Tepecik Eğit. ve Araşt. Hast. Dergisi 2015; 25(1):49-54
doi:10.5222/terh.2015.049

Klinik Araştırma

Diagnostic value of cytokeratin 7/20 expression in primary and metastatic liver tumors

Primer ve metastatik karaciğer tümörlerinde sitokeratin 7/20 ekspresyonunun tanısal değeri

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ABSTRACT

Objective: Analyzing the expression of cytokeratin 7 and 20 plays an important role in the discrimination between primary and metastatic liver tumors. The cytokeratin 7/20 phenotype of tumors is one of the most frequently used criterion for differential diagnosis.
Methods: In 72 liver specimens (59 resection material and 13 core needle biopsies) evidence of intrahepatic cholangiocarcinoma (n=25), colorectal adenocarcinoma metastases (n=28), 13 pancreaticobiliary adenocarcinoma (n=13), gastric adenocarcinoma metastases (n=4), lung adenocarcinoma metastasis, (n=1), and breast carcinoma metastasis ((n=1) were investigated In all cases location of the primary focus was determined. Immunohistochemical analysis was performed with two monoclonal cytokeratin antibodies, cytokeratin 20 and cytokeratin 7 which were tested in 72 tumor sections.
Results: The positivity rate and intensity of immunostaining were evaluated. Cytokeratin 7(+)/20(-) phenotype was seen in 76% (19/ 25) of the cases with intrahepatic cholangiocarcinoma with 89% specificity, 76% sensitivity and 79% positive predictive value. The cytokeratin 7(+)/20(+) phenotype was detected in 69% (9/13) of the cases with pancreaticobiliary adenocarcinomas with 76.3% specificity, 69.2% sensitivity, and 39.1% positive predictive value. The cytokeratin 7(-)/20(+) phenotype was observed in 82% (23/28) of the cases with colorectal adenocarcinoma metastases with 97.7% specificity, 82.1% sensitivity, and 95.8% positive predictive value.
Conclusion: In conclusion, our study shows that cytokeratin 7/20 phenotype has diagnostic value in the differential diagnosis of primary and metastatic liver tumors.

Key words: Cytokeratin 7, cytokeratin 20, liver, metastases

ÖZET

Amaç: Primer ve metastatik karaciğer tümörlerinin tanısında sitokeratin 7 ve 20 ekspresyon analizinin önemli rolü vardır. Tümörlerde sitokeratin 7/20 fenotipi ayırıcı tanılarda en sık kullanılanlardan biridir.
Yöntemler: Yirmi beş intrahepatik kolanjiokarsinom, 28 kolorektal adenokarsinom metastazı, 13 pankreatikobilier adenokarsinom, 4 gastrik adenokarsinom metastazı, 1 akciğer adenokarsinom metastazı, 1 meme karsinom metastazını içeren 72 karaciğer materyali (59 rezeksiyon materyali ve 13 iğne biyopsi) araştırıldı. Tüm olgularda primer tümör lokalizasyonu belirlendi. İmmunohistokimyasal çalışma 72 tümörlü kesitte sitokeratin 7 ve 20 monoklonal antikorları ile yapıldı.
Bulgular: İmmun pozitiflik oranı ve boyanma yoğunluğu değerlendirildi. Sitokeratin 7(+)/20(-) fenotipi intrahepatik kolanjiokarsinom olgularının %76'sında (19/25), %76 duyarlılık, %89 seçicilik ve %79 pozitif tahmin değerleri ile saptandı. Sitokeratin 7(+)/20(+) fenotipi 13 pankreatikobilier adenokarsinomun 9'unda (%69) saptandı. Seçicilik %76,3, duyarlılık %96,2 ve pozitif tahmin değeri %39,1 olarak bulundu. Sitokeratin 7(-)/20(+) fenotipi ise kolorektal adenokarsinom metastazı olgularının %82'sinde (23/28) saptandı (seçicilik %97,7, duyarlılık %82,1, pozitif tahmin değeri %95,8).
Sonuç: Sonuç olarak çalışmamız, sitokeratin 7/20 fenotipinin primer ve metastatik karaciğer tümörlerinin ayırıcı tanısında önemli yeri olduğunu göstermektedir.

Anahtar kelimeler: Sitokeratin 7, sitokeratin 20, karaciğer, metastaz

Alındığı tarih: 19.03.2015
Kabul tarihi: 07.04.2015

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INTRODUCTION

The liver is a very common site for metastatic tumors. Hepatic metastases frequently originate from primary tumors of the lungs, breast and gastrointestinal tract [1]. Colorectal carcinoma metastases (CRM) are the most commonly seen metastases in the gastrointestinal tract [1,2]. The origin of the primary tumor is frequently unknown at initial presentation. However, exact identification of the primary origin of the metastasis has prognostic and therapeutic value [3,4]. Differential diagnosis of the metastases to the liver encounters difficulties because of the similarities in the histological appearance of the adenocarcinomas. As there is considerable histologic overlap between adenocarcinomas from different organs, immunohistochemical phenotyping of metastatic adenocarcinomas in the liver and other organs is mandatory [4,5]. There have been some conspicious successes with site-specific antibodies such as prostate specific antigen [6] and thyroglobulin [7] but most antibodies are not proven to be site-specific like carcinoembryonic antigen [8].

The development of monoclonal antibodies specific to individual cytokeratin (CK) molecules has begun to help to distinguish epithelial tumors that originate from different organs [9,10]. There are 20 subtypes of CKs according to their molecular weight and isoelectric pH [9,10]. CK7 is typically found in the epithelium of gastrointestinal tract, including gall bladder [11], hepatic ducts [12], and pancreatic ducts [13], female genital tract, breast, urinary tract, and lung [11]. CK20 is found in epithelium of gastrointestinal tract (incl. gastric and intestinal mucosa), genitourinary tract (urothelium), squamous epithelium of any anatomical site, and Merkel cells [10,14]. These CKs usually retain their tissue specificity in their neoplastic counterparts. Coordinated expression of these two CKs has been found to be relevant in identifying the site of origin of various metastatic carcinomas [15,16].

In the liver, administration of CK7 and CK20 to the primary and metastatic carcinomas can be widely applied for differential diagnosis [10,15-18]. CK7(-)/CK20(+) phenotype of the liver metastasis indicates with a 78% probability that the primary site of the tumor is colon or rectum, while CK7(+)/CK20(+) phenotype is associated with a 74% probability of pancreaticobiliary origin of the metastasis [19]. Cholangiocarcinomas (CC) often show CK7(+)/CK20(-) phenotype but CK20 positivity can be seen occasionally and its differential diagnosis can be difficult.

Therefore, we carried out this study to assess CK7 and CK20 expression in primary and metastatic liver adenocarcinomas and also to test the significance of these immunostaining in the discrimination between these two entities.

MATERIAL and METHODS

We evaluated 72 specimens of liver tumors (59 surgical specimens and 13 needle biopsies) consisting of 28 colorectal adenocarcinomas, 25 cholangiocarcinomas (CC), 13 pancreaticobiliary carcinomas (7 pancreatic adenocarcinomas, 6 extrahepatic CC), 4 gastric adenocarcinomas, 1 lung adenocarcinoma and 1 breast carcinoma. Primary tumor localizations could be established in all tumors based on clinical (medical history and follow-up), radiological, histological and laboratory data.

All needle biopsies and the most representative paraffin blocks of the surgical specimens were stained for CK7 and CK20. Five-micrometer-thick sections from parafin-embedded tissues were laid on positively charged slides and deparaffinized in xylene and rehydrated. Immunostaining was performed using an automated immunohistochemical stainer according to the manufacturer's guidelines (IVIEWTM DAB, BenchMarkXP, Ventana, USA). Protease digestion for CK20 (Biogenex, KS20.8, diluted 1/200) and microwaving for CK7 (Neomarkers, OV-TL 12/30, dilution: 1/200) were performed before incubation with the primary monoclonal antibodies. The reaction product was developed using 3,3-diaminobenzidine tetrahydrochloride (DAB) and sections were counterstained with hematoxylin.

Bile duct sections prepared from the non-tumorous part of the liver tissue, and also colonic epitelium served as positive controls for CK7 and CK20, respectively.

Evaluation of immunostaining and statistics

The staining intensity of CK7 and 20 expressions was semiquantitatively graded as 0 (negative or less than 10% of the tumor cells), 1+ (positivity between 10%-50% of tumor cells), 2+ (positivity between 50%-75% of tumor cells), and 3+ (more than 75% of tumor cells).

The positivity rate and intensity of immunostaining defined in different groups of tumors were compared using Fisher's exact test. The diagnostic values of each possible CK7/CK20 phenotypes were analyzed according to their specificity, sensitivity and positive predictive value.

RESULTS

The median age of the patients was 59 years (range, 41-78 years) including 44 (61.1%) male and 28 (38.9%) female patients.

The intensity of CK7 and CK20 expression and CK7/CK20 phenotype in primary and metastatic adenocarcinomas are summarized in Tables 1 and 2.

CK7 was expressed in 100% (25/25) of intrahepatic CC preparations with a cytoplasmic pattern, and strong staining ntensity. Pancreaticobiliary adenocarcinoma preparations also showed 100% (13/13) strong positivity and intensity with CK 7. The proportion of CK20 positivity in CC was 24% (6 /25) with a low staining intensity while this ratio rised to 69% (9/13) in pancreaticobiliary adenocarcinomas with variable staining intensities (24% vs 69%; $p<0.001$, Fisher's exact test).

CK20 was positive in all CRM (100%, 28 of 28) preparations mostly with high staining intensity. CK7 immunostaining was seen only in 5 cases (18%) with a low intensity.

CK7(+)/CK20(-) phenotype

This phenotype was present in 76% of intrahepatic CC, 31% of pancreaticobiliary carcinomas and 100% of breast carcinoma. CK7(+)/CK20(-) pheno-

Table 1. The intensity of CK7 and 20 expression in the primary and metastatic adenocarcinomas.

CK7	Colorectal n	IntrahepaticCC n	Pancreaticobiliary n	Gastric n	Breast n	Lung n
0	23	0	0	2	0	0
(+)	5	0	0	0	0	0
(++)	0	10	2	0	0	1
(+++)	0	15	11	2	1	0
Total positivity	5 (18%)	25 (100%)	13 (100%)	2 (50%)	1 (100%)	1 (100%)
CK20	**n**	**n**	**n**	**n**	**n**	**n**
0	0	19	4	2	0	0
(+)	0	5	1	1	0	0
(++)	11	1	4	0	0	1
(+++)	17	0	4	1	0	0
Total positivity	28 (100%)	6 (24%)	9 (69%)	2 (50%)	0	1 (100%)

Table 2. Distribution of carcinomas according to CK7/CK20 phenotype.

	CK7(+)/CK20(-)	CK7(+)/CK20(+)	CK7(-)/CK20(+)	CK7(-)/CK20(-)	Total
Colorectal	0	5 (18%)	23 (82%)	0	28
IntrahepaticCC	19 (76%)	6 (24%)	0	0	25
Pancreaticobiliary	4 (31%)	9 (69%)	0	0	13
Gastric	0	2 (50%)	1 (25%)	1 (25%)	4
Breast	1 (100%)	0	0	0	1
Lung	0	1 (100%)	0	0	1
Total	24 (33%)	23 (32%)	24 (33%)	1 (2%)	72

type had a 89% specificity, 76% sensitivity and 79% positive predictive value for intrahepatic CC (Figure 1,2). In pancreaticobiliary adenocarcinomas. While its specificity, positive predictive value and sensitivity were 66, 16, and, 30.7%, respectively .

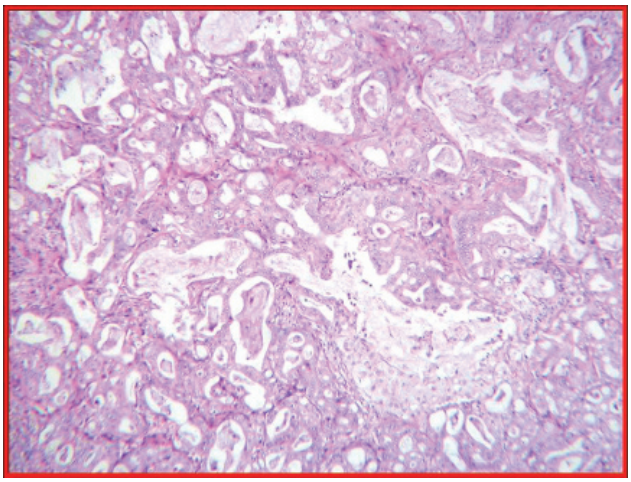

Figure 1. Metastatic extrahepatic cholangiocarcinoma in liver (H&Ex40).

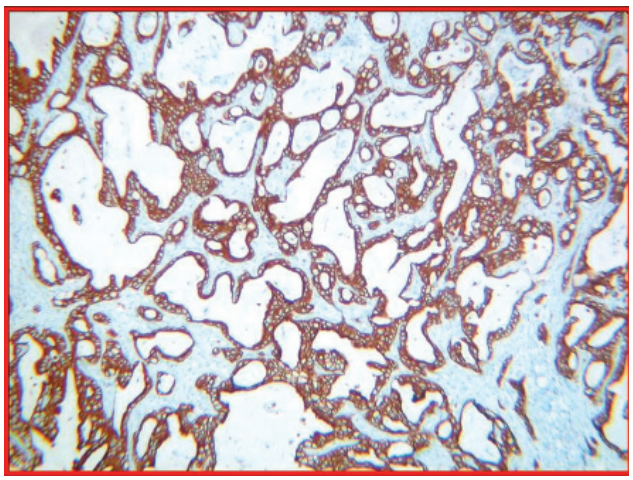

Figure 2. CK7 positivity in metastatic extrahepatic cholangiocarcinoma (x40).

CK7(+)/CK20(+) phenotype

Five CRM (18%), 6 intrahepatic CC (24%), 9 pancreaticobiliary carcinoma (69%), 2 gastric adenocarcinoma (50%), and one lung adenocarcinoma (100%) specimens were included in this group. The most relevant diagnostic value of this phenotype was seen in pancreaticobiliary adenocarcinomas (76.3% specificity, 69.2% sensitivity, and 39.1% positive predictive value) (Figure 3-5).

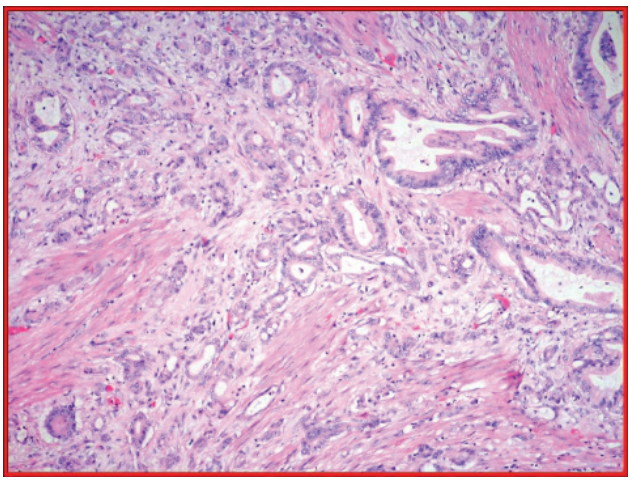

Figure 3. Metastatic pancreatic adenocarcinoma in liver (H&Ex100).

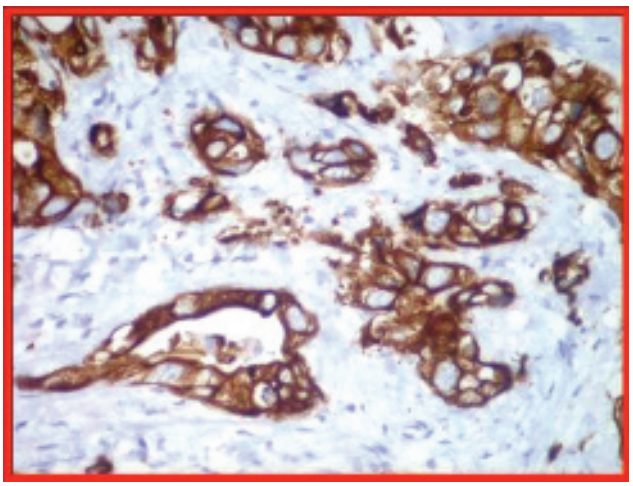

Figure 4. CK7 positivity in metastatic pancreatic adenocarcinoma (x200).

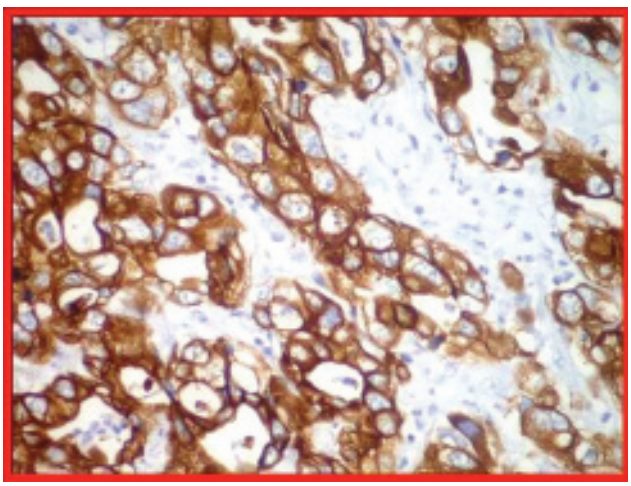

Figure 5. CK20 positivity in metastatic pancreatic adenocarcinoma (x400).

CK7(-)/CK20(+) phenotype

Most of the CRM specimens exhibited CK7(-)/CK20(+) phenotype (82%, 23 of 28) (Figure 6, 7). A single case of gastric adenocarcinoma exhibited this phenotype (25%, 1 of 4 cases). The CK7(-)/CK20(+) phenotype had a superior diagnostic value in detecting CRM (97.7% specificity, 82.1% sensitivity, and 95.8% positive predictive value).

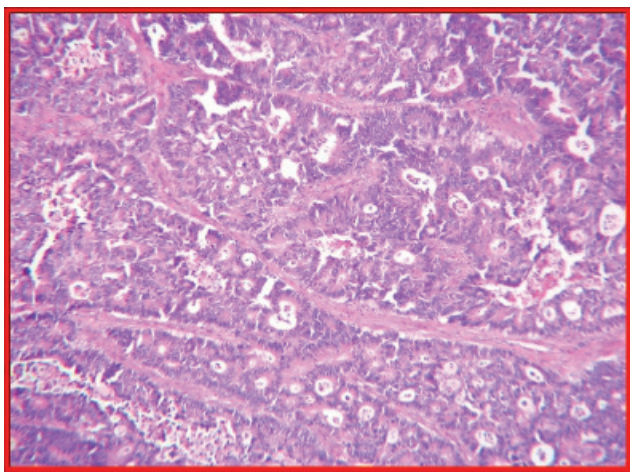

Figure 6. Metastatic colorectal carcinoma in liver (H&Ex100).

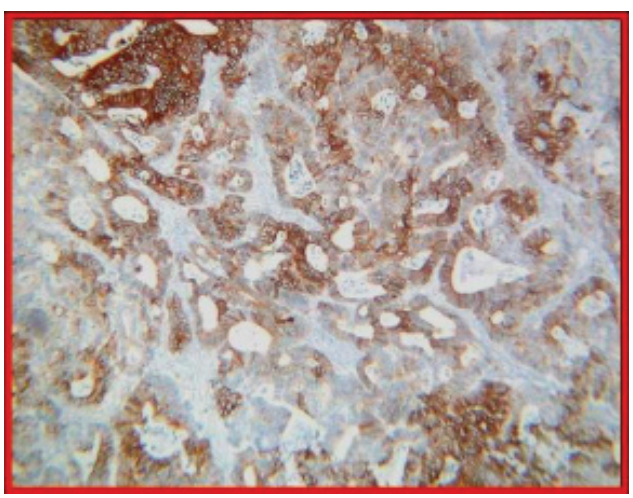

Figure 7. CK20 positivity in metastatic colorectal carcinoma (x200).

DISCUSSION

As many as 60% of all cases of metastatic adenocarcinomas are of unknown origin at the time of initial clinical presentation. Liver is one of the most common site for metastatic tumors and the differential diagnosis is a main problem for pathologists because of the similarities in their histological characteristics [15-18]. Immunohistochemical phenotyping of metastatic tumors in the liver has become a routine procedure in many centers. Use of CK7/CK20 seems to be a very useful profile in determining CRM because of the specificity of CK7(-)/CK20(+) phenotype [9,15-21]. On the other hand, CC, and primary liver adenocarcinomas, often display CK7(+)/CK20(-) phenotype. Rullier et al. [20], showed that CK7(+)/CK20(-) phenotype is specific for CC (100% specificity) whatever their location in the biliary tree. CC tends to show CK20 positivity when the location shifts towards extrahepatic bile ducts [20].

In our study, we showed that intrahepatic CC is always CK7(+) with a strong immunostaining intensity in keeping with published literature [9,15-22]. CK20 positivity in CC was low (24%, 6/25) and the immunostaining intensity was weak. These data were comparable with the outcomes of series published by Maeda (40%) [17] but differed from other studies in which higher CK20 positivity was detected [10,20].

In pancreaticobiliary adenocarcinomas, we saw 100% strong positivity and staining intensity with CK7. The rate of CK20 positivity was 69%. This ratio was statistically high when compared with intrahepatic CC (24% vs 69%; $p<0.001$, Fisher's exact test). The CK7(+)/CK20(+) phenotype was analyzed in 69% (9/13) of the cases with 76.3% specificity, 69.2% sensitivity, and 39.1% positive predictive value in our study. The frequency of CK7(+)/CK20(+) metastatic pancreaticobiliary adenocarcinomas was also 69% in the series of Tot et al. [21]. This ratio was higher in their other study (74%) [19]. Wang et al. [16] reported that 65% of the cases with pancreaticobiliary carcinomas showed this phenotype. On the other hand, some reports have indicated very low CK20 positivity or negativity in pancreaticobiliary carcinomas [23]. Our pancreaticobiliary subgroup included extrahepatic CC and pancreas adenocarcinomas, and exhibited a similar distribution of CK phenotype.

In the CRM subgroup, results were as expected: CK20 positivity was seen in all cases of CRM (100%, 28/28) mostly with high staining intensity. Only 5

cases (18%) showed CK7 positivity with a low staining intensity. However, we decided that application of CK20 alone was not adequate for the differential diagnosis between CRM and pancreaticobiliary adenocarcinomas. The CK7(-)/CK20(+) phenotype had a superior diagnostic value in detecting CRM (97.7% specificity, 82.1% sensitivity, and 95.8% positive predictive value).

The frequency of CK7 expression was very high in our study group except CRM. Intrahepatic CC, pancreaticobiliary adenocarcinoma, lung and breast adenocarcinoma specimens showed 100% and gastric adenocarcinomas displayed 50% positivity with CK7. However, the number of metastatic gastric, breast and lung adenocarcinoma specimens was very low in our series so that it would be inaccurate to discuss the CK7/CK20 phenotype.

In conclusion, our study confirms that combined application of CK7 and 20 has a diagnostic value in the determination of primary and metastatic liver adenocarcinomas. We observed that CK7(+)/CK20(-) phenotype with high intensity indicates the diagnosis of intrahepatic CC, CK7(+)/CK20(+) phenotype favors the diagnosis of pancreaticobiliary adenocarcinomas. The most relevant and specific immunohistochemical parameter is CK7(-)/CK20(+) phenotype. This parameter is highly accurate for the determination of colorectal origin.

Conflict of interest statement - We declare that we have no conflict of interest.

REFERENCES

1. Craig JR, Peters RL, Edmonson HA. Atlas of tumor pathology, 2nd series, fascicle 26. Washington DC. Armed Forces Institute of Pathology 1989.
2. Melato M, Laurino L, Mucli E, et al. Relationship between cirrhosis, liver cancer and hepatic metastases: an autopsy study. *Cancer* 1989;64:455-459. http://dx.doi.org/10.1002/1097-0142(19890715)64:2<455::AID-CNCR2820640219>3.0.CO;2-C
3. Altman E, Camdan E. An analysis of 1539 patients with cancer of unknown primary site. *Cancer* 1986;57:120-124. http://dx.doi.org/10.1002/1097-0142(19860101)57:1<120::AID-CNCR2820570124>3.0.CO;2-M
4. Ma CK, Zarbo RJ, Frierson HF Jr, et al. Comparative immunohistochemical study of primary and metastatic carcinoma of the liver. *Am J Clin Pathol* 1993;99:551-557.
5. Brown RW, Campagna LB, Dunn JK, et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. *Am J Clin Pathol* 1997;107:12-19.
6. Nadji M, Tabei SZ, Castro A, et al. Prostatic origin of tumors: an immunohistochemical study. *Am J Clin Pathol* 1980;73:735-739.
7. De Micco C, Ruf J, Carayon P, et al. Immunohistochemical study of thyroglobulin in thyroid carcinomas with monoclonal antibodies. *Cancer* 1987;59:471-476. http://dx.doi.org/10.1002/1097-0142(19870201)59:3<471::AID-CNCR2820590320>3.0.CO;2-S
8. Fenoglio CM, Crum CP, Pascal RR, et al. Carcinoembryonic antigen in gynecologic patients. II. Immunohistological expression. *Diagn Gynecol Obstet* 1981;3:291-299.
9. Moll R, Franke WW, Schiller DL, et al. The catalog of the human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982;31:11-24. http://dx.doi.org/10.1016/0092-8674(82)90400-7
10. Moll R, Löwe A, Laufer J, et al. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992;140:427-447.
11. Bartek J, Vojtesek B, Staskova Z, et al. A series of 14 new monoclonal antibodies to keratins: characterization and value in diagnostic histopathology. *J Pathol* 1991;164:215-224. http://dx.doi.org/10.1002/path.1711640306
12. Van Eyken P, Sciot R, Callea F, et al. The development of the intrahepatic bile ducts in man: a keratin-immunohistochemical study. *Hepatology* 1988;8:1586-1595. http://dx.doi.org/10.1002/hep.1840080619
13. Kasper M, von Dorsche H, Stosiek P. Changes in the distribution of intermediate filament proteins and collagen IV in fetal and adult human pancreas. *Histochemistry* 1991;96:271-277. http://dx.doi.org/10.1007/BF00271547
14. Berezowski K, Stastny JF, Kornstein MJ. Cytokeratins 7 and 20 and carcinoembryonic antigen in ovarian and colonic carcinoma. *Mod Pathol* 1996;9: 426-429.
15. Leroy X, Copin MC, Boman F, et al. Cytokeratins 7 and 20: aid in tumor typing. *Ann Pathol* 1998;18:103-109.
16. Wang NP, Zee S, Zarbo RJ, et al. Coordinate expression of cytokeratins 7 and 20 defines unique subsets of carcinomas. *Appl Immunohistochem* 1995;3:99-107.
17. Maeda T, Kajiyama K, Adachi E, et al. The expression of cytokeratins 7, 19 and 20 in primary and metastatic carcinomas of the liver. *Mod Pathol* 1996;9:901-909.
18. Choi JE, Noh SJ, Lee JH, et al. Expression of keratin 20 and its clinicopathological significance in intrahepatic cholangiocarcinoma. *Oncol Lett* 2012;4(3):534-540.
19. Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. *Cancer* 1999;85:171-177. http://dx.doi.org/10.1002/(SICI)1097-0142(19990101)85:1<171::AID-CNCR24>3.0.CO;2-V
20. Rullier A, Le Bail B, Fawaz R, et al. Cytokeratin 7 and 20 expression in cholangiocarcinomas varies along the biliary tract but still differs from that in colorectal carcinoma metastasis. *Am J of Surg Pathol* 2000;24:870-876. http://dx.doi.org/10.1097/00000478-200006000-00014
21. Tot T, Samii S. The clinical relevance of cytokeratin phenotyping in needle biopsy of liver metastasis. *APMIS* 2003;111:1075-1082. http://dx.doi.org/10.1111/j.1600-0463.2003.apm1111201.x
22. Nanashima A, Tobinaga S, Araki M, et al. Intraductal papillary growth of liver metastasis originating from colon carcinoma in the bile duct: report of a case. *Surg Today* 2011;41(2):276-280. http://dx.doi.org/10.1007/s00595-009-4235-y
23. Duval JV, Savas L, Banner BF. Expression of Cytokeratins 7 and 20 in Carcinomas of the Extrahepatic Biliary Tract, Pancreas, and Gallbladder. *Arch Pathol Lab Med* 2000;124:1196-1200.