RESEARCH ARTICLE

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Immunohistochemical Characterization Improves the Reproducibility of the Histological Diagnosis of Ovarian Carcinoma

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Abstract

Background: Ovarian cancer is the leading cause of gynecologic cancer-related death. Histological assessment remains the standard clue for the diagnosis of ovarian carcinoma. Misinterpretation and inconsistent application of histological criteria may lead to significant interobserver variability and poor reproducibility of the diagnosis. In this study, we investigated the discrepancy in histological diagnosis and the significance of a designed panel of immunohistochemical markers for the improvement of the diagnostic reproducibility of ovarian carcinomas. **Methods:** We performed a retrospective study on 74 ovarian carcinomas. All tumor slides were independently reviewed by two pathologists. The results for seven available immunomarkers as p53, WT-1, $p16^{INK4A}$, CK7, CK20, and estrogen and progesterone receptors were determined for all cases by immunohistochemistry. **Results:** The histological diagnosis review performed using standard histology showed a concordance of diagnoses in 86% of cases with Cohen's kappa of 0.80. Immunohistochemical results increased significantly the diagnosis reproducibility with a concordance of 91% and a Cohen's kappa of 0.86 (P = 0.001). **Conclusion:** Although the histological diagnosis remains reliable, the use of a designed panel of immunohistochemical markers improves significantly the interobserver concordance and the classification accuracy of ovarian carcinomas.

Keywords: Carcinoma- ovary- diagnosis- histology- immunohistochemistry- reproducibility

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Introduction

Ovarian cancer is the leading cause of gynecologic cancer related-death among women with 151,917 estimated deaths in 2012 worldwide (Globocan, 2012; Siegel et al., 2014; Torre et al., 2018). In Tunisia, ovary cancer is the fourth most common cancer in women with 4.6 new cases per 100,000 and it is responsible for 5.5% of cancer mortality (Globocan, 2012; Missaoui et al., 2010). These significant incidence and mortality rates can be explained by the absence of clinical symptoms in the early stages as well as the non-availability of screening strategies and effective and reliable treatments. Therefore, the prognosis of ovarian cancer remains very poor, thus this cancer is considered as a challenge for pathologists and oncologists (Gilks and Prat, 2009; Kipps et al., 2013; Siegel et al., 2014; Xu et al., 2017; Zhang et al., 2017).

In the recent era, five major histological types have been delineated: high-grade serous, low-grade serous, mucinous, clear cell and endometrioid carcinoma (Bian et al., 2014; Gilks and Prat, 2009; Kipps et al., 2013). Currently, histological assessment remains the relevant clue for the diagnosis of ovarian tumors. Misinterpretation and inconsistent application of the morphological criteria may lead to significant intra- and interobserver variability and poor reproducibility of the diagnosis (Abdelaal et al., 2016; Bian et al., 2014; Cymbaluk-Ploska et al., 2016; Gilks et al., 2008; Köbel et al., 2008; Köbel et al., 2010a; Köbel et al., 2010b; Köbel et al., 2016; Köbel et al., 2018; Seidman et al., 2015). Diagnosis problems include the distinction between primary and metastatic carcinoma, the distinction between low-grade and high-grade serous carcinoma, the distinction between serous carcinoma and endometrioid carcinoma, and the distinction of clear cell carcinoma from other types of carcinoma containing clear cells (Abdelaal et al., 2016; Bian et al., 2014; Cymbaluk-Ploska et al., 2016; Gilks et al., 2008; Köbel et al., 2008; Köbel et al., 2010a; Köbel et al., 2010b; Köbel et al., 2016; Köbel et al., 2018; Seidman et al., 2015). To avoid these subjective discrepancies and to improve

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diagnostic accuracy, a variety of methods, including molecular diagnosis techniques, have been recently investigated. Classification based on the histology, the immunophenotype and the genotype of ovarian carcinomas could be highly reproducible.

The present study aimed to review the histological diagnosis of ovarian carcinoma by two pathologists and to evaluate the contribution of immunohistochemistry to the diagnosis and the classification of ovarian carcinoma by analyzing the tumor expression of Wilm's Tumor-1 protein (WT-1), p53, $p16^{INK4A}$, cytokeratin 7 (CK7), cytokeratin 20 (CK20), and estrogen and progesterone receptors (ER and PR).

Materials and Methods

Seventy-four specimens were retrieved from the surgical pathology files of the Department of Pathology of Farhat Hached University Hospital, Sousse, Tunisia, between 2009 and 2014. The studied cases were distributed into the following groups, according to the World Health Organization (WHO) Classification of Tumors of the Breast and Female Genital Organs: high-grade serous carcinomas (n=48), low-grade serous carcinomas (n=8), endometrioid carcinomas (n=12), mucinous carcinomas (n=5) and clear cell carcinoma (n=1) (Tavassoli and Devilee, 2003). All selected cases were non-metastatic primary ovarian cancers and were diagnosed in patients who have not received chemotherapy. The slides were reviewed by two independent pathologists.

All tissues had been routinely fixed in 10% buffered formalin and paraffin embedded. One or two paraffin blocks containing representative portions of the tumors were selected for each case, and 4-µm-thick sections were obtained. The study was approved by the local Human Ethics Committee and it conformed to the provisions of the Declaration of Helsinki.

Formalin-fixed, paraffin-embedded tissues were deparaffinized in xylene, rehydrated through serial dilutions of alcohol and washed in phosphate–buffered saline (pH 7.2). After pre-treatment with the antigen retrieval solution at 95°C for 40 minutes (Table 1), endogenous peroxidase activity was blocked in 3% hydrogen peroxide. Slides were then incubated with primary antibody at room temperature (20-25°C) for 30 min (Table 1). Diaminobenzidine was used as the chromogen for the immunostaining. Finally, sections were counterstained with hematoxylin and mounted (Missaoui et al., 2018). Specific positive controls were used for each antibody. Negative controls were obtained by excluding the primary antibody. Images were captured by the microscopic digital camera Olympus system. Immunohistochemistry evaluation was independently performed by two pathologists. All immunostaining were scored as positive or negative as indicated in Table 1.

Interobserver concordance and Cohen's kappa were calculated using the SPSS software Version 22.0. Probability values (P) of 0.05 or less were considered statistically significant.

Results

A total of 15 cases were reclassified by the two pathologists. Thus, the concordance degree between the first and the second histological review was 86% and the Cohen's kappa was 0.80. Among 49 high-grade serous carcinomas, the diagnosis was confirmed for 35 cases and reclassified for 14 cases. The Cohen's kappa

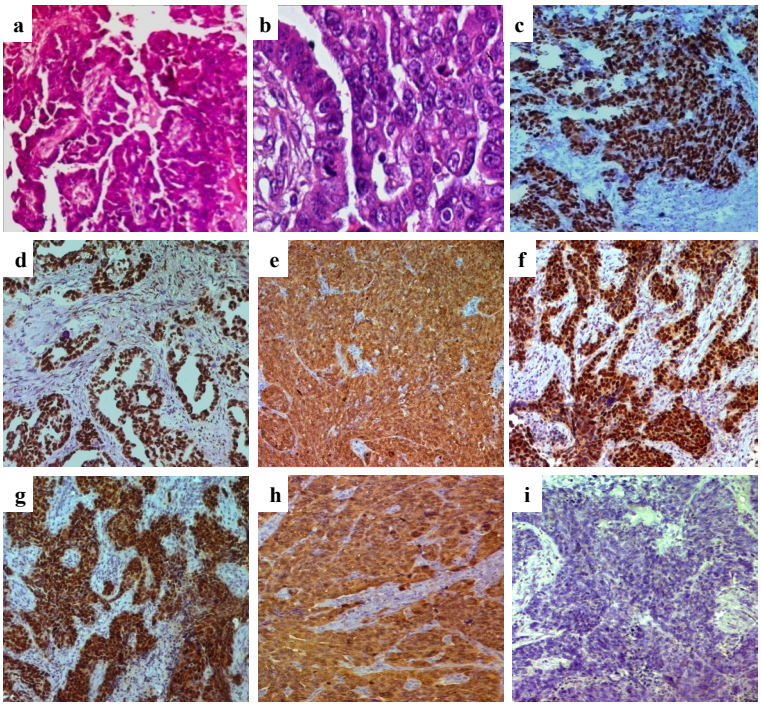

Figure 1. Ovarian High-grade Serous Carcinoma, Histology and Immunophenotype. Hematoxylin and eosin staining [Mx100] (a) and [Mx400] (b). Positive expression of p53 (c), WT-1 (d), $p16^{INK4A}$ (e), ER (f), PR (g), and CK7(h). No CK20 expression (i).

Table 1. Immunohistochemistry Conditions and Evaluation

Marker	Clone	Provenance	Dilution	Retrieval solution	Positive immunostaining
p53	SP5	SpringBio	1/100	Citrate 0.01M, pH 6.0	Nuclear staining in ≥ 10% of tumor cells
WT-1	6F.H2	Dako	1/50	Trypsin	Nuclear staining in ≥1% of tumor cells
$p16^{INK4A}$	E6H4	Ventana	Ready for use	Citrate 0.01M, pH 6.0	Nuclear staining in ≥ 90% of tumor cells
ER	1D5	Dako	1/40	Citrate 0.01M, pH 9.0	Nuclear staining in ≥1% of tumor cells
PR	PgR636	Dako	1/40	Citrate 0.01M, pH 9.0	Nuclear staining in ≥1% of tumor cells
CK7	RN-7	NovoCastra	1/50	Citrate 0.01M, pH 6.0	Cytoplasm staining in≥50% of tumor cells
CK20	PW-31	NovoCastra	1/50	Citrate 0.01M, pH 6.0	Cytoplasm staining in≥50% of tumor cells

WT-1, Wilm's Tumor-1 protein; CK7, Cytokeratin 7; CK20, cytokeratin 20; ER, estrogen receptor; PR, progesterone receptor.

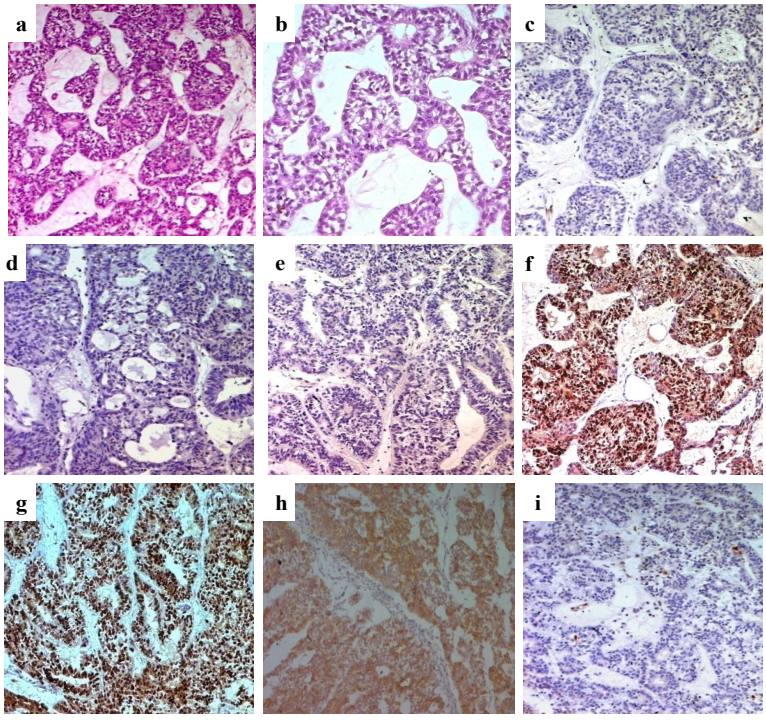

Figure 2. Ovarian Endometroid Carcinoma, Histology and Immunophenotype. Hematoxylin and eosin staining [Mx100] (a) and [Mx400] (b). No expression of p53 (c), WT-1 (d), $p16^{INK4A}$ (e), and CK20 (i). Diffuse expression of ER (f), PR (g), and CK7 (h).

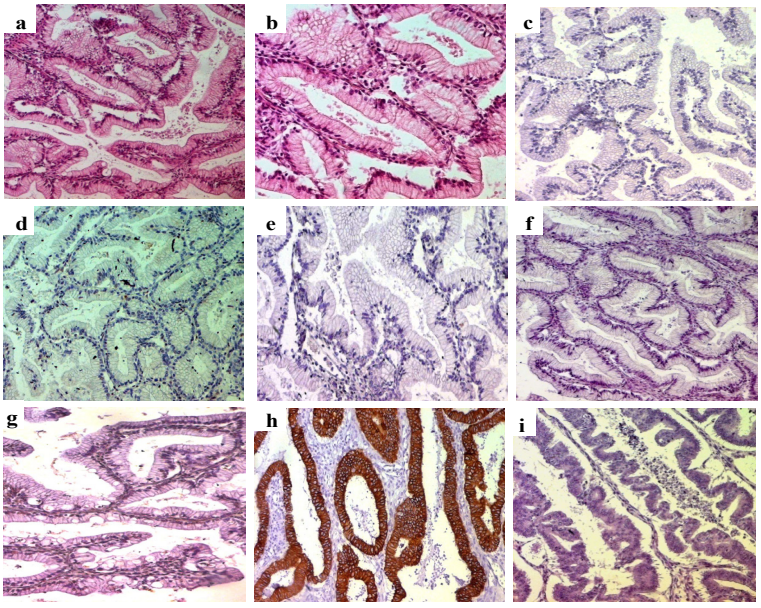

Figure 3. Ovarian Mucinous Carcinoma, Histology and Immunophenotype. Hematoxylin and eosin staining [Mx100] (a) and [Mx400] (b). No expression of p53 (c), WT-1 (d), $p16^{INK4A}$ (e), ER (f), PR (g), and CK20 (i). Positive CK7 expression (h).

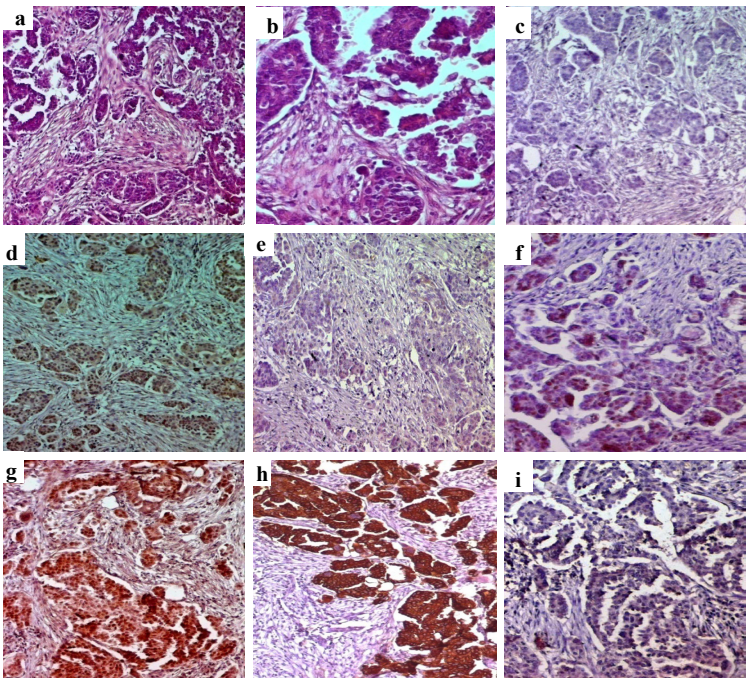

Figure 4. Ovarian Low-grade Serous Carcinoma, Histology and Immunophenotype. Hematoxylin and eosin staining [Mx100] (a) and [Mx400] (b). No expression of p53 (c), $p16^{INK4A}$ (e) and CK20 (i), positive expression of WT-1 (d), ER (f), PR (g), and CK7 (h).

was 0.72. The reviewed cases were classified into seven endometrioid carcinomas, six undifferentiated carcinomas and one clear cell carcinoma. For mucinous carcinomas, the diagnosis was confirmed in four cases and only one case was reclassified as clear cell carcinoma. The Cohen's kappa was 0.73. However, for all endometrioid, clear cell, and low-grade serous carcinomas analyzed, the histological diagnosis was confirmed by the two pathologists and the Cohen's kappas were 0.70, 0.82 and 1, respectively.

Immunohistochemical results were shown in Figure 1-5. Overall, the concordance between reviewed histological diagnosis and immunohistochemical results was 91% and Cohen's kappa was 0.86 (P = 0.001). Among the 35 reviewed high-grade serous carcinomas, only one case was reclassified as clear cell carcinoma

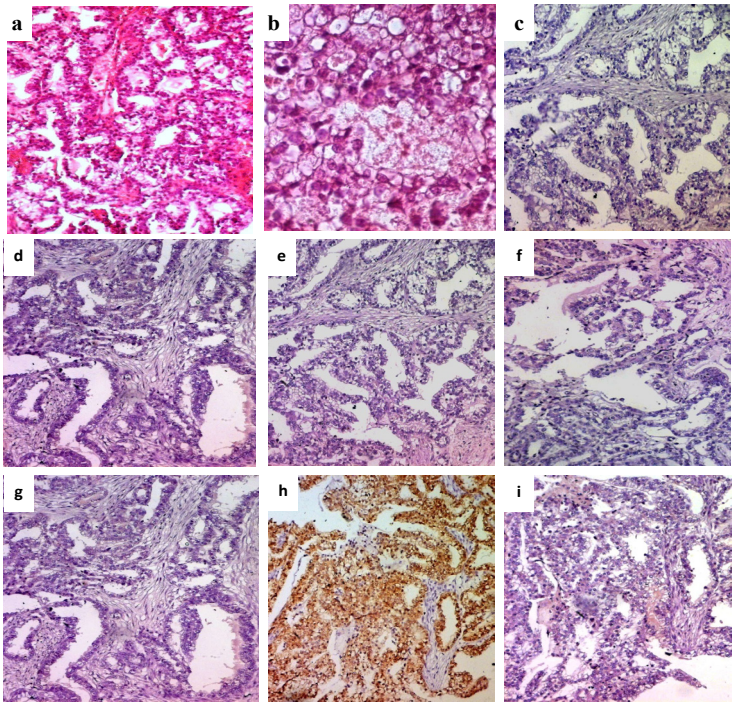

Figure 5. Ovarian Clear Cell Carcinoma, Histology and Immunophenotype. Hematoxylin and eosin staining [Mx100] (a) and [Mx400] (b). No expression of p53 (c), WT-1 (d), $p16^{INK4A}$ (e), ER (f), PR (g), and CK20 (i). Positive CK7 expression (h).

(Figure 1). The Cohen's kappa was 0.78. For endometrioid carcinomas, six cases were reclassified as high-grade serous carcinomas and the Cohen's kappa was 0.75 (Figure 2). No reclassification was made for mucinous and low-grade serous carcinomas and the Cohen's kappa values were 1 in both cases (Figure 3 and 4). For the undifferentiated carcinomas, four cases were reclassified as high-grade serous carcinomas. Among the three reviewed cases of clear cell carcinomas, two cases were reclassified as high-grade serous carcinomas and the Cohen's kappa was 0.86 (Figure 5).

Discussion

The diagnosis of ovarian carcinoma types is based on morphological features (Gilks and Prat, 2003). However, misinterpretation and inconsistent application of the morphological criteria may lead to a significant intra- and interobserver variability and a poor reproducibility of the diagnosis. In this paper, we confirmed that the histological diagnosis of some ovarian carcinoma types based on the WHO criteria remains a challenge for pathologists mainly regarding the distinction between high-grade serous carcinomas, endometrioid carcinomas and clear cell carcinomas and the differentiation between the primary and metastatic ovarian carcinomas. Among the 74 cases studied, 16 cases were difficult to classify. The interobserver concordance was 86% and the Cohen's kappa was 0.80. The inter-pathologist diagnosis variability requires the application of additional diagnosis tools leading to an accurate diagnosis. The use of immunohistochemistry in the diagnosis and classification of ovarian carcinoma types could be of major contribution since an accurate and effective classification improves the treatment strategies (Köbel et al., 2016; Köbel et al., 2018; Malpica et al., 2007; McCluggage, 2004; O'Neill et al., 2007). We reported here a significant improvement of the histopathological diagnosis accuracy of ovarian carcinoma using immunohistochemistry.

Several studies examined the reproducibility of the histological classification of ovarian carcinomas. Köbel et al. analyzed the reproducibility of cell type diagnosis among six pathologists across Canada (Köbel et al., 2010a). The median inter-pathologist concordance was 92.3% and the median Cohen's kappa was 0.89. This excellent inter-pathologist reproducibility of cell type diagnosis resulted from a brief training exercise and the use of defined criteria for ovarian carcinoma typing. Thus, immunostaining results did not significantly improve diagnosis reproducibility of ovarian carcinomas (Köbel et al., 2010a). However, Brugghe et al. found a total agreement between observers in only 61% studied cases (Brugghe et al., 1995). McCluggage reported that histological diagnosis is reproducible only in some cases and it was difficult in other cases, mainly regarding the differentiation between high-grade serous carcinomas and endometrioid carcinomas and between clear cell carcinomas and undifferentiated carcinomas (McCluggage, 2004). Recently, Köbel et al., (2014) showed that the revaluation of an initial diagnosis by eight pathologists showed an agreement degree of only 78%, suggesting serious problems in the histological diagnosis of ovarian carcinomas. Previously, Lund et al., (1991) studied the reproducibility of the WHO classification of ovarian carcinomas criteria and reported a moderate interobserver agreement with a concordance ranging from 56% to 68%. To improve diagnostic accuracy of ovarian carcinomas, Kalloger et al., (2011) investigated the immunohistochemical expression of 22 biomarkers on tissue microarrays constructed from 322 archival samples from the British Columbia Cancer Agency archives and an independent set of 242 cases of ovarian carcinoma from the Gynecologic Tissue Bank at Vancouver General Hospital. The researchers reported nine useful biomarkers for the classification of the five major subtypes of ovarian carcinoma, including CDKN2A, DKK1, HNF1B, MDM2, PGR, TFF3, TP53, VIM and WT-1. In this respect, Köbel et al., (2013) considered that biomarker-based classification of ovarian carcinomas is feasible, improves comparability of results across research studies, and can reclassify cases which lack reliable original histopathological diagnosis.

In the current study, we found no difficulties in the distinction between low-grade and high-grade serous carcinomas. The expression of p53 protein was observed in all high-grade serous carcinoma cases as reported previously (Armes et al., 2005; Giordano et al., 2008; Köbel et al., 2014; McCluggage, 2008). However, no specific p53 expression was observed in low-grade serous carcinomas. McCluggage (2008) considered the p53 expression as a surrogate marker for the differentiation of high-grade serous carcinomas from other ovarian carcinomas. Moreover, WT-1 and $p16^{INK4A}$ expression was observed in all serous carcinoma cases as described (Giordano et al., 2008; Köbel et al., 2014; Nazlioglu et al., 2010). The WT-1 expression is characteristic of serous subtypes of ovarian carcinomas and it is useful in precision of the origin of the primary or secondary serous carcinoma (McCluggage, 2004). In the initial study of interobserver diagnosis variability of ovarian carcinomas, Malpica et al., (2007) has shown that the distinction between low-grade and high-grade serous carcinomas was highly reproducible. Subsequently, this distinction has been identified as the second most common problem in ovarian carcinoma diagnosis after the distinction of endometrioid from high-grade serous carcinomas (Köbel et al., 2010b; Köbel et al., 2014).

In our study, the distinction of endometrioid from high-grade serous carcinomas was an important challenging area of differential diagnosis of ovarian carcinoma. Six endometrioid carcinomas were reclassified as high-grade serous carcinomas using immunohistochemistry. No positive immunostaining was observed for the p53 and WT-1 proteins as reported previously (Köbel et al., 2014; McCluggage, 2004; McCluggage, 2008). Köbel et al., (2014) considered that the use of WT-1 as a marker of serous cell type can be of great help. They suggested that the immunohistochemical testing should improve interobserver reproducibility in cases with a glandular pattern, severe nuclear atypia and an absence of squamous differentiation.

The differential diagnostic problems associated with ovarian clear cell carcinoma are well-established and include primitive germ cell tumor, sex cord stromal tumor, and metastasis. Distinction from other types of surface epithelial carcinoma may also pose a diagnosis challenge. Using immunohistochemistry, two clear cell carcinomas were reclassified as high-grade serous carcinomas and only one high-grade serous carcinoma was reclassified as clear cell carcinoma. The immunostaining results were negative for all antibodies except for CK7. Similarly, no expression of p53, $p16^{INK4A}$, WT-1 and hormone receptors (ER and PR) was reported by the study of Köbel et al., (2014) including 14 clear cell carcinomas. According to the Sangoi et al., (2008) as clear cell and low-grade serous carcinomas exhibit significantly different immunoreactivity for WT-1 and ER, these markers may be useful adjunctive tests in problematic cases. HNF1B is considered also as a useful biomarker for clear cell ovarian carcinoma diagnosis (Köbel et al., 2014; McCluggage, 2005). In the study of DeLair et al., (2011) clear cell carcinoma has characteristic morphological features and a specific immunophenotype in the vast majority of the cases, including HNF positive expression, and ER, PR, WT-1 and p53 negative expression. The authors suggested that clear cell-rich tumors with features that differ from the classical morphological appearances should suggest the possibility of an alternative diagnosis.

Currently, immunohistochemical study of mucinous carcinomas was negative for all markers except for CK7 as described (Köbel et al., 2008; Kurman and Shih, 2010; Seidman et al., 2004). According to some studies, immunohistochemistry does not seem very useful in the diagnosis of mucinous carcinomas since morphology is reproducible (Köbel et al., 2008; Kurman and Shih, 2008; Sangoi et al., 2008). However, Shin et al., (2010) considered that CK7, CK20, CDX2 and MUC2 immunostaining is a useful additional diagnostic tool to differentiate metastatic colorectal adenocarcinoma involving ovaries from primary ovarian mucinous adenocarcinoma in addition to clinical history and gross and microscopic findings. Vang et al., (2006) investigated the expression of CDX2 and CK20 in conjunction with the coordinate expression of CK7 in mucinous tumors involving the ovary. CDX2 provided some advantage over CK20 for distinguishing primary ovarian mucinous tumors from metastases of upper, but not lower, gastrointestinal tract origin. Among these markers, CK7 provides the predominant discriminatory value, although it is limited to the distinction of primary ovarian tumors from metastases of the lower gastrointestinal tract origin (Vang et al., 2006).

In conclusion, although the histological diagnosis of ovarian carcinoma remains reliable, our study showed a significant improvement of histological diagnosis accuracy and reproducibility as a result of the use of designed immunohistochemical markers.

Compliance with Ethical Standard

This study was approved by the local Human Ethics Committee at the Farhet Hached University Hospital of Sousse (Tunisia) and it conformed to the provisions of the Declaration of Helsinki.

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None.

Conflict of interest

The authors have no conflicts of interest to declare.

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