Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(2):496-502

Immunohistochemical profile in malignant ovarian tumors operated in our center and its discriminative importance

Demet Baloglu[1], Ebru Inci Coskun[2], Saadet Alan[3], Ercan Yilmaz[2], Koray Kutluturk[4]

[1]Izmir Urla State Hospital, Clinic of Gynaecology and Obstetrics, Izmir Turkey
[2]Inonu University Faculty of Medicine, Department of Gynaecology and Obstetrics, Malatya, Turkey
[3]Inonu University Faculty of Medicine, Department of Pathology, Malatya, Turkey
[4]Inonu University Faculty of Medicine, Department of General Surgery, Malatya, Turkey

Received 12 May 2020; Accepted 03 June 2020
Available online 07.06.2020 with doi: 10.5455/medscience.2020.09.9254

Abstract

The aim of this study is to determine the correlation of histopathological subtype with frozen section examination and the importance of immunohistochemical analysis of ovarian malignancies which also include metastatic ones. This study is a retrospective study that have included 55 patients who have been operated for adnexial tumor and have the diagnosis of malignancy during surgery by frozen section or after surgery by final pathologic examination done by immunohistochemistry (IHC). The mean age of the patients with malignant ovarian tumor is 52.33 ± 15.5 years. When the pathologic diagnosis reports examined it has been found that 34 of 55 patients (61.8 %) have had epithelial type, 9 have had sex cord stromal tumor, 3 have had germ cell type and 9 of the patients have had metastatic ovarian cancer. Survival rates have been found as 70.6 % for epithelial ovarian cancer, 100 % for sex cord stromal tumors, 100 % for germ cell tumor and 44.4 % for metastatic tumors. Despite the rapid development in examination and imaging methods, histopathology is the pivotal issue in the diagnosis and also in sub-type diagnosis of pelvic mass lesions. The improvement is better in patients evaluated and operated in gynecologic oncology centers. The two important factor in this subject is the experience of the surgeon for maximum salvage from the tumoral burden and the well examination by frozen section the immunohistochemical methods for the discrimination of gastrointestinal tumors which can mimic ovarian primary tumors. Immunohistochemical methods have very important progression in diagnosis of cancer, its origin and subtypes and however IHC also could have a key role in treatment of cancer by targeted therapy.

Keywords: Immunohistochemistry, ovarian cancer, metastatic ovarian tumors, frozen section, gastrointestinal tumors

Introduction

According to the data reported in 2018 by World Health Organization (WHO)' s International Agency for Research on Cancer, ovarian cancer has been in the eighth order in the ranking [1]. It has been sixth in Turkey [2]. Ovarian cancer has sustained to be the most mortal gynecologic cancer among women in our country and all over the world since the ovarian cancer does not have any spesific symptom and there has not been an effective screening method for the diagnosis. The overall risk for a woman for her lifetime is 1/63 [1,2]. Since the prognosis is poor, the 5 years survival rate is under 50 % [3,4]. The early, certain diagnosis and dynamic management of the treatment have been quite important also in avoidance of recurrence.

***Coresponding Author:** Ebru Inci Coskun, Inonu University Faculty of Medicine, Department of Gynaecology and Obstetrics, Malatya, Turkey
E-mail: ebruincicoskun@gmail.com

Epidemiologic risk factors are advanced age, genetic and hormonal factors. Many studies revealed that the age of woman has been an important risk factor. Incidence has been increased every decade. It has been known that the prognosis has been worsened as the age increases and the prognosis has been better relatively under age of 50 [5]. In 10 % of ovarian cancers genetic factors such as are claimed in etiology. It has been reported that the patients which have BRCA 1 gene mutation and high risk in family history have a risk for ovarian cancer as 29-46 %, and as 12-20 % in patients with BRCA 2 gene mutation [6]. Early menarrche, late menopause are important risk factors because of these increase in exposure to ovulation. However the estrogen replacement therapy in postmenopausal period cause increased risk [7].

Epithelial ovarian tumors originated from coelomic epithelium of the ovarian surface. Ninty percent of the malign ovarian tumors are originated from the surface epithelium of the ovary

[8]. In the time of diagnosis usually found extended beyond the ovary and in the abdominal cavity [9]. Mostly seen subtype is serous carcinomas and secondly mucinous carcinomas are seen [10]. Mucinous tumor cells are like endocervical or intestinal epithelium [11]. Thus, it is difficult to make a differential diagnosis between mucinous ovarian tumor and intestinal tumor metastasis to ovaries according to the basic histopathologic methods. That' s why there is a need for advanced methods like immunohistochemistry has found a place in ovarian tumors. Other subtypes of epithelial tumors are endometrioid and clear cell type [11].

Sex cord stromal tumors are originated from matrix cells which have potential of hormone production. That' s why symptoms of excess estrogen and androgen have been seen in these patients. According to the response of the target organ to this excess of hormones, changes with the age of the patient. Granulosa cell tumors are subtype of the sex cord stromal tumors and constitutes 70 % of them. Granulosa cell tumors are divided into two other groups as adult type and juvenil type granulosa cell tumors (95 % and 5 %, respectively). Juvenil type arises from the nongerminative tissues of the ovary and seen in the first two decades of life [12].

Two-five percent of all ovarian malignancies and also 70 % of the ovarian tumors seen before age of 20, are germ cell tumors. One third of these tumors in childhood and adolescents are malignant. 40 % of these malign germ cell tumors are disgerminomas, the mostly seen histologic type. Most frequently seen malign germ cell tumors are immature teratomas (40-50 %) and it is consisted of every three germ cell layer and the survival rate is determined by the histologic grade of the tumor. A mixed pattern in cellular differentiation has been seen in 25-30 % of the germ cell tumors and in this case the mostly seen component is disgerminomas. It has been seen typically with immature teratoma or yolk sac tumor or with both of them [13].

Metastatic ovarian tumors are reported between the ratios as 4-12.5 % in studies [14]. Gastrointestinal system tumors and breast cancer are mostly seen metastatic origins. Hodgkin lymphoma and genital tract malignancies could make metastasis to the ovaries, too. A rare metastatic origin is bilier cancer for ovary but has been reported in between 0.9-2.7 % [15]. Krukenberg tumor in which signet ring cells has been seen in ovaries are mostly bilateral and constitutes 1-2 % of the all ovarian tumors [16]. Clinical symptoms of metastatic ovarian tumors are like primary ovarian tumors and the prognosis is poor since they are rarely determined before the primary lesion advances.

Variety of ovarian tumors sometimes make the diagnosis harder. The subtype should be determined precisely since the prognosis and treatment varies according to the subtypes of the ovarian tumor. In recent years, immunohistochemical methods have an important place in the diagnosis of ovarian tumors. Therefore, histopathologic determination of the subtype and the exclusion of metastatic tumors have a pivotal role in the management of treatment so the morbidity and the mortality could be affected in a big scale. Immunohistochemistry (IHC) is also valuable to determine the origin of the tumor which is not defined properly by the morphologic and clinical aspects. So it can be used in peritoneal biopsies and also in fluid specimens for these purpose.

The aim of this study is to determine the correlation of histopathological subtype with frozen section examination and the importance of immunohistochemical analysis of ovarian malignancies which also include metastatic ones.

Material and Methods

Ethical approval for this study has been taken from Inonu University Health Sciences Non-interventional Clinical Research and Publish Ethics Committee. The medical records of 55 patients who have undertaken surgery because of a pre-diagnosis of adnexial mass lesion with a malignancy report after or during surgery, between January 2014 and November 2017. Operation history, laboratory results and pathologic frozen and final diagnosis reports have been evaluated retrospectively. Pathologic results which reported 'malign' and also have immunohistochemical evaluation of the specimens have been determined and studied. 55 patients have been evaluated in these terms. These patients have been consisted of 34 epithelial ovarian cancer, 9 sex cord stromal ovarian tumor, 3 germ cell tumor and 9 metastatic ovarian tumors.

Frozen Section Evaluation

Intraoperative consultation for pathologic examination (frozen section examination) has been done as soon as possible so the patients have the least amount of anestesia. Frozen section generally has been done in the cases of unexpected findings, surgical edge evaluation, lymph node metastasis evaluation (sentinel lymph node), and in the purpose of being sure that the sampling has been proper and enough. The instructions about the whole process about intraoperative consultation like acceptance terms, frozening, cutting and staining steps, people in charge of the equipment and the declaration of the results have been well organized. The time period of intraoperative consultation has been noted. All of the results and decisions have been documented. 90 % of the intaoperative consultations have been reported in 20 minutes they received to the laboratory. It has been told to the surgical team if there has not been decided malignant or benign clearly or the origin of the malignancy could not been determined, in those cases, the decision should be done finally after the paraffin section examinations. If the morphological characteristics are not enough to make the certain diagnosis, the pathologist apply immunohystochemical staining methods. The microscopic examination directs the pathologist about which immunohystochemical marker should be chosen. Because of this algorithm and also cost effectiveness, immunohystochemistry does not have been undertaken for all of the specimens. Clinics could have their own IHC panel protocols in terms of diagnosis and education as well. The more accurate the choice, the more accurate the contribution has been reached, actually this issue is the most pivotal decision of this process. This also presents the importance of the collaboration of the pathologists and the surgical team clearly.

Immunohystochemical (IHC) Analysis

Specimens taken during the surgical procedures has been examined in Inonu University Medical Faculty Department of Pathology. Sections in 4-5 micrometer thickness has been taken from the tissue samples embedded to paraffin fixed by formaldehyde, to the positive charged lams for the immunohistochemical examination.

IHC steps

Lams have been heated at 72 °C for deparaffinization and incubated 20 minutes in Ultra CC1 (EDTA) solution for antigen retrival. It has been incubated 32 minutes by using primary antibody MMP-9 (92 kDa Collagenase IV, 1:50 dilution, Thermo Scientific) in 1:50 proportion. For background staining, incubation with Haematoxilen for 24 minutes and with Bluing reagent for 4 minutes has been done. These immunohystochemical staining procedures has been done with Ventana Benchmark XT ve Ventana Benchmark Ultra devices. Lams passed through washing steps has been externed from the devices and washed with detergeant water, has been waited in 96 % alcohol. After drying, closing has been done with Leica CV5030 device so the immunhystochemical procedure has been ended. The antibody clones used in our pathology department laboratory are: Estrogen: SP1, Progesterone: 16, Ki67: SP6, p53: DO-7/BPS-12, WT1: 6f-H2, CK7: OU-TL 12/30, CK20: IT KS20.8, Kalretinin: SP13, İnhibin: BC/RI, Pax8: RTU MRQ-SO, Moc31: RTU, Panck: AEI/AE3, AFP: C3.

Statistical analysis

Data collected retrospectively has been analyzed by IBM SPSS Statistics 22.0 program. Data have been summarized as median values and values, ± standard deviations, median (min-max) and digital (%) values. No variable evaluation needed.

Results

The median age of patients are 52.33 ± 15.5. After the pelvic surgeries of 55 patients have been undertaken, 34 of 55 (61.8 %) patients were diagnosed as epithelial type, 9 patients had sex cord stromal type and 3 patients (5.4 %) had germ cell type and 9 patients (19.3 %) had metastatic ovarian cancer (Table 1).

Table 1. Distrubition according to the histologic subtypes

Tumor - Histologic subtype	Patients count	%
Epithelial	34	61.8
Sex cord stromal tumor	9	16.4
Germ cell tumor	3	5.4
Metastatic	9	16.4
Total	55	100

Survival rates of subtypes are lower and the prognosis is poor in the metastatic patients, even in some cases with primary gastrointestinal tumors some can be inoperable. But gastrointestinal tumors and adnexal tumors can not be eradicated in some cases, even preoperative endoscopic interventions for the differential diagnosis have been undertaken. The best survival rates are in sex cord stromal tumor and germ cell tumors (Table 2).

Table 2. Age distrubition and survival rates according to histological subtypes

	Epithelial (n=34) 61.8 %	Sex cord stromal tumor (n=9) 16.3 %	Germ cell tumor (n=3) 5.4 %	Metastatic (n=9) 16.3 %
Age	58.56 ± 11.72	41.33 ± 16.92	22.67 ± 8.08	49.67 ± 11.32
Survival rates	Living: 24 (72.7 %)	Living:9 (100 %)	Living:3 (100 %)	Living:4 (44.4 %)

Two patients had the 'benign' diagnosis in frozen section but the final pathologic report of one patient is sex cord stromal tumor and the other one is granulosa cell tumor. Sex cord stromal tumor has been diagnosed by the immunohistochemical markers as vimentin, Ki67 (%15-20 positive), pancytokeratin, kalretinin, inhibin and CD99. Granulosa cell tumor has been diagnosed by the immunohistochemical markers as vimentin, Ki67 (%15-20 positive), S100, actin and CD10.

Frozen section examination of five patients could not been reported as a certain diagnosis if it is malignant or benign disease. One of these is diagnosed as steroid cell tumor (NOS=Not otherwise specified), one is diagnosed as mixed germ cell tumor (yolk sac 40 5, mature teratom 60 %), one as high grade clear cell and two are diagnosed as endometrioid carcinoma by the final pathologic examination with immunohistochemical analysis. Steroid cell tumor has been stained positive for the immunohistochemical markers such as vimentin, progesteron, Ki67(%10), chromogranin A and CD 99. Mixed germ cell tumor has been stained positive for pancytokeratin and CD 10. Patients diagnosed with final pathologic reports as endometrioid carcinoma, the specimens have been found positive for p53 in common, but one of the cases the specimens also have positive staining for p16, p53, pax-8, CA 125 and ER (Estrogen receptor).

Frozen has revealed 8 metastatic tumors and 4 of them were originated from gastrointestinal system, 1 gastric, 1 rectum and 2 of them were originated from colon. One patient has been diagnosed as granulosa cell tumor in frozen section but it has been revealed by the final pathologic report by the immunohistochemical analysis as metastasis of breast cancer. It has been found positive for vimentin, progesterone, Ki67 (70 % nuclear positive), p16, p53, pancytokeratin, CK7, EMA, CD56.

Immunohistochemical markers which have been usually used are as following in the table in our patient group. AFP is almost spesific for germ cell tumors and inhibin for sex cord stromal tumors. P53 is usually seen positive in epithelial tumors and also in metastatic tumors. CK7 is also positive for many epithelial and metastatic tumors (Table 3).

Table 3. Distribution of malignant ovarian tumors according to their immunohistochemical characteristics

Immunohistochemical marker		Epithelial	Sex cord stromal tumor	Germ cell tumor	Metastatic
Estrogen	Negative	4	1	0	3
	Positive	17	0	0	0
Progesterone	Negative	5	1	0	2
	Positive	5	1	0	1
Ki-67	Negative	0	0	0	0
	Positive	13	5	1	2
P53	Negative	5	0	0	0
	Positive	21	0	0	3
WT-1	Negative	6	1	0	3
	Positive	18	2	0	1
CK-7	Negative	0	4	0	2
	Positive	16	0	0	5
CK-20	Negative	15	1	0	2
	Positive	1	0	0	4
Calretinin	Negative	6	0	0	1
	Positive	0	4	0	1
Inhibin	Negative	0	0	0	1
	Positive	0	6	1	0
PAX-8	Negative	0	0	0	0
	Positive	8	0	0	0
MOC-31	Negative	0	0	0	0
	Positive	1	0	0	0
PAN-CK	Negative	0	0	0	0
	Positive	0	0	1	1
AFP	Negative	0	0	0	0
		0	0	3	0

Discussion

Ovarian cancer has a diagnostic difficulty for women all around the world, and also have high mortality. Despite the advanced imaging methods, the primary factor for diagnosis and treatment plan is histopathology of the tumor. It has been known that the results of patients operated in gynecologic oncology centers have been better. The factors related to this concept are surgical experience for total resection of tumoral burden and a pathologic examination and experience which has been consisted of frozen and immunohistochemistry.

Immunohistochemistry has been used not only for defining subtypes but also for finding the primary origin of the metastatic tumors. Ovaries are common sites for metastasis [17]. The metastasis can occur by blood, lymphatics, direct or transperitoneal extension. The most metastatic non-gynecologic tumors seen in ovary are, gastric, colon and breast cancer. The incidence of metastatic tumors are approximately 10 % of ovarian tumors. The gastrointestinal system especially colon is the most seen metastatic tumor in ovary and the second one is breast cancer. These are the non-gynecologic primary sites, if the gynecologic primary site is considered, endometrium cancer is in the first rank [18]. Ovarian carcinomas and gastrointestinal carcinomas have common morphologic features those cause a diagnostic dilemma, so the discrimination by immunohistochemistry is pivotal since the treatment protocols, metastasis patterns, prognosis and the sensitivity to chemotherapy are so different. That's why the diagnosis should be done precisely. Many metastatic adenocarcinomas can mimic primary ovarian tumors easily. Metastatic ovarian tumors reveal some characteristics in general such as a nodular pattern of ovarian involvement, bilaterality, infiltrative stromal invasion, lymphovascular invasion especially seen in hilus of the ovary. However these are not also patognomonic.

A few immunhistochemical markers used more frequently could be mentioned in some detail. Ovarian tumors are mostly stained positive with cytokeratins (CK7 and CK20) which are found in epithelial cells. These are used widely to diagnose a tumor if it is primary or not [19]. Figure 1 shows an IHC staining sample for CK 7 in clear cell cancer. These cytokeratines are also used for ascit investigation as well. Cytokeratins maybe the first and mostly studied IHC staining methods for distinguishing primary ovarian and metastatic ovarian carcinomas [20]. One of these cytokeratins can be negative in both primary and secondary ovarian tumors and this can be sometimes confusing though. This confusion has been seen especially in mucinous tumors. In such conflicted cases β-catenin and CDX-2 can be used, these reveal nuclear positivity in colorectal adenocarcinomas but ovarian mucinous tumors are negative, mostly. However endometrioid adenocarcinomas can have β-catenin positivity, too [21]. CDX-2 is a highly sensitive marker for gastrointestinal adenocarcinomas and other carcinomas like pancreas, bile duct, bladder, ovary, with intestinal differentiation [21]. It has also reported that CDX-2 has a high sensitivity and specifity for neuroendocrine neoplasms [23]. PAX8 is especially useful for the differential diagnosis of primary ovarian carcinomas and breast carcinomas. It has been highly sensitive [24]. Ren et al. have also reported usage of PAX8 IHC staining in ascit and pleural fluid. They reported that high grade serous carcinomas had positive results for PAX8 staining [25].

CEA (Carcinoembryogenic antigen) is an oncofetal glycoprotein and it has been expressed in adenocarcinomas, especially in gastrointestinal adenocarcinomas. It has been used for the discrimination of colonic (CEA diffuse-intense positive) and ovarian adenocarcinoma (CEA negative or focal weak positive). It is usually also used for mesothelioma, as a negative marker [26].

Inhibin-α, a peptide hormone, secreted by ovarian granulosa cells and has been a highly spesific marker for ovarian sex cord stromal tumors. Calretinin has a high sensitivity for sex cord stromal tumors but not so spesific like inhibin-α, since it can be stained in epithelial tumors, too [27,28]. It is also a good marker (~100 % sensitivity) for malignant mesothelioma, so it is used to make a diffential diagnosis between adenocarcinoma and mesothelioma, studied together with cytokeratins and EMA (epithelial membrane antigen) [29].

EMA is used to in addition to cytokeratines to detect the epithelial differentiation in sarcomatous lesions which are not stained for cytokeratines or only stained focally, in this manner EMA is very useful [30]. It is not only satining positive is important but also staining negative is important for diagnosis in some undifferentiated tumors. It provides exclusion diagnosis. Especially the undiffentiated tumors are hard to make diagnosis without immunohistochemistry [31].

Vimentin is also an intermediate filament especially mesenchymal cells carries. However, epithelial ovarian tumors express cytokeratin in a homogenous way but vimentin has been expressed in different patterns according to the type of the carcinoma. Serous carcinomas for instance express vimentin higher such as 62.5 % in a study reported by Goel and friends [32]. Figure 2 shows an IHC staining sample for vimentin in serous carcinoma of the ovary. In the same study it has been also reported that the vimentin expression has been positively correlated with the histologic grade of the serous tumors. In mucinous ovarian tumors it is more challenged to make a diffential diagnosis, since the immunhistochemical stains are less helpful [33].

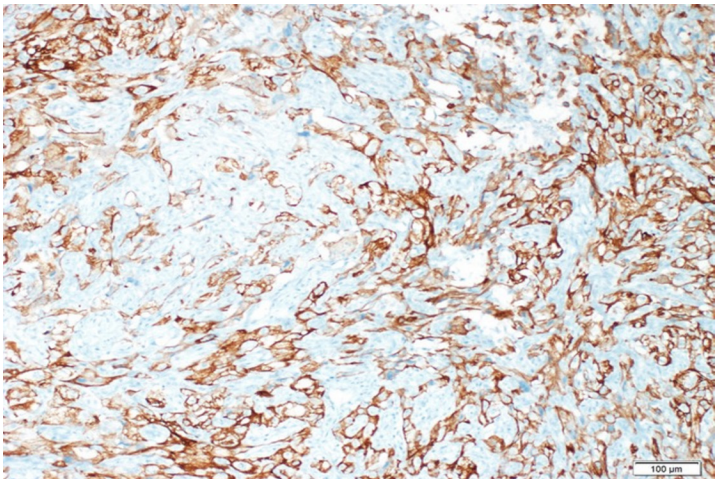

Figure 1. IHC staining sample for CK 7 in clear cell cancer

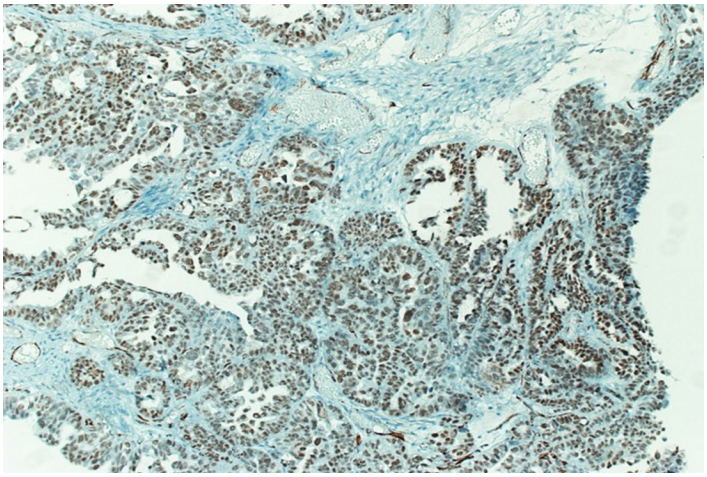

Figure 2. IHC staining sample for vimentin in serous carcinoma of the ovary

Willm's tumor associated protein (WTAP), is a nuclear protein defined as a cancer suppressor and normally have an essential role in urogenital development. It has been first determined by its relation with WT-1. Recent studies revealed that it is also associated with metastatic ovarian tumors [34]. Yu and colleagues reported a correlation between this marker and survival of the high grade serous ovarian cancer. WT-1 itself has been observed in most of the primary ovarian carcinomas, especially in serous tumors [35].

Ki-67 is an important marker that shows the poor prognosis if the expression is high. This indication of proliferation potency is not only for ovarian but also many kind of tumors in the human body [36]. P16 is a cyclin-dependent kinase inhibitor, and has a role in cell cycle. It has been expressed in serous ovarian carcinomas especially high grade ones [37]. Intense staining for p53 is a strong marker for high grade serous ovarian carcinomas as well [38].

Immunohistochemistry has an essential role in discrimination of primary and secondary ovarian tumors. Besides this role, it has been used in diagnosis of synchronous tumors. It is important

to define if it is a metastasis or a synchronous tumor. If the two tumors have the same molecular alterations it means a primary lesion exists and the second tumor is its metastasis [19]. There should be an intense caution about that some synchronous tumors could have similar molecular characteristics, may be because of the same carcinogenic agent. In uterine and ovarian synchronous tumors for instance, the origin tumor site should be determined for different treatment modalities and the clinical manifestations in the beginning should also help in this manner. Staging also should be undertaken subsequently, according to the Federation of Gynecology and Obstetrics (FIGO). Moreover, not only the differential diagnosis but also a target should be defined for potential treatment trials when the immunhistochemical characteristics are revealed in the cancer tissue. For instance there has been p53 targeted vaccine trials ongoing for ovarian cancer (https://clinicaltrials.gov/).

Besides the frequently used markers, some other markers recommended in the literature, for instance napsin A. It has been found sensitive and specific for clear cell carcinoma in particular. It seems important since the clear cell carcinoma has the poorest prognosis among the all ovarian cancers so it is crucial to make the correct diagnosis [39].

Some triple or more markers are used as diagnostic panels to make the diagnosis easily. In this manner some algorithms are developed but there is no certain algorithm for ovarian cancer yet, maybe it could not be. Many combinations of markers can be used in time, and many others can be added to the panels. The morphological features of the specimens will lead the pathologist to make the proper IHC staining choices and panels. In this pathway it should be remembered that the clinical characteristics, surgical exploration and macroscopic features can be very useful for the differential diagnosis. Tumor size is useful in this regard, primary ovarian tumors usually larger than metastatic ones. In addition bilaterality is a feature usually for metastatic tumors. Gross exploration and first microscopic examination are crucial as leading also to choose the proper immunohistochemical markers for the certain diagnosis. Therefore the communication between clinics and laboratory disciplines are pivotal for sharing all data both macroscopic and microscopic [40].

The total evaluation should be done by a multidisciplinary and interdisciplinary gynaecologic oncology meetings. Conflicts about the certain diagnosis should be overcome and the decision about the treatment modality and follow up process should be made in consensus. It has been recommended that the medical oncology, pathology, gynecologic oncology, radiation oncology and if needed other clinical or pre-clinical departments should be involved in the council. It has been done in this way at our department so the final decision are made by considering all clinical and pathologic-immunhistochemical features of the cases in order to maintain the best management of this patient group.

Conclusion

Several markers have been used for immunohistochemistry widely in diagnosis of neoplasms. Each day there has been a new marker in this field of investigation. A marker used in a organ system could be investigated in another system and sometimes a brand new diagnostic criteria occurs. Since different behaviour characteristics and different treatment ways have been seen in different organ tumors it is essential to determine the primary tumor for survival issues. As the biologic pathways have been enlighted in detail by the time, the more success in the understanding cancer mechanisms will be achieved and potential treatment targets will be discovered.

Conflict of interests
We declare that we have no conflict of interest.

Financial Disclosure
This study received no financial support.

Ethical approval
Inonu University Scientific Research and Publication Ethics Committee (Health Science Non-interventional Clinical Research Ethics Council) Approval No: 2018/2-17

References

1. Globocan (2012), Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2018: https://gco.iarc.fr/
2. T.C. Sağlık Bakanlığı. Türkiye Halk Sağlığı Genel Müdürlüğü. Kanser Dairesi Başkanlığı. Türkiye Kanser İstatistikleri: https://hsgm.saglik.gov.tr/tr/kanser-istatistikleri
3. National Cancer Institute: SEER Cancer Statistics Review (CSR) 1975–2014. https://seer.cancer.gov/archive/csr/1975_2014/.
4. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017;67:7–30.
5. Goodman MT, Shvetsov YB. ‘ Incidence of ovarian, peritoneal, and fallopian tube carcinomas in the United states, 1995-2004’. Cancer epidemiol Biomarkers Prev. 2009;18:132-9.
6. Vencken PM, Kriege M, Hoogwerf D, et al. ‘Chemosensitivity and outcome of BRCA1 and BRCA2 associated ovarian cancer patients after first line chemotherapy compared with sporadic ovarian cancer patients’ Ann Oncol. 2011;22:1346-52.
7. Purdie DM, Bain CJ, siskind V, et al. Ovulation and risk of epithelial ovarian cancer. Int J Cancer. 2003;104:228-32.
8. Berek SJ, Berek and Novak’s Gynecology. 2007. p. 1457-9.
9. Seidman JD, Russel P, Kurman RJ (2002). Surface epithelial tumours of the ovary. In: Kurman RJ (edn): Blaustein’s Pathology of the female genital tract, (5 th edn), Springer-Verlag, New York.
10. Kurman RJ, Shih I. The origin and pathogenesis of epithelial ovarian cancer: A proposed unifying theory, Am J surg Pathol. 2010;34:433-43.
11. Chen VW, ruiz B, Kılleen JL, et al. Pathology and classification of ovarian tumors. Cancer. 2003;15(10 suppl):2631-42.
12. Schumer ST, Cannistra SA. ‘Granulosa cell tumor of the ovary’,J clin Oncol. 2003;21:1180-9.
13. Talerman A, Path FRC.(2002), Germ cell tumors of the ovary. In Kurman R (ed): Blaustein’ s Pathology of the Female Genital Tract, 5 th ed. New York, Springer-Verlag, p. 967-1034.
14. Turan T, Aykan B, Koç S, et al. Analysis of metastatic ovarian tumors from extra-genital primary site. Tumori. 2006;92:493-7.
15. Moore RG, Chung M, Granai GO, et al. Incidence of metastasis to the ovaries from nongenital tract tumors. Gynecol Oncol. 2004;93: 87-91.
16. Ümmugul Ü, Arslan ŞH, et al. Isolated ovarian metastasis of gastric cancer. Krukenberg Tumor Contemp Oncol (Pozn). 2013;17:515-9.
17. Asotra S, Sharma J, Sharma N. Metastatic ovarian tumor. J Cytol. 2009;26:144-5.
18. Di Saia, Creasman. Clinical Gynecologic Oncology 8th Ed. 2012;Chapter 12, p. 354. Elsevier Saunders

19. McCluggage WG, Wilkinson N. Metastatic neoplasms involving the ovary: a review with an emphasis on morphological and immunohistochemical features. Histopathology. 2005; 47:231-247.

20. Kriplani D, Patel MM. Immunohistochemistry: a diagnostic aid in differentiating primary epithelial ovarian tumors and tumors metastatic to the ovary. South Asian J Cancer. 2013;2:254-8.

21. Chou YY, Jeng YM, Kao HL, Chen T, Mao TL, Lin MC. Differentiation of ovarian mucinous carcinoma and metastatic colorectal adenocarcinoma by immunostaining with β-catenin. Histopathology. 2003;43:151-6.

22. Moskaluk CA, Zhang H, Powell SM, et al. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. Mod Pathol. 2003;16:913-9.

23. Kyriakopoulos G, Mavroeidi V, Chatzellis E, Kaltsas GA, Alexandraki KI. Histopathological, immunohistochemical, genetic and molecular markers of neuroendocrine neoplasms. Ann Transl Med. 2018;6:252.

24. Nonaka D, Chiriboga L, soslow RA. Expression of pax8 as a useful marker in distinguishing ovarian carcinomas from mammary carcinomas. Am J surg Pathol. 2008;32:1566-71.

25. Ren S, Klump W. Gynecologic serous carcinoma An Immunohistochemical analysis of malignant body fluid specimens. Arch Pathol Lab Med. 2019;143:677-82.

26. Berezowski K, Stastny JF, Kornstein MJ Cytokeratins 7 and 20 and carcinoembryonic antigen in ovarian and colonic carcinoma. Mod Pathol. 1996;9:426-9.

27. McCluggage WG, Maxwell P Immunohistochemical staining for calretinin is useful in the diagnosis of ovarian sex cord-stromal tumours. Histopathology. 2001;38:403-8.

28. Deavers MT, Malpica A, Liu J, Broaddus R, Silva EG Ovarian sex cord-stromal tumors: An immunohistochemical study including a comparison of calretinin and inhibin. Mod Pathol. 2003;16:584-90.

29. Ordóñez NG The diagnostic utility of immunohistochemistry in distinguishing between epithelioid mesotheliomas and squamous carcinomas of the lung: A comparative study. Mod Pathol. 2006;19:417-28.

30. Mouhamed HA. The diagnostic utility of immunohistochemistry in undifferentiated ovarian carcinoma. Arch Cancer Res. 2017;5:143.

31. Bahrami A, Truong LD, Ro JY. Undifferentiated tumor: True identity by immunohistochemistry. Arch Pathol Lab Med. 2008;132:326-48.

32. Goel A, Rao NM, Santhi V, Byna SS, Grandhi B, Conjeevaram J. Immunohistochemical characterization of normal ovary and common epithelial ovarian neoplasm with a monoclonal antibody to cytokeratin and vimentin. Iran J Pathol. 2018;13:23-29.

33. Hart WR. Diagnostic challenge of secondary (metastatic) ovarian tumors simulating primary endometrioid and mucinous neoplasms. Pathology International 2005;55:231-243.

34. Carter JH, Deddens JA, Mueller G, et al. Transcription factors WT1and p53 combined: a prognostic biomarker in ovarian cancer. Br J Cancer. 2018;119:462–70.

35. Liliac L, Carcangiu ML, Canevari S, et al. The value of PAX8 and WT1 molecules in ovarian cancer diagnosis. Rom J Morphol Embryol. 2013;54:17-27.

36. Mahadevappa A, Krishna SM, Vimala MG. Diagnostic and prognostic significance of Ki-67 immunohistochemical expression in surface epithelial ovarian carcinoma. J Clin Diagn Res J Clin Diagn Res. 2017;11:8-12.

37. Manu V, Hein TA, Boruah D, et al. Serous ovarian tumors: Immunohistochemical profiling as an aid to grading and understanding tumorigenesis. Med J Armed Forces India. 2020;76:30-6.

38. Vassilev LT, Vu BT, Graves B, et al. In Vivo Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2. Science. 2004;303:844–8.

39. Yamashita Y, Nagasaka T, Naiki-Ito A, et al. Napsin A is a spesific marker for ovarian clear cell adenocarcinoma. Modern Pathol. 2015;28:111-7.

40. Seidman JD, Kurman RJ, Ronnett BM. Primary and metastatic mucinous adenocarcinoma of the ovaries: incidence in routine practice with a new approch to improve intraoperative diagnosis. Am J Surg Pathol. 2003;27:985-93.