GE* Glandular epithelial cells

Table 3 The original results by cytological diagnosis in 82 cases of endometrial carcinomas

Diagnostic parameters	n	Cytological		Diagnosis	
		AC	SAC	AH	GE
Significantly Enlarged Nuclei	17	17	0	0	0
Multistage Papillary A	21	15	6	0	0
Large and Numerous Nucleoli	10	8	2	0	0
Neutrophils in the Cytoplasm	8	6	2	0	0
Lobulated Arrangements	18	0	12	6	0
Escaped Arrangements	8	0	0	4	4
Total	82	46	22	10	4

Multistage Papillary A: multistage papillary arrangements; Neutrophils in the Cytoplasm: large vacuoles containing neutrophils in the cytoplasm

AC Adenocarcinoma cells, *SAC* Suspected for adenocarcimoma cells, *AH* Atypical hyperplasia cells, *GE* Glandular epithelial cells

methods for diagnosing benign and malignant endometrial lesions have been continuously reported [23, 24], there are few reports on the cytological screening criteria for endometrial lesions, especially the lack of reports on the comparison of morphological characteristics and arrangements of benign and malignant endometrial cells. The cytological diagnosis of endometrial carcinoma cells reported in previous literature only focused on the morphological characteristics of the cells, without the arrangements of the cells. In this study, we found that both morphological characteristics and arrangements between endometrial polyps and the normal glandular epithelial cells were similar (Fig-1A). The epithelial cells were round and oval, with same size and equal cell spacing, evenly distribution with obvious two-dimensional flat sheet arrangements.

Both simple hyperplasia and complex hyperplasia of endometrium showed obvious hyperplasia of glands and even though the structure of glands changed, but the glandular epithelial cells did not have atypia [25]. In this study, we found that the morphological characteristics of endometrial hyperplasia cells were still similar compared with those of normal epithelial cells, although the number of epithelial cells were significantly increased and the cell spacing is significantly reduced. The epithelial cells were still arranged in a two-dimensional flat sheet.

The atypical hyperplasia is not only an important factor for the progression to endometrial cancer, but also a risk factor for endometrial hyperplasia patients complicated with endometrial cancer [26, 27]. In this study, we found that the morphological characteristics and arrangements of atypical glandular cells were changed. The nuclei increased and the nucleocytoplasmic ratio increased, and the extent of nuclear enlargement was within three times compared with that of normal endometrial nuclei. In the arrangement modes, the cells are crowded and overlapped with each other, and the two-dimensional flat sheet distribution has disappeared, showing a three-dimensional arrangement tendency (Fig-1B).

It is well known that it is difficult to distinguish benign and malignant endometrial cells by cytological diagnosis. It is generally believed that high level of expertise is needed for the interpretation, especially to distinguish atypical hyperplasia cells from

adenocarcinoma cells [28]. Norimatsu et al. by systematically describing the morphological features and arrangements of cells believed that LBC provides an opportunity to reassess the role of endometrial cytology [29] and is a useful tool in the cellular diagnosis and follow-up of endometrial lesions [30].

In this study, we found that the premise of accurate interpretation of endometrial carcinoma cells is to quantify the differences in morphological characteristics and arrangements between endometrial carcinoma cells and atypical hyperplasia cells. Due to the enlargement of the nucleus and corresponding increase in cytoplasm in endometrial cancer cells, we found that determining the increase in nuclear to cytoplasmic ratio was not as accurate as determining the extent of nuclear enlargement between benign and malignant cells or among malignant cells. To avoid false negative results, first we need carefully observe the extent of nuclear enlargement. When we compared with surrounding benign endometrial cell nuclei or with surrounding malignant cell nuclei, five times nuclear enlargement usually is considered to be an adenocarcinoma cell rather than an atypical proliferative cell (Fig-1C). In this study, 17 cases of adenocarcinoma cells were diagnosed using this criterion. Among these 17 cases, 2 were initially diagnosed as severe atypical hyperplasia during histological biopsy, while the cytological reports were adenocarcinoma cells. The final postoperative pathological diagnoses (resection) were consistent with cytological diagnoses. These results suggest that the enlargement of nucleus is the most reliable parameter to judge adenocarcinoma cells. If the extent of nuclear enlargement is between 3–5 times, we should consider the likelihood that these cells are adenocarcinoma cells. In this study, we found a total of 26 cases of such cells, including 22 cases of adenocarcinoma cells and 4 cases of atypical hyperplasia cells. Therefore, the likelihood of adenocarcinoma cells (84.62%) is significantly higher than that of atypical hyperplasia cells (15.38%) ($p < 0.01$). In practical work, such cells should be considered as suspicious adenocarcinoma cells first, and then carefully observe the changes of other parameters. If large and numerous nucleoli are found in the nucleus or large vacuoles containing neutrophils are found in the cytoplasm at the same time, such cells should be diagnosed as adenocarcinoma cells (Fig-1E and F).

In addition to the morphological characteristics of cells, it is also important to carefully observe cellular arrangements. The multistage papillary arrangements are another reliable parameter (Fig-1D). Our results suggest that although cytology cannot observe the tissue structure, the cellular arrangements can indirectly reflect the characteristics of tissue structure. In addition, we found a hidden cell arrangement in 18 cases of adenocarcinoma cells, that is, lobulated arrangement (Fig-1G). At scanning power, many cells look like belonging to same cluster of cells. However, careful observation shows cells of the "same" cluster demonstrates a tendency to differentiate into multiple small cell clusters, and there are still connections between the small clusters, which may be the early manifestation of multistage papillary arrangements. This arrangement has not been reported yet. The most easily missed arrangements in this study are escaped arrangements. A few cells escaped from the original cell clusters in the shape of bird tail or chicken feather blanket (Fig-1H). The enlargement of the nucleus is often not obvious, which is mainly manifested in the elongation of the nucleus, and is perpendicular to the cell cluster. In this study, all the 8 cases of adenocarcinoma cells by escaped arrangements were missed, which gave us a profound lesson.

The cellular arrangements were easily to be ignored by cytologists and rarely reported in the literature. Most cytologists thought that cytological diagnosis was only based on the morphological characteristics of cells. However, the results of this study showed that 57.32% of endometrial adenocarcinoma cells were interpreted according to multiple papillary arrangements, lobulated arrangements, and escaped arrangements. Twenty-two cases of suspected cancer cells, 10 cases of atypical hyperplasia cells, and 4 cases of glandular epithelial cells in the Table 3 were also due to the neglect of cell arrangements. The results suggest that the cellular arrangements have an irreplaceable role in the interpretation of endometrial adenocarcinoma cells. The cellular arrangements may be consistent with the cytoarchitecture mentioned by Norimatsu et al [28]. The limitations of this study are the relatively small sample size and retrospective nature of the study design. It is necessary to further expand the sample size and conduct in-depth research in the future.

Conclusion

The cytological diagnosis of endometrial cancer cells not only depends on the morphological characteristics of individual cells, but also on careful observation of cellular arrangements. Significantly enlarged nuclei, multistage papillary arrangements, large and numerous nucleoli, and large vacuoles containing neutrophils in the cytoplasm were diagnostic parameters that are easy to understand and recognize. Lobulated arrangements and escaped arrangements are the main reasons for missed diagnosis of endometrial carcinoma cells by cytology.

Statement of human and animal rights

All procedures in this study were conducted in accordance with the First Hospital of China Medical University's (APPROVAL NUMBER/2023-189) approved protocols. This article does not contain any studies with animals.

Statement of informed consent
Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Authors' contributions
Ming-Zhe Wu, Dong-Ge Liu, Mu-Lan Jin, Yi Zhang, Hong-Tao Xu, and Guang-Ping Wu contributed to study design and conduct. Ming-Zhe Wu, Na-Jin Gu, Ming-Ming Xiao, Xu-Yan Liu, and Jian Wang analysed the data and provided statistical support. Ming-Zhe Wu and Guang-Ping Wu contributed to language editing in this article. All authors made substantial contributions to interpretation of results, were involved in drafting the manuscript and revising it critically for important intellectual content, approved the final versin for submission and agree to be accountable for all aspects of work.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained for the experimental procedures by the Ethics Committee of the First Hospital of China Medical University, Shenyang, China.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Gynecology, The First Hospital of China Medical University, Shenyang 110001, China. [2]Department of Pathology, The Second Hospital of Dalian Medical University, Dalian 116027, China. [3]Department of Pathology, The People'S Hospital of Liaoning Province, Shenyang 110016, China. [4]Department of Pathology, Beijing Hospital, National Geriatric Center, Beijing 100730, China. [5]Department of Pathology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China. [6]Department of Pathology, General Hospital of Benxi Iron & Steel Industry Group of Liaoning Health Industry Group, Benxi 117000, China. [7]Department of Pathology, The First Hospital of China Medical University, Shenyang 110001, China.

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