Iraqi Journal of Cancer and Medical Genetics

Diagnostic value of immunohistochemical panel (Cytokeratin CK 7, Cytokeratin CK20, High molecular weight cytokeratin HMWCK (clone CK34βE12) and Prostatic specific antigen (PSA) in differentiation between poorly differentiated prostatic and urothelial carcinoma

Ikram A. Hasan, Hiba A. Gaidan, Methaq M. Al-kaabi

Mustansiriyah University / College of Medicine Pathology Department

Abstract:

Background: Prostatic adenocarcinoma may spread to bladder or vice versa, this is because of the anatomical proximity of these two organs. The differentiation between these two tumors is critical for therapeutic and prognostic implication.
Aim of study: Evaluate the usefulness of a panel of immunohistochemical markers (CK7, CK20, HMWCK34 βE12 and PSA) in differentiation between challenging cases of high grade urothelial and poorly differentiated prostatic carcinoma with morphological overlapping.
Material and methods: A total of 40 cases (20 cases poorly differentiated prostatic adenocarcinoma and 20 cases high grade urothelial carcinoma) were collected from archive of teaching laboratory of Al Yarmouk teaching hospital and private laboratories for the period from January 2015 to July 2017.All formalin fixed paraffin embedded tissue block were stained immunohistochemically with a panel of marker (CK7, CK20, HMWCK34 βE12 and PSA) and scoring was performed.
Results: For prostatic adenocarcinoma, 17 out 20 (85%) were positive for PSA, while only two cases (10%) of urothelial carcinoma cases showed weak and focal staining for this marker (the pvalue was <0.0001). In contrast, 16 out of 20(80%) of the urothelial carcinoma cases were positive for CK34βE12 in comparison to only one case (5%)of prostatic carcinoma showed positive expression for this marker (the p value was highly significant <0.0001).
Regarding CK7and CK20: combined expression of both markers was noticed in 17 cases (85%) of urothelial carcinoma compared to only 2 cases(10%) of prostatic adenocarcinoma and the difference was highly significant(p value <0.0001). negative expression for both markers was noticed in 18 cases (90%) of prostatic adenocarcinoma compared to only 2 cases (10%) of urothelial carcinoma and the difference was highly significant(p value < 0.0001).CK7 positivity alone was noted in 17 cases(85%) of urothelial carcinoma while only 2 cases (10%) of prostatic carcinoma show positivity for this marker and the p value was highly significant (p value < 0.0001).18 cases (90%) of urothelial carcinoma showed positive expression for CK20 alone compared to only to 3 cases (15%) of prostatic carcinoma (p value < 0.0001).
Conclusion: Using CK7 or CK20 alone will not be helpful for differentiation between prostatic carcinoma and high grade urothelial carcinoma, while combined expression of both markers(CK7 and CK20) is very useful in ruling out carcinoma of the prostate, since, it is very rare for both markers to show positive expression in prostatic carcinoma.In difficult cases that show negative expression of both marker, CK34βE12 remain the most valuable marker for urothelial origin while, PSA immunohistochemical marker remains the most helpful marker to prove the prostatic origin of metastatic carcinoma.

Key words: *high grade urothelial carcinoma, poorly differentiated prostatic carcinoma, CK7, CK20, CK34βE12, PSA immunohistochemistry*

Corresponding Address:
Ikram A. Hasan
Mustansiriyah University / College of Medicine Pathology Department
Email: ikram.alhadithi@uomustansiriyah.edu.iq

Introduction:

"Poorly differentiated urothelial carcinoma and prostatic carcinoma may share similar clinical and morphological features. Differentiation between well differentiated variants of prostatic adenocarcinoma and urothelial carcinoma may be easy, but the morphological features alone may be not sufficient to differentiate between the poorly differentiated forms of these two tumors"(1). "This differentiation is quite important because it has staging and prognostic implication" (2).

"Moreover, distinction between these two entities is important be¬cause the treatment for urothelial carcinoma is different from that of invasive prostatic adenocarcinoma (3). Advanced urothelial carcinoma is generally treated with chemo¬therapy whereas; advanced prostatic adenocarcinoma is often treated with anti-an¬drogen hormone therapy"(3). "So in morphologically difficult cases, immunohistochemical stains are necessary to establish the diagnosis (1)."Involvement of prostate by urothelial carcinoma can occur from direct invasion of urothelial carcinoma into the stroma of prostate or from intraductal extension of urothelial carcinoma with or without subsequent invasion of prostatic stroma "(4).

"The involvement of the urinary bladder by prostate adenocarcinoma as direct extension or by metastases is the second most common origin of bladder cancer and occurs in 12% of all secondary bladder tumors "(5)

"To date, no marker is sufficiently specific and/or sensitive to determine the urothelial or prostatic origin of poorly differentiated carcinoma" (1).So using a immunohistochemical panel is very useful for this purpose.

"Cytokeratin CK7, CK20 and high molecular weight cytokeratin, (HMWCK 34(clone βE12) have been used as potential urothelial marker although they are not specific entirely for urothelial carcinoma" (6)

"Cytokeratin 7 (CK7): Cytokeratins are intermediate filament proteins present in different epithelial cells. They are expressed in normal organs and the tumors that arise from them in a tissue-specific manner. It is usually negative in prostatic carcinoma and positive in urothelial tumors" (7).

"Cytokeratin 20 (CK20) belongs to cytoskeleton associated with intermediate filaments, cytokeratin 20 is specifically expressed in superficial and in some intermediate cells of normal urothelium but its expression beyond these limits may suggest progression to urothelial carcinoma"(8)

"Cytokeratin 34βE12 is a High Molecular Weight cytokeratin that reacts with all squamous and ductal epithelium and stains carcinomas. This antibody recognizes cytokeratins 1, 5, 10, and 14 that are found in complex epithelia.Also called CK903, high molecular weight keratin "(9)

"Immunohistochemical expression of PSA is used widely to help in the diagnosis of prostatic carcinoma that metastasize to other organs"(10).

"Prostate-specific antigen (PSA) is a 33-kDa serine protease that is secreted by prostatic epithelium and non-prostate tissues, such as epithelial lining of the periurethral and perianal glands " (11).

The aim of this study is to evaluate the usefulness of apanel of immunohistochemical markers (CK7, CK20, HMWCK34 βE12 and prostatic specific antigen PSA) in differentiation between challenging cases of high grade urothelial and poorly differentiated prostatic carcinoma with morphological overlapping.

Materials and methods:

Atwenty cases of prostatic biopsy (chips) diagnosed as poorly differentiated prostatic carcinoma (all of the cases were high grade Gleason).Twenty cases of transurethral resection (TURT) and radical cystectomy diagnosed as high grade urothelial carcinoma .these cases were collected from archive of teaching laboratory of Al Yarmouk teaching hospital and private laboratories for the period from January 2015 to July 2017.

Hematoxilin and eosin (H&E) slides were reviewed to verify the histological finding. Immunohistochemical stain of specific HRP/DAB (ABC) as following: Deparafinize rehydrate formalin fixed parafin embedded blocks were sectioned then enough drops of hydrogen peroxide block were added to cover the sections. Incubation of the slides for 10 minutes. Protein block had been used and the slides incubated for 10 minutes at room temperature to block nonspecific background staining. The next step was addition of primary antibody and incubate over night for CK34 be12 only, while the other three markers needs only one hour incubation of primary antibody according to manufacture's instructions (Dako). After that, application of biotinylated gout antimouse and incubation for 10 minutes. This followed by application ofStreptavidin Peroxidase and incubation for 15 minutes .Addition of DAB Chromogen to tissue with incubation for 1-10 minutes. Application of counterstain (hematoxylin) for 2 minutes and coverslips.

After each of the previous steps we wash 4 times in buffer solution.

The positive and technical negative control slides were included in each run.

Scoring of the immunohistochemical was performed by two independent pathologists according to the following criteria:

CK7 &CK20:

" Focal positive expression is considered when less than 10% of the tumor cells were stained by these, whereas equal or more than 10% staining is considered diffuse "(8)

Regarding PSA staining: Since there was no apparent difference of staining intensity, a three-category scoring system was modified from previous studies:

"High expression (++)" is considered when more than 10% of the tumor cells revealed PSA staining; while low expression (+)", is between 0% and10% immunoreactivity; and "negative (−)", no immunoreactivity for PSAwas detected"(12)

"For scoring of Ck34βE12Immunoreactivity:

"No staining (0%),

"Partial staining (<60%)",

"Diffuse staining (≥60)"

(13 -15)

Statistical analysis:

The statistical analysis was done using SPSS 24. We used Pearson Chi square (X2) test and Fisher exact test when indicated (if the number of the cases was less than 5).

Table 1: Immunohistochemical markers that used in this study

Antibody	Antigen retrieval method	Dilution of primary Antibody	manufacturer
CK34BE12	Microwave	1:500	Abcam
PSA	Microwave	1:25	DAKO
CK7	Microwave	1:50	DAKO
CK20	Microwave	1:50	DAKO

Results:

The results of staining of immunohistochemical panel are summarized in table 2.

CK 34βE12 was positive in 16 out of 20 (80%) of the urothelial carcinoma (UC) cases. The intensity of the staining was as the following: 10 cases (50%) show strong and diffuse cytoplasmic and membranous positivity, while 6 cases (30%) were partially stained.

In contrast, only one case (5%) of prostatic carcinoma shows partial positivity for this marker (table 1).

There was a highly significant difference between these two tumors (p value is <0.0001).

PSA positivity was noticed in the cytoplasm of 17 out of 20(85%) of prostatic carcinoma cases, 14 cases (70%) show high expression (diffuse and strong positivity ≥10%). While the low expression was noticed in 3 cases (15%).In contrast 18 case of (90%) urothelial carcinoma cases were negative for this marker.

Only 2 (10%) cases of urothelial carcinoma show low expression.

There was highly significant difference between these two tumors stained with this marker (p value is <0.0001).

Regarding CK7:

Eighteen cases (90%) of prostatic carcinoma were negative and only 2 cases show focal staining. While, 17 out of 20 (85%) of urothelial carcinoma cases were positive including 12 cases show diffuse and strong positivity and 5 cases were weakly and focally stained.

CK20:

Eighteen out of twenty (90%) of urothelial carcinoma cases are positive for CK 20 including 11 cases(55%) show strong and diffuse positivity and 7 cases (35%) were focally and faintly stained.

We noticed coexpression of CK7 and CK20 in 17 cases of urothelial carcinoma and 2 cases of prostatic carcinoma.

This difference between both tumors regarding coexpression of CK7/CK20 was highly significant (p value <0.0001)

Both markers were negative in 18 cases of prostatic carcinoma and 2 cases of urothelial carcinoma. These two cases were positive for CK34βE12 and negative for PSA (Figure 1).

Table 2: Immunophenotype of Poorly Differentiated Prostatic Carcinomas and High-Grade Urothelial carcinoma (cases using mmunohistochemical panel (CK7, CK20, CK34βE12 and PSA

Marker	Prostatic carcinoma	Urothelial carcinoma	P value
CK34βE12	(5%)1/20	(80%)16/20	0.0001>
0	(95%)19/20	(4l20(20%	
60%>	1/20	(30%)6/20	
60%≤	-	(50%)10/20	
PSA	(85%)17/20	(10%)2/20	0.0001>
0	(15%)3	(90%)18/20	
10%>	(15%)3	(10%)2/20	
10%≤	(55%)14	0	
CK7	(10%)2/20	(85%)17/20	0.0001>
0	18	(15%)3	
10%>	(10%)2/20	(25%)5/20	

10%≤	0	(60%)12/20	
CK20	(15%)3/20	(90%)18/20	0.0001>
0	17/20	(10%)2	
10%>	(15%)3/20	(35%)7	
10%≤	0	(55%)11	

P value < 0.05 is significant

Table 3: Immunostaining of CK-7 and CK-20 in poorly differentiated Prostatic adenocarcinoma andhigh grade urothelial Carcinomas

Marker	Prostatic adenocarcinoma	Urothelial carcinoma	P value
CK7+/CK20+	2	17	0.0001>
CK7-/CK20-	18	2	0.0001>
CK7+ ONLY	2	17	0.0001>
CK20+ONLY	3	18	0.0001>

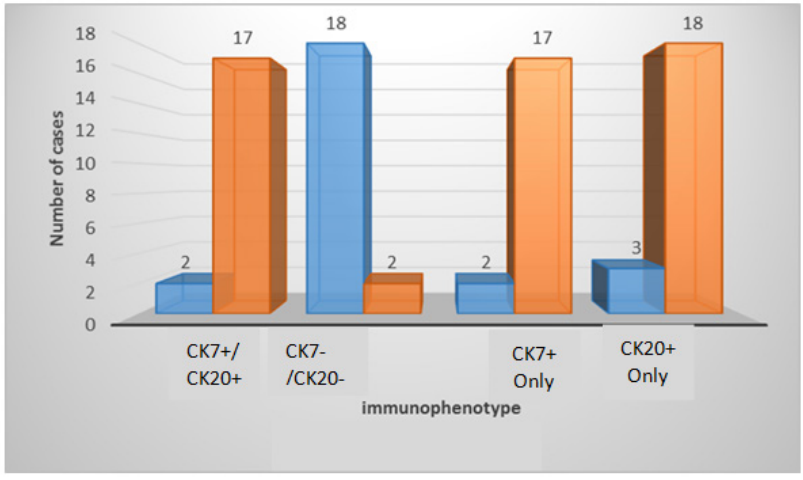

Figure 1: immunohistochemical expression of cytokeratin (CK7 and CK20) in poorly differentiated prostatic (blue bar) and urothelial carcinoma (orange bar).

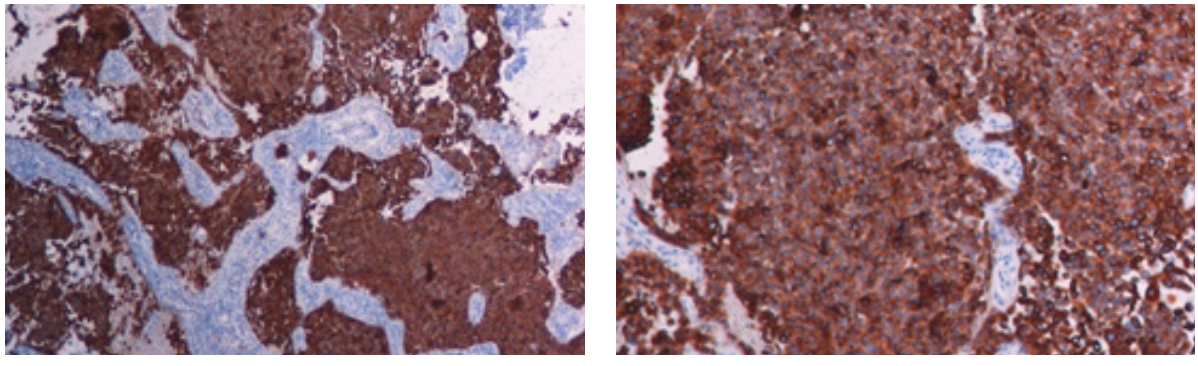

Figure 2: A photomicrograph showing positive staining of urothelial carcinoma with CK7 in low power 4X (on the left) and high power10X (on the right)

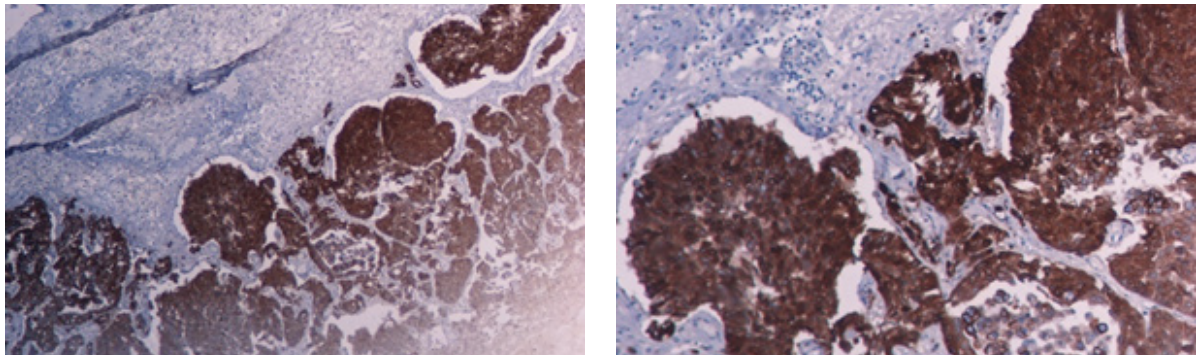

Figure 3: A photomicrograph showing positive staining of urothelial carcinoma with CK20 in low power (on the left) and high power (on the right)

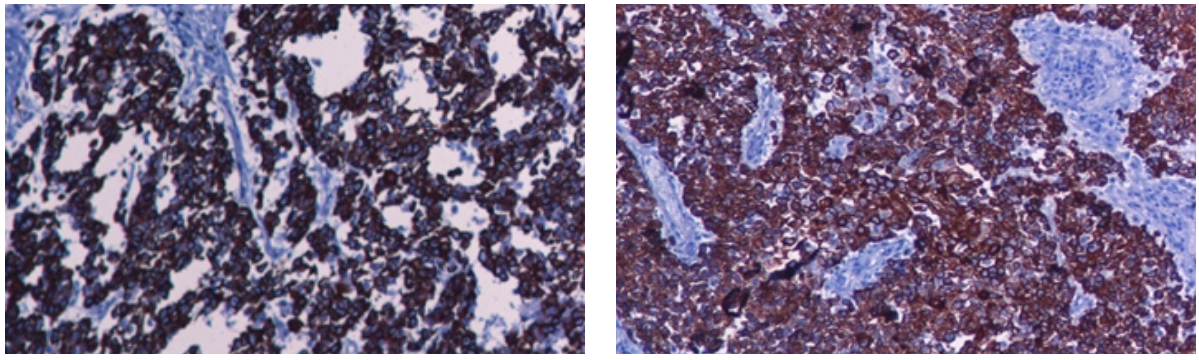

Figure 4: A photomicrograph showing positive staining of urothelial carcinoma with CK34 beta E12 in low power (on the left) and high power (on the right)

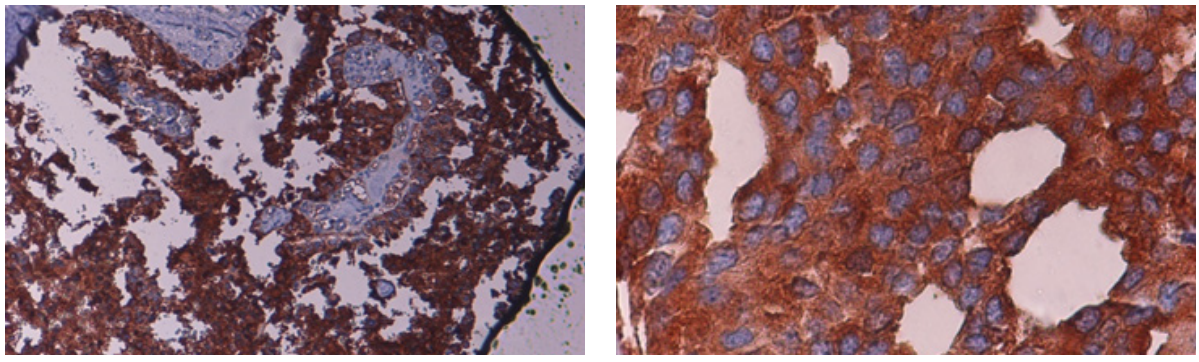

Figure 5: A photomicrograph showing positive staining of prostatic carcinoma with PSA in low power (on the left) and high power (on the right)

Discussion:

A common diagnostic dilemma that faces the pathologist is to differentiate between poorly differentiated prostatic carcinoma arising in the neck of the urinary bladder and high grade urothelial carcinoma with extension to prostate due to the overlapping morphological criteria and the similarity in the clinical manifestation in these two entities.

It is crucial to distinguish between these two tumors, because the staging, treatment and prognostic implication of infiltrating urothelial carcinoma is different from prostatic carcinoma.

Therefore, it is necessary to use confirmatory immunohistochemical markers that have the ability to distinguish between these tumors.

In this study a panel of immunohistochemical markers which include CK34βE12, PSA, CK7 and CK20 was examined.

This study shows that CK34BE12 was strong and diffusely positive in 80% of high grade urothelial carcinoma. This result is in line with previous studies like Woo et al(3) , Chuang AY et al (16) , Kunju LP et al(17) and Genega EM et al (18) who show that CK34BE12 was sensitive marker in 75.4%, 91.4%, 97% and 65.2% of cases of urothelial carcinoma respectively.

Only one case (5%) of prostatic carcinoma that show focal and weak staining for CK34βE12. This case was positive for PSA and negative for both CK7 and CK20.This result clarify the importance of PSA expression as valuable and useful marker to prove the prostatic origin of the tumors and this was in agreement with Lakshmi et al (1) (95% of prostatic carcinoma cases were positive), Woo Jin Oh et al (89.5% of prostatic carcinoma cases were positive) (3) and Ming et al (10) who shows that PSA is widely used to help in the diagnosis of metastatic prostatic carcinoma.

PSA was diffuse and strongly positive in 85% of prostatic carcinoma cases. In contrast to negative staining in 18 (90%) cases of urothelial carcinoma.

This was in agreement with other studies like Lakshmi et al(1) , Genega et al ,(18), Mhawech et al (19) and Bassily et al (20) who show positive expression of PSA in 95%, 94%, 95% &90% of cases of prostatic carcinoma respectively .

18 (90%) cases of prostatic carcinoma were negative for both CK7 and CK20, while, 17(85%) of urothelial carcinoma cases show combined expression for CK7and CK 20.

Other studies agree with this study like Lakshmi et al (1) who show that 86% of prostatic carcinoma cases were negative for both markers versus 50% of urothelial carcinoma cases were positive for both markers, Mhawech P et al (19) who show that 72% of prostatic carcinoma cases were negative for both markers versus 62% of urothelial carcinoma cases which were positive for both markers and Bassily et al (20) who show that 81% of prostatic carcinoma were negative for both markers versus 60% of urothelial carcinoma cases which were positive for both markers.

Conclusion:

Using CK7 or CK20 alone will not be helpful for differentiation between prostatic carcinoma and high grade urothelial carcinoma, while combined expression of both markers (CK7 and CK20) is very useful in ruling out carcinoma of the prostate, since, it is very rare for both markers to show positive expression in prostatic carcinoma.

In difficult cases that show negative expression of both markers CK34βE12 remain the most valuable marker for urothelial origin.

PSA immunohistochemical marker remains the most helpful marker to prove the prostatic origin of metastatic carcinoma.

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