# Immunocytologic Identification of Serous and Mucinous Cystadenocarcinoma Types of Ovarian Carcinoma that Have Metastasised to Serous Fluids

## Seröz Sıvılara Metastaz Yapan Seröz ve Müsinöz Over Kistadenokarsinom Tiplerinin İmmünositolojik Tanımlaması

Rabia PİŞİRİCİLER, MD,[a]
Meral ATAY, MD,[b]
Esin ÇALIŞKAN AK, MD,[a]
Ziya ATAY, MD[b]

[a]Department of Histology and Embryology, Marmara University Faculty of Dentistry, İstanbul
[b]Cytopathology Labor, Hannover, Germany

Geliş Tarihi/*Received:* 16.09.2009
Kabul Tarihi/*Accepted:* 23.11.2009

Yazışma Adresi/*Correspondence:*
Rabia PİŞİRİCİLER, MD
Marmara University Faculty of Dentistry, Department of Basic Science, İstanbul, TÜRKİYE/TURKEY
rpisiriciler@marmara.edu.tr

**ABSTRACT Objective:** Most cases of neoplastic deaths in females are due to ovarian carcinoma. Ovarian carcinomas are generally primary tumors and they cause plevral, peritoneal and pericardial effusion after metastasis. Subclassification of ovarian carcinoma is important both biologically and therapeutically. We aimed to demonstrate the specificity of several reactives in the serous fluids in detection of serous and mucinous metastatic ovarian adenocarcinomas. **Material and Methods:** Eight hundred fifty serous fluid specimens admitted to Hannover Cytopathology Laboratory with suspected ovarian carcinoma metastasis were stained with May Grünwald-Giemsa and 127 of them were diagnosed as metastatic carcinoma. In order to detect the primary site, alkaline phosphatase (ALP), cancer antigen 125 (CA 125) and cancer antigen 15-3 (CA 15-3) reactives were applied to these 127 serous fluids and 116 of them were diagnosed as ovarian carcinoma metastasis. For differentiation of the serous and the mucinous types, alcian blue (AB), vimentin (VIM) and cytokeratin 20 (CK20) reactives were applied to these samples. **Results:** AB and CK20 positive reactivities were found to be more significant in mucinous cystadenocarcinomas than in serous cystadenocarcinomas (93 vs. 7% for AB and 95.5 vs. 4.5% for CK20, respectively $p< 0.001$). However, VIM was found to reflect negative reactivity in mucinous cystadenocarcinomas (92.3%) comparing with serous cystadenocarcinomas (7.7 %) ($p< 0.001$). **Conclusion:** Positive AB and CK20 reactivity has a higher predictive value for detection of mucinous cystadenocarcinomas in patients with serous fluid metastasis, whereas negative VIM reactivity may have similar importance for cytological differentiation of serous ovarian cystadenocarcinomas.

**Key Words:** Ovarian carcinoma, adenocarcinoma, pleura-ascites-pericardial fluid, serous-mucinous identification, immunocytochemistry

**ÖZET Amaç:** Kadınlarda kanserden ölümle sonuçlanan olguların çoğu over karsinom kaynaklıdır. Over karsinomlar genellikle primer orijinlidir ve metastaz sonrası plevra, periton ve perikardiyum boşluklarında sıvı birikimine neden olur. Over karsinomların alt sınıflandırılmasının hem biyolojik hem de terapötik açıdan önemli olduğu gösterilmiştir. Bu çalışmada seröz sıvılara metastaz yapan seröz ve müsinöz over kistadenokarsinomların tanımlanmasında kullanılan bazı reaktiflerin özgünlüğü ve duyarlılığını göstermeyi amaçladık. **Gereç ve Yöntemler:** Laboratuvara over karsinom metastaz şüphesi ile gönderilen 850 seröz sıvı örneği May Grünwald-Giemsa ile boyandı ve 127 tanesine metastatik karsinom tanısı konuldu. Primer odağı saptamak amacıyla alkalen fosfataz (ALP), Kanser antijeni 125 (CA 125) ve kanser antijeni 15-3 (CA 15-3) reaktifleri 127 seröz sıvıya uygulandı ve 116 tanesine over karsinom metastazı tanısı kondu. Seröz ve müsinöz tiplerin ayırımı için bu örneklere "Alcian" mavisi (AB), vimentin (VIM) ve sitokeratin 20 (CK20) reaktifleri uygulandı. **Bulgular:** AB ve CK20 pozitivitesi seröz kistadenokarsinomlara kıyasla müsinöz kistadenokarsinomlarda daha anlamlı bulundu. (AB için sırasıyla %93'e karşı %7, CK20 için %95.5'e karşı %4.5; $p <0.001$) bulundu. Bununla birlikte VIM'in seröz kistadenokarsinom (%7.7) ile karşılaştırıldığında ($p <0.001$), müsinöz kistadenokarsinomda negatif reaktiviteyi (%92.3) yansıttığı bulundu. **Sonuç:** Pozitif AB ve CK20 reaktivitesi, seröz sıvı metastazı olan hastalarda müsinöz kistadenokarsinom tespiti için yüksek prediktif değerlere sahiptir. Bununla birlikte negatif VIM reaktivitesi, seröz over kistadenokarsinomların sitolojik diferansiyasyonu için benzer öneme sahip olabilir.

**Anahtar Kelimeler:** Over karsinomu, adenokarsinom, plevra-asit-perikardiyal sıvı, seröz-müsinöz tanımlama, immünositokimya



**Turkiye Klinikleri J Med Sci 2009;29(6):1435-40**

Ovarian carcinoma is the most lethal gynecological malignancy and 90% of them originate from the surface epithelium of ovarian gland. These neoplasms are classified into distinct morphologic categories based on the appearance of the epithelium: serous, mucinous, endometrioid, clear cell, transitional, squamous, mixed or undifferentiated types.[1-3] The morphology-based prognostic factors of the ovarian carcinoma are the histological subtype, tumour grading and perhaps the receptor status. A correct histological classification with consideration of all morphology related prognostic factors is necessary for oncologists to choose the optimal individual therapeutic option.[4]

Ovarian carcinomas are generally of primary origin and cause fluid accumulation in the pleura, peritoneum and pericardium. In the last 20 years, immune-stains applied with various antibodies in serous fluids became very important because they increased the diagnostic accuracy of differentiation and type detection of metastatic carcinomas of unknown primary site.[5-7]

The numerical diversity of tumours due to cellular and morphological diversity of the ovary results in confusion, and leads to difficulties in diagnosis. Since both primary and metastatic ovarian carcinomas mostly display the same ultrastructural structure as that of adenocarcinomas, in differential diagnosis, tumour markers, cytochemistry and immunocytochemical reactives facilitate the diagnosis.[8-10] Numerous immunocytochemical reactives improve the chances of type detection, early diagnosis, treatment and cure.[6,8-12]

In the literature, even though there are several cytological markers that have been proposed for differentiation of serous and mucinous types of ovarian carcinoma, none of them has been proven to be superior to each other currently. Herein, therefore, we intended to demonstrate the specificity of AB, ALP, CA 125, CA 15-3, VIM and CK20 reactives in detection of serous and mucinous ovarian adenocarcinomas that were metastasized to serous fluids.

## MATERIAL AND METHODS

The study materials were 850 serous fluids sent to the Hannover Cytopathology Laboratory in Germany between November 2005 and May 2006 with suspected ovarian carcinoma metastasis. This study is a part of routine clinical screening procedure for patients suspected of ovarian carcinoma. For this reason, ethical approval was not included. The age of the subjects ranged between 40 and 94 years. The fluids admitted to the laboratory were centrifuged for 10 minutes at 1500 rpm and 8-12 smear preparations were prepared from each material and fixed by air drying. Serous and mucinous ovarian cystadenocarcinomas were identified by three steps: 1. For morphological assessment, two of these preparations were stained with May Grünwald-Giemsa method. According to morphological features, serous fluids were classified into five stages. Stage IV and V (127 serous fluids) were accepted as a metastatic carcinoma and these fluids also clinically diagnosed as metastatic ovarian carcinoma. 2. In order to detect the primary site of the metastatic carcinoma ALP, CA 125 and CA15-3 were applied to 127 serous fluids with metastatic carcinoma (stage IV and V) and 116 of them were diagnosed as ovarian carcinoma. 3. For serous and mucinous type detection of the 116 serous fluids diagnosed with ovarian carcinoma, VIM, CK 20 and AB were separately applied to the other preparations. In order to attain the degree of positivity in serous and mucinous cystadenocarcinomas, VIM, CK 20, AB, ALP, CA 125 and CA15-3 were used. The reactives applied to all of the fluids diagnosed as ovarian adenocarcinoma could not be assessed in some of the smear preparations due to the lack of enough material or tumour cells.

Primary antibodies (CA125, CA15-3, VIM, CK20) were visualized with detection DAKO LSAB 2 System using peroxidase labelled streptavidin biotin secondary detection kit (code no K0675; Dako, Glastrup, Denmark). After each application the preparations were washed with Tris-Buffered Saline. They were then stained with the opposite stain Hemalaum and covered with glycerine.[13]

Pearson chi-square and Fisher's exact methods were used to compare of serous and mucinous positive results.

## RESULTS

According to morphological feature, 850 serous fluids are classified into five stages. Distribution of these samples according to stage I-II, stage III, stage IV and stage V were 690, 33, 9, and 118, respectively. Of the 127 serous fluid samples with carcinoma in stage IV and V, 95 were pleural fluids (75%), 29 were ascites (23%) and 3 were pericardial fluids (2%) (Table 1).

The primary sites of a total of 127 serous fluids with stage IV and V carcinomas were evaluated as ovarian in 116, endometrial in 5 and ovarian metastatic adenocarcinoma in 6 samples with the reactives (ALP, CA 125 and CA15-3) applied (data not shown).

In the morphological identification of 116 serous fluids diagnosed as ovarian carcinomas, 83 the of had characteristics mucinous and 33 had serous cystadenocarcinoma. Mucinous and serous cystadenocarcinomas were grouped according to their degree of positivities. While in mucinous cystadenocarcinomas AB, VIM and CK20 were found to be 97, 33 and 82% positive, respectively, in serous cystadenocarcinomas AB, VIM and CK20 were found to be 29, 84 and 13% positive, respectively (Figure 1 C, D) (Table 2). There were no differences in ALP, CA125 and CA15-3 reactives between serous and mucinous cystadenocarcinomas samples (Figure 1 A, B and E) (Table 2).

When compared to serous cystadenocarcinomas, AB and CK20 positive reactivity were found to be more significant in mucinous cystadenocarcinomas (7 vs. 93% for AB and 4.5 vs. 95.5% for

**TABLE 1:** The distribution of 850 serous fluids according to stages.[1]

| Serous fluids | Stage I-II | Stage III | Stage IV | Stage V | Total |
|---|---|---|---|---|---|
| Pleura | 561 | 20 | 9 | 86 | 676 |
| Ascites | 119 | 6 | - | 29 | 154 |
| Pericardium | 10 | 7 | - | 3 | 20 |
| **Total** | **690** | **33** | **9** | **118** | **850** |

[1]Stage I-II: Normal and inflammation, Stage III : Suspected, Stage IV: Strongly suggesting malignancy Stage V: Definitely malignant.

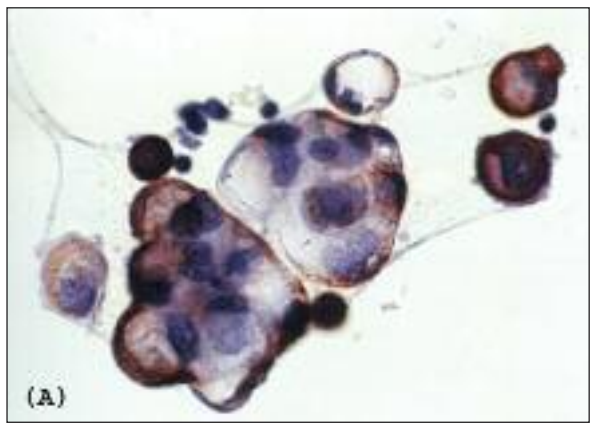


**FIGURE 1A:** Diffuse cytoplasmic and membranous positivity in mucinous cystadenocarcinomas (CA125 x 600).

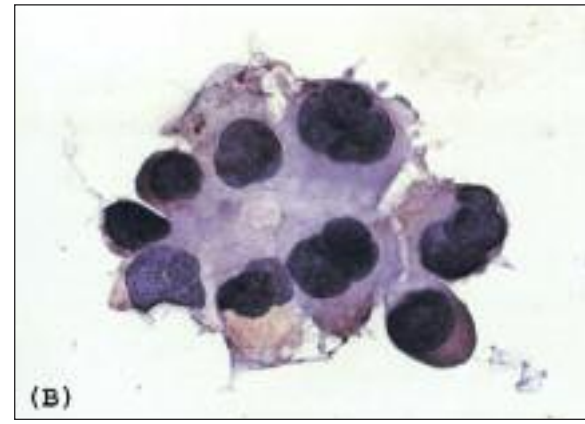


**FIGURE 1B:** Cytoplasmic reaction and more than one nucleus in serous cystadenocarcinomas (CA15-3 x 600).

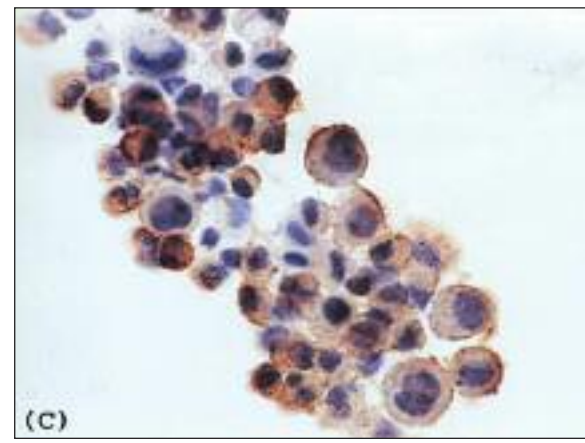


**FIGURE 1C:** Intensive cytoplasmic reaction in serous papillary cystadenocarcinomas. Some cells have two nuclei (VIM x 600).

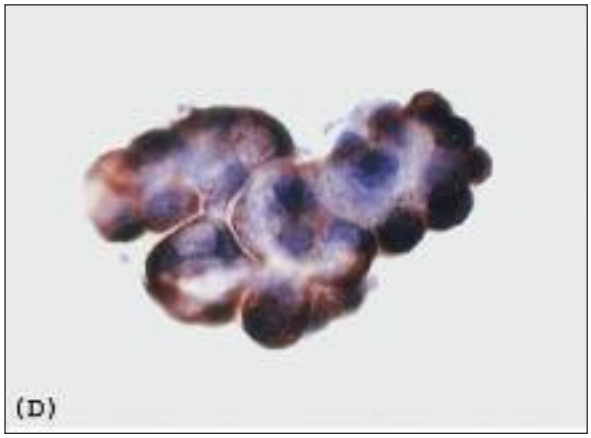


**FIGURE 1D:** Diffuse CK20 positivity in mucinous cystadenocarcinomas (CK20 x 600).

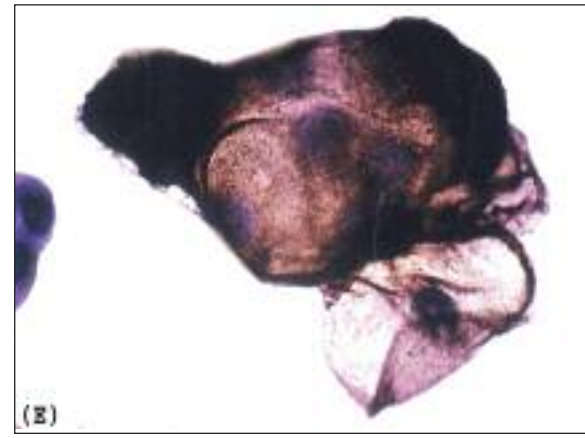


**FIGURE 1E:** Diffuse ALP positivity in ovarian adenocarcinomas (ALP x 600).

CK20, p< 0.001). However, VIM was more negative in mucinous cystadenocarcinomas than the serous ones (92.3 vs. 7.7%, p< 0.001; Table 3).

## DISCUSSION

Since primary and metastatic ovarian carcinomas display the same structure as the adenocarcinoma, tumour markers and immunological reactives are being used in the diagnosis and tumour type detection.[5, 8,12,14] CA 125 positivity has been investigated without distinguishing between serous and mucinous types, and the positivity rates were reported to vary from 28.6 to 96%.[5,6,8,11,15,16] CA 125 was previously thought to be specific to ovarian carcinomas in numerous studies however it is positive in all epithelial ovarian tumours and normal structures originating from breast, gastrointestinal system, thyroid, kidney and even coelomic epithelium; but it is more frequent in serous ovarian tumours.[5, 6, 14] In our study CA125 positivity was 95% in mucinous carcinomas and 93% in serous carcinomas.

Similarly, CA 15-3 is mostly specific for breast cancers in women.[7, 10] CA 15-3 can also be positive in ovarian, lung and prostate tumours. It is positive in non-cancerous conditions (benign breast, ovarian disease, endometriosis, pelvic inflammation and hepatitis), as well. It has been reported that

**TABLE 2:** The degrees of positivity in mucinous and serous cystadenocarcinomas.

| | | 0 | 1+ | 2+ | 3+ | Total positive |
|---|---|---|---|---|---|---|
| | Reactive | n (%) | n (%) | n (%) | n (%) | n (%) |
| | AB | 2 | 18 (26) | 35 (51) | 13 (19) | 66 (97) |
| Mucinous | ALP | 11 | 9 (11) | 30 (37) | 31 (38) | 70 (86) |
| Cystadenocarcinoma | CA 125 | 3 | 9 (11) | 38 (46) | 32 (39) | 79 (96) |
| | CA 15-3 | 19 | 18 (27) | 25 (38) | 14 (2) | 57 (75) |
| | VIM | 48 | 15 (21) | 7 (9) | 2 (3) | 24 (33) |
| | CK20 | 14 | 15 (19) | 37 (48) | 11 (14) | 63 (82) |
| | AB | 11 | 2 (13) | 3 (16) | - | 5 (29) |
| | ALP | 3 | 3 (10) | 21 (68) | 4 (13) | 28 (9) |
| Serous | CA 125 | 2 | 6 (19) | 15 (48) | 8 (26) | 29 (93) |
| Cystadenocarcinoma | CA 15-3 | 5 | 4 (14) | 14 (50) | 5 (18) | 23 (82) |
| | VIM | 4 | 7 (28) | 9 (36) | 5 (20) | 21 (84) |
| | CK20 | 21 | 1 (4) | 2 (8) | - | 3 (13) |

**TABLE 3:** The comparison of positivity of reactives in serous and mucinous cystadenocarcinomas.

| | Marker[1] | Serous | Mucinous | Significance[2] |
|---|---|---|---|---|
| AB | 0 | 84.6% | 15.4% | |
| | 1 | 7% | 93.0% | * |
| ALP | 0 | 21.4% | 78.6% | |
| | 1 | 28.6% | 71.4% | NS |
| CA 125 | 0 | 40.0% | 60.0% | |
| | 1 | 26.9% | 73.1% | NS |
| CA 15-3 | 0 | 20.8% | 79.2% | |
| | 1 | 28.8% | 71.3% | NS |
| VIM | 0 | 7.7% | 92.3% | |
| | 1 | 46.7% | 53.3% | * |
| CK 20 | 0 | 60.0% | 40.0% | |
| | 1 | 4.5% | 95.5% | * |

[1]0: negative; 1: positive (+1, +2, +3).
[2] NS: nonsignificant, *: p <0.0001.

it can also be positive in pregnancy and lactation. CA 15-3 is always positive in clear cell carcinomas of the adrenal gland and it is always negative in primary adrenocortical neoplasms, which is significant in diagnosis. CA 15-3 antibody is used to detect the primary site of the metastatic carcinoma in serous fluids in our study. CA 15-3 positivity was reported to be 75% in mucinous carcinomas and 82% in serous carcinomas.[10]

In recent years the importance of vimentin and cytokeratin has been emphasised in tumour differentiation.[7,9-12,16-18] VIM and CK20 are cytoplasmic and intermediary filament proteins constituting an important portion of the cellular skeleton. VIM is also expressed in mesenchymal cells, lymphoma and extra-muscular sarcomas. In various studies, VIM positivity has been reported be 30-31% in non-mucinous ovarian carcinomas, 59% in the ones without serous mucinous differentiation and 17% in mucinous ovarian carcinomas.[10,18,19] In our study, VIM was more positive in serous carcinomas (84%) than in mucinous carcinomas (32%).

CK 20 is essentially a Type I acidic cytokeratin found in urothelium and Merkel cells in gastric and intestinal epithelium. It is also expressed in colon, stomach, pancreas, ovarian tumours and transitional and Merkel cell carcinomas.[7,9,16,17,19-21] Squamous cell carcinomas, breast, lung and endometrial adenocarcinomas, non-mucinous ovarian tumours and small cell carcinomas do not express CK 20.[18,20,22] Although non-mucinous ovarian tumours do not express CK 20, the CK20 expression rate of mucinous ovarian tumours has been reported to vary from 0 to 69%.[10,11,17,19,20,22] Cytokeratin is useful in the detection of tumours origins provided that they are collected in adequate numbers in mucinous tumours.[23] In these studies the number of cases ranges between 14-77. In this study, a higher number of cases with CK20 positivity was found in mucinous carcinomas when compared to serous carcinomas (82 vs 13%).

AB positivity is specific in mucinous cystadenocarcinomas.[24-30] AB positivity (97%) in the present study is in agreement with literature. ALP positivity is specific in the differential diagnosis of adenocarcinoma.[24] ALP is negative in epidermoid carcinoma. Since poorly differentiated adenocarcinomas are morphologically similar to poorly differentiated epidermoid carcinomas, ALP is used in the differentiation of these two tumours.[12,24,31]

In the literature research we noted that various immunocytochemical panels have been applied for the detection of the primary sites of adenocarcinomas that metastasised to serous fluids, however we did not encounter any studies aiming to cytologically identify serous and mucinous ovarian cystadenocarcinomas. In conclusion, for the detection of the type of adenocarcinomas (serous and mucinous) metastasised to serous fluids, use of positive CK20 and AB reactivities and negative VIM reactivity, in addition to conventional cytochemical and immunocytochemicals, can be considered.

## REFERENCES


1. Bell DA. Origins and molecular pathology of ovarian cancer. Mod Pathol 2005;18 (Suppl 2):S19-32.
2. Christie M, Oehler MK. Molecular pathology of epithelial ovarian cancer. J Br Menopause Soc 2006;12(2):57-63.
3. Güneş N, Yılmaz F, Uzunlar AE .[Grading of the ovarian serous cystadencarcinomas, and its correlation with BRCA1 and P53 immunoreactivity]. Turkiye Klinikleri J Med Sci 2007;27(3):335-43.
4. Horn LC, Fricke K, Krugmann J. [Histologic classification and morphologic prognostic factors in malignant ovarian tumors]. Zentralbl Gynakol 1995;117(7):335-45.
5. Pomjanski N, Grote HJ, Doganay P, Schmiemann V, Buckstegge B, Böcking A. Immunocytochemical identification of carcinomas of unknown primary in serous effusions. Diagn Cytopathol 2005;33(5):309-15.
6. Cuijpers VM, Boerman OC, Salet van de Pol MR, Vooijs GP, Poels LG, Ramaekers FC. Immunocytochemical detection of ovarian carcinoma cells in serous effusions. Acta Cytol 1993;37(3):272-9.
7. Bedrossian CW. Special stains, the old and the new: the impact of immunocytochemistry in effusion cytology. Diagn Cytopathol 1998;18(2):141-9.
8. Longatto Filho A, Alves VA, Kanamura CT, Nonogaki S, Bortolan J, Lombardo V, et al. Identification of the primary site of metastatic adenocarcinoma in serous effusions. Value of an immunocytochemical panel added to the clinical arsenal. Acta Cytol 2002;46(4):651-8.
9. Bedrossian CW. Diagnostic problems in serous effusions. Diagn Cytopathol 1998; 19(2):131-7.
10. Kaufmann O, Fietze E, Dietel M. [Immunohistochemical diagnosis in cancer metastasis of unknown primary tumor]. Pathologe 2002; 23(3):183-97.
11. Lagendijk JH, Mullink H, van Diest PJ, Meijer GA, Meijer CJ. Immunohistochemical differentiation between primary adenocarcinomas of the ovary and ovarian metastases of colonic and breast origin. Comparison between a statistical and an intuitive approach. J Clin Pathol 1999;52(4):283-90.
12. Pisiriciler R, Yerci O, Topalidis T, Atay Z. [Cytochemical studies for determining the histogenesis of anaplastic and poorly differentiated lung tumors]. Pneumologie 1994;48(9):718-20.
13. Battmann A, Pollex U, Schäffer R. [The diagnostic value of the immunochemistry in evaluation of pleural cancers]. Verh Dtsch Ges Path 1997;81:636.
14. Welander CE. What do CA 125 and other antigens tell us about ovarian cancer biology? Acta Obstet Gynecol Scand Suppl 1992; 155:85-93.
15. Yang W, Zhang T, Fan J. [The role of CA125 in the differential diagnosis of primary ovarian carcinoma and metastatic ovarian carcinoma originated from the gastrointestinal tract] Zhonghua Fu Chan Ke Za Zhi 2001;36(5):302-3.
16. McCluggage WG. Recent advances in immunohistochemistry in the diagnosis of ovarian neoplasms. J Clin Pathol 2000;53(5): 327-34.
17. Azumi N, Battifora H. The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms. A comprehensive immunohistochemical study on formalin- and alcohol-fixed tumors. Am J Clin Pathol 1987;88(3):286-96.
18. Lagendijk JH, Mullink H, Van Diest PJ, Meijer GA, Meijer CJ. Tracing the origin of adenocarcinomas with unknown primary using immunohistochemistry: differential diagnosis between colonic and ovarian carcinomas as primary sites. Hum Pathol 1998;29(5):491-7.
19. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000;13(9):962-72.
20. Ji H, Isacson C, Seidman JD, Kurman RJ, Ronnett BM. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. Int J Gynecol Pathol 2002;21(4):391-400.
21. Moll R, Löwe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol 1992; 140(2):427-47.
22. Vang R, Gown AM, Barry TS, Wheeler DT, Yemelyanova A, Seidman JD, et al. Cytokeratins 7 and 20 in primary and secondary mucinous tumors of the ovary: analysis of coordinate immunohistochemical expression profiles and staining distribution in 179 cases. Am J Surg Pathol 2006;30(9):1130-9.
23. Cathro HP, Stoler MH. Expression of cytokeratins 7 and 20 in ovarian neoplasia. Am J Clin Pathol 2002;117(6):944-51.
24. Atay Z, Topalidis T. [Cyto and immunocytochemistry methods]. Cytodiagnostik der Serösen Höhlen. Atlas und Lehrbuch. 1st ed. Berlin: Wolfgang Pabst Verlag; 1994. p.354-7.
25. Sakayori M, Nozawa S, Udagawa Y, Chin K, Lee SG, Sakuma T, et al. [Biological properties of two newly established cell lines (RMUG-S, RMUG-L) from a human ovarian mucinous cystadenocarcinoma] Hum Cell. 1990;3(1):52-6.
26. Baker PM, Oliva E, Young RH, Talerman A, Scully RE. Ovarian mucinous carcinoids including some with a carcinomatous component: a report of 17 cases. Am J Surg Pathol 2001;25(5):557-68.
27. Wan Q, Xu D, Li Z. [Establishment and characterization of a cell line derived from human ovarian mucinous cystadenocarcinoma] Zhonghua Fu Chan Ke Za Zhi 2001;36(7):421-3.
28. Karseladze AI. [Morphology of borderline mucinous ovarian tumors] Arkh Patol 1989; 51(5):40-6.
29. Aalto ML. Mucosubstances in classification of serous and mucinous ovarian tumors: a morphometrical study. Eur J Obstet Gynecol Reprod Biol 1986;22(3):139-44.
30. Kidera Y, Yoshimura T, Ohkuma Y, Iwasaka T, Sugimori H. [Establishment and characterization of a cell line derived from mucinous cystadenocarcinoma of human ovary]. Nippon Sanka Fujinka Gakkai Zasshi 1985;37(9): 1820-4.
31. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984;32(2):219-29.