ROLE OF CYTOKERATINS (PANCK, CK5/6, CK7, CK20) AS AN IMMUNOHISTOCHEMISTRY MARKERS IN DIAGNOSIS OF EPITHELIAL MALIGNANCY (A STUDY OF 100 CASES)

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ABSTRACT

Background:

Epithelial malignancies constitute the majority of human cancers and frequently pose diagnostic challenges, particularly in small biopsies, poorly differentiated neoplasms, and cases presenting as metastasis without a known primary site. Conventional morphology alone is often insufficient to determine epithelial lineage or predict the primary origin. Cytokeratins (CKs), a family of intermediate filament proteins expressed in epithelial cells, play a crucial diagnostic role owing to their characteristic and lineage-specific expression patterns. Pan-cytokeratin (PanCK) serves as a broad-spectrum epithelial marker, whereas CK7, CK20, and CK5/6 exhibit organ-specific and differentiation-specific profiles that help refine tumor classification. The CK7/CK20 immunophenotypic algorithm is especially valuable in metastatic adenocarcinomas and carcinoma of unknown primary (CUP).

Aim:

To evaluate the diagnostic utility and expression patterns of PanCK, CK7, CK20, and CK5/6 in 100 histologically confirmed epithelial malignancies and to assess their contribution in identifying tumor lineage and predicting the primary site, particularly in CUP and poorly differentiated tumors.

Materials and Methods:

A prospective–retrospective study was conducted on 100 epithelial malignancies diagnosed on routine H&E sections. Representative paraffin blocks underwent immunohistochemistry using primary antibodies PanCK, CK7, CK20, and CK5/6. Staining intensity, proportion of positive tumor cells, and distribution pattern were assessed. Clinical details, tumor site, and histologic subtype were correlated with CK expression. CK7/CK20 profiles were used to generate organ-specific diagnostic predictions. Special focus was placed on CUP cases to evaluate the effectiveness of CK profiles in suggesting probable primaries.

Results:

PanCK was positive in 98% of cases, confirming its high sensitivity for epithelial differentiation. CK7 was predominantly expressed in tumors of the lung, breast, ovary, endometrium, and pancreatobiliary tract, while CK20 expression was characteristic of colorectal adenocarcinoma, gastric intestinal-type carcinoma, and urothelial carcinoma. CK5/6 positivity was largely restricted to squamous cell carcinomas of the esophagus, cervix, and kidney, as well as mucoepidermoid carcinoma and a subset of urothelial carcinomas, highlighting its value in recognizing squamous or basal differentiation. The CK7/CK20 immunoprofile provided significant diagnostic clues: CK7+/CK20− strongly suggested lung, breast, female genital tract, and pancreatobiliary primaries; CK7−/CK20+ was highly specific for colorectal carcinoma; CK7+/CK20+ supported urothelial and some upper GI origins; and CK7−/CK20− favored prostate and hepatocellular carcinoma. In CUP cases, cytokeratin profiling allowed identification of a probable primary in a majority of patients, with lung being the most common inferred site.

Conclusion:

Cytokeratin immunohistochemistry using PanCK, CK7, CK20, and CK5/6 is an indispensable component of the diagnostic armamentarium for epithelial malignancies. PanCK confirms epithelial lineage, while CK7 and CK20, especially in combined profiles, provide powerful organ-specific patterns. CK5/6 reliably identifies squamous and basal differentiation. CK-based immunoprofiling significantly enhances diagnostic accuracy in challenging cases, particularly CUP, and should be integrated into routine diagnostic algorithms to guide clinical management and targeted therapy.

Keywords: Cytokeratin, PanCK, CK7, CK20, CK5/6, immunohistochemistry, epithelial malignancy, carcinoma of unknown primary

INTRODUCTION

Epithelial malignancies, or carcinomas, account for more than 80% of all cancer diagnoses worldwide. Their burden is particularly high in organs such as the lung, breast, gastrointestinal tract, hepatobiliary system, genitourinary tract and female genital tract. In routine practice, histopathology with haematoxylin and eosin staining is the cornerstone of diagnosis; however, tumours that are poorly differentiated, present as small biopsies or occur as metastases with an unknown primary site often defy precise classification on morphology alone. Cytokeratins (CKs) are intermediate filament proteins expressed in epithelial cells with organ- and cell-type–specific patterns. Tumours of epithelial origin usually retain these CK profiles, which can be exploited diagnostically. Pan-CK confirms epithelial lineage, whereas CK7 and CK20, used in combination, are powerful tools for predicting the site of origin of adenocarcinomas. CK5/6 serves as a marker of squamous or basal differentiation and is useful in identifying squamous cell carcinoma, basal-like breast carcinoma and certain salivary gland tumours. Carcinoma of unknown primary (CUP) comprises approximately 3–5% of all invasive cancers and remains a major diagnostic challenge. In these cases, cytokeratin profiling plays a central role in the immunohistochemical algorithm, guiding the use of

site-specific markers such as TTF-1, CDX2, GATA3, PSA, WT1 and PAX8. The present study was undertaken to systematically evaluate the expression patterns of Pan-CK, CK7, CK20 and CK5/6 across a spectrum of epithelial malignancies and to assess their diagnostic utility, particularly in metastatic and CUP settings.

MATERIAL AND METHODS

This prospective and retrospective study was conducted in the Department of Pathology at a tertiary care centre over a period of 24 months. A total of 100 cases of epithelial malignancies were included. Patients of all age groups and both sexes were represented. Cases were selected on the basis of histomorphological diagnosis of epithelial malignancy on routine H&E; slides. Both primary and metastatic lesions, as well as cases presenting as CUP, were included. Formalin-fixed paraffin-embedded tissue blocks were retrieved. Sections of 3–4 µm thickness were cut and stained with H&E; for confirmation of diagnosis. Immunohistochemistry was performed using a polymer-based detection system. Primary antibodies included Pan-CK, CK7, CK20 and CK5/6. Heat-induced epitope retrieval was carried out in Tris-EDTA buffer (pH ~8.4) in a pressure cooker, followed by blocking of endogenous peroxidase and protein blocking. Sections were incubated with primary antibodies for 60–120 minutes as per manufacturer's recommendations, followed by HRP-labelled secondary reagent and DAB chromogen. Slides were counterstained with haematoxylin and mounted. Cytoplasmic staining for all CK markers was considered positive. Necrotic areas, tissue folds and non-neoplastic elements were excluded. The proportion of positive tumour cells and staining intensity were assessed. Clinical details, site, histological type and grade were recorded for all cases. The CK expression patterns were correlated with tumour type and site. Descriptive statistics were used to summarise the data.

METHOD:

Immunohistochemical staining will done following standard procedures as mentioned below.

IHC PROCEDURE:

- Put slides in oven at 70 degrees centigrade for 1Hr-for deparaffinization.
- Dip in to xylene-1(15 min), xylene-2(10 min)
- Dip in absolute alcohol (5 min).
- Put in distilled water (5min)
- Antigen retrieval in pressure cooker (1 whistle)
- Take out the jar from cooker and allow it to cool
- When liquid in the jar is clear, take out the slide and dip in to distilled water -1(5 min) & distilled water -2 (5 min)
- Put the slide in to moist chamber and pour with wash buffer
- Switch off the light and pour the slide with H2O2 (5 min)
- Peroxide block-10 min (5 min)
- Wash Phosphate buffer 3 times
- Wipe the slide with tissue but

DON'T ALLOW TISSUE TO DRY

- Circle sections with paraffin marker
- Immediately apply primary antibody Ki-67- Biocare (120min)
- Wash slides with phosphate buffer 3 times and apply enhancer (10 min)
- Wash slides with phosphate buffer 3 times and apply secondary antibody for 30 min (POLYHRP1SS LABELLED)
- Wash slides with phosphate buffer 3 times and apply DAB (10 min)
- Wash slides with phosphate buffer 3 times and apply improver (3 min)
- Wash slides with phosphate buffer 3 times then apply Counter stain with Haematoxylin (1-2 min) and wash with tap water then dry.
- Put the slide in absolute alcohol (5 min)
- Dry it and dip in xylene then mount with DPX.

All immunostained sections for PanCK, CK5/6, CK7, and CK20 were examined systematically under light microscopy. Initial screening was performed at **100× magnification** to identify the **most representative and strongly immunoreactive areas** of each tumor. Detailed assessment and scoring of cytokeratin expression were subsequently carried out at **400× magnification**.

For each case, multiple high-power fields were evaluated to ensure adequate representation of the tumor. **Only viable tumor cells were included** in the assessment. **Vascular endothelial cells, lymphocytes, stromal cells, inflammatory infiltrates, and necrotic foci were excluded** from interpretation.

Cytokeratin expression was assessed based on **cytoplasmic staining pattern** and quantified as the **percentage of tumor cells showing positive immunoreactivity**. Staining intensity (mild, moderate, strong) and distribution (focal or diffuse) were recorded wherever applicable.

For interpretative purposes, immunostaining was categorized as:

- **Negative expression:** 0–9% of tumor cells showing cytokeratin positivity
- **Positive expression:** 10–100% of tumor cells showing cytokeratin positivity

This scoring approach allowed standardized evaluation of CK7, CK20, CK5/6, and PanCK expression across epithelial malignancies and facilitated comparison between tumor types and grades.

RESULTS

The mean age of patients was in the sixth decade, with the highest proportion of cases (30%) in the 51–60 year age group, followed by 22% in the 61–70 year group. Only 16% of cases occurred below 40 years of age, highlighting the predominance of epithelial malignancies in middle-aged and elderly patients. Pan-CK was diffusely positive in 98% of cases, confirming epithelial differentiation in the vast majority of tumours. Two cases with absent or focal Pan-CK expression were later confirmed as non-epithelial on extended immunohistochemistry. CK7 expression was predominantly observed in lung adenocarcinoma, breast carcinoma, ovarian serous carcinoma, endometrial carcinoma, pancreatobiliary adenocarcinoma and urothelial carcinoma. CK7 negativity was typical of colorectal adenocarcinoma, prostatic adenocarcinoma and conventional hepatocellular carcinoma. CK20 expression was characteristically positive in colorectal adenocarcinoma and a subset of gastric and urothelial carcinomas, while being negative in lung, breast, ovarian serous, pancreatic ductal, prostatic and hepatocellular carcinomas. The CK7/CK20 immunoprofiles followed well-recognised patterns: CK7+/CK20− in lung, breast, ovary and endometrium; CK7−/CK20+ in colorectal carcinoma; CK7+/CK20+ in urothelial and some upper gastrointestinal/pancreatobiliary tumours; and CK7−/CK20− in prostate and conventional hepatocellular

carcinoma. These profiles were highly informative in metastatic cases and in CUP. CK5/6 expression was strongly positive in squamous cell carcinomas of the esophagus, cervix and kidney, as well as in mucoepidermoid carcinoma. A proportion of urothelial carcinomas showed basal CK5/6 expression, reflecting their heterogeneous differentiation. Prostatic adenocarcinoma and adenoid cystic carcinoma were uniformly negative for CK5/6, supporting its role as a squamous/basal marker. In 16 cases that initially presented as CUP, a stepwise approach starting with Pan-CK followed by CK7/CK20 and CK5/6, and then organ-specific markers, allowed a probable primary site to be assigned in the majority. Lung was the most common inferred primary, followed by lower gastrointestinal tract, prostate and breast.

Table 1. Age distribution of epithelial malignancies (n=100)

Age group (years)	Number of patients	Percentage (%)
20–30	5	5
31–40	11	11
41–50	20	20
51–60	30	30
61–70	22	22
>70	12	12

Figure - Age-wise distribution of epithelial malignancies.

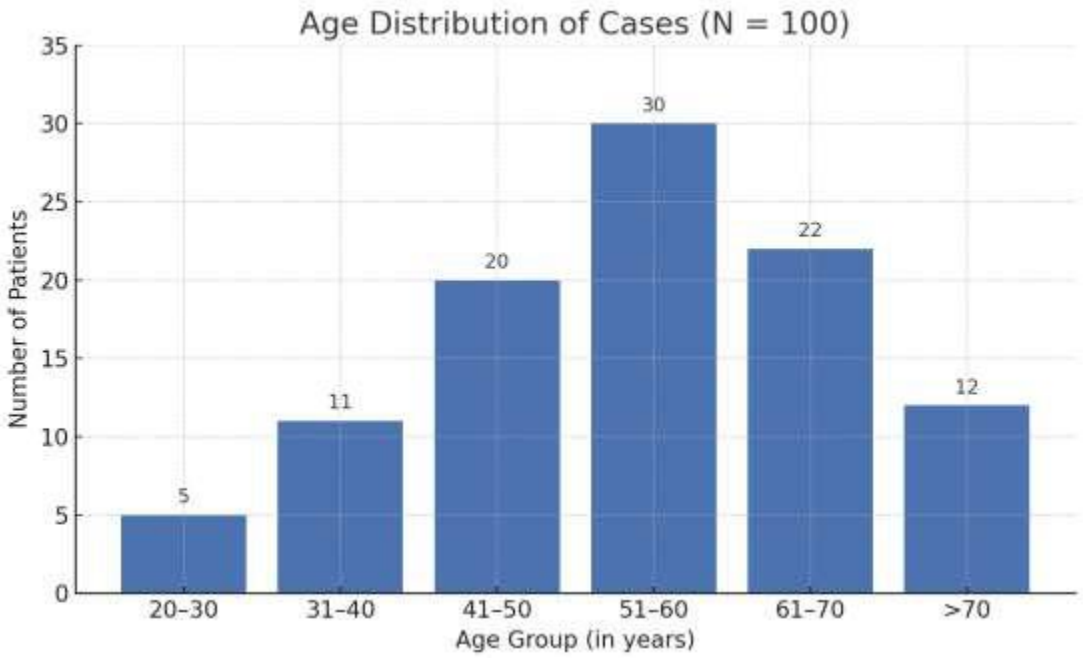


Table 2. Histopathological diagnosis distribution (n=100)

Histopathological diagnosis	Number of cases	Percentage (%)
Adenocarcinoma	82	82
Squamous Cell Carcinoma	8	8
Transitional Cell Carcinoma	5	5
Mucoepidermoid Carcinoma	5	5
Total	100	100

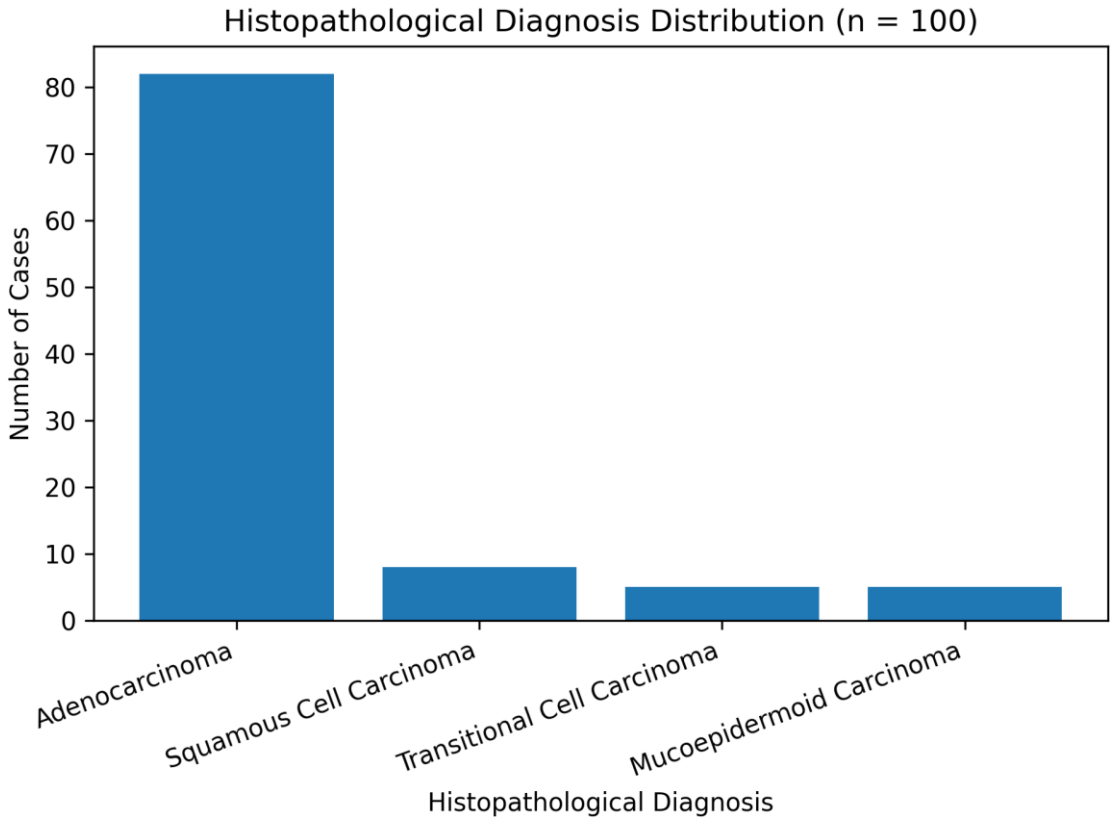


Table 3. Primary site distribution among CUP cases (n=16)

Primary site	Number of cases	Percentage (%)
Lung	7	43.75
Lower GI (Colorectal)	3	18.75
Prostate	2	12.5
Breast	2	12.5
Ovary	1	6.25
Kidney	1	6.25

Figure - Primary site distribution in carcinoma of unknown primary (CUP).

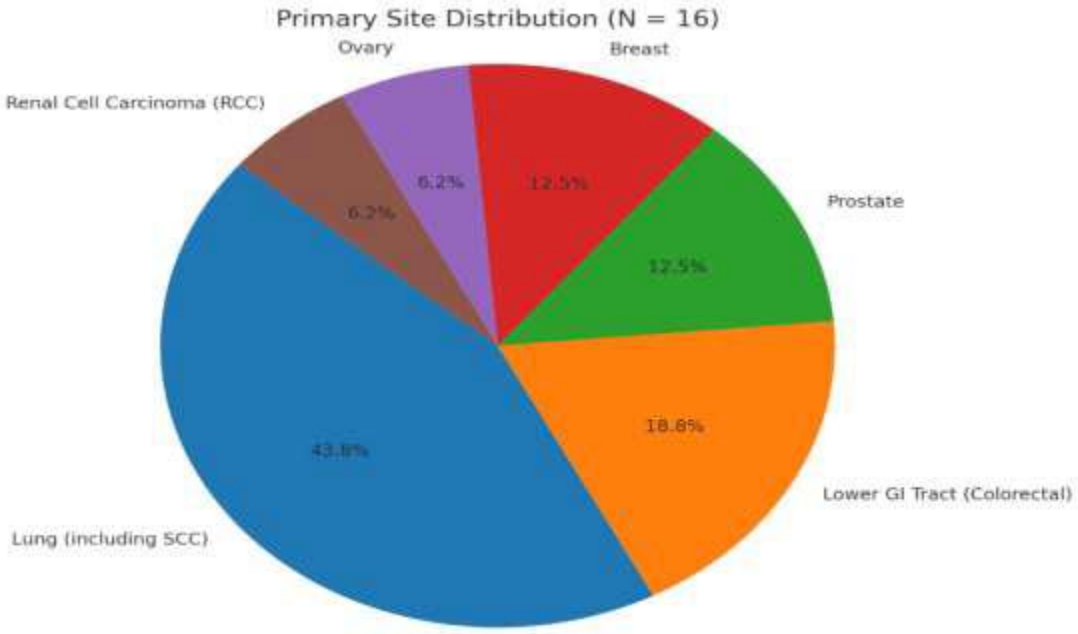
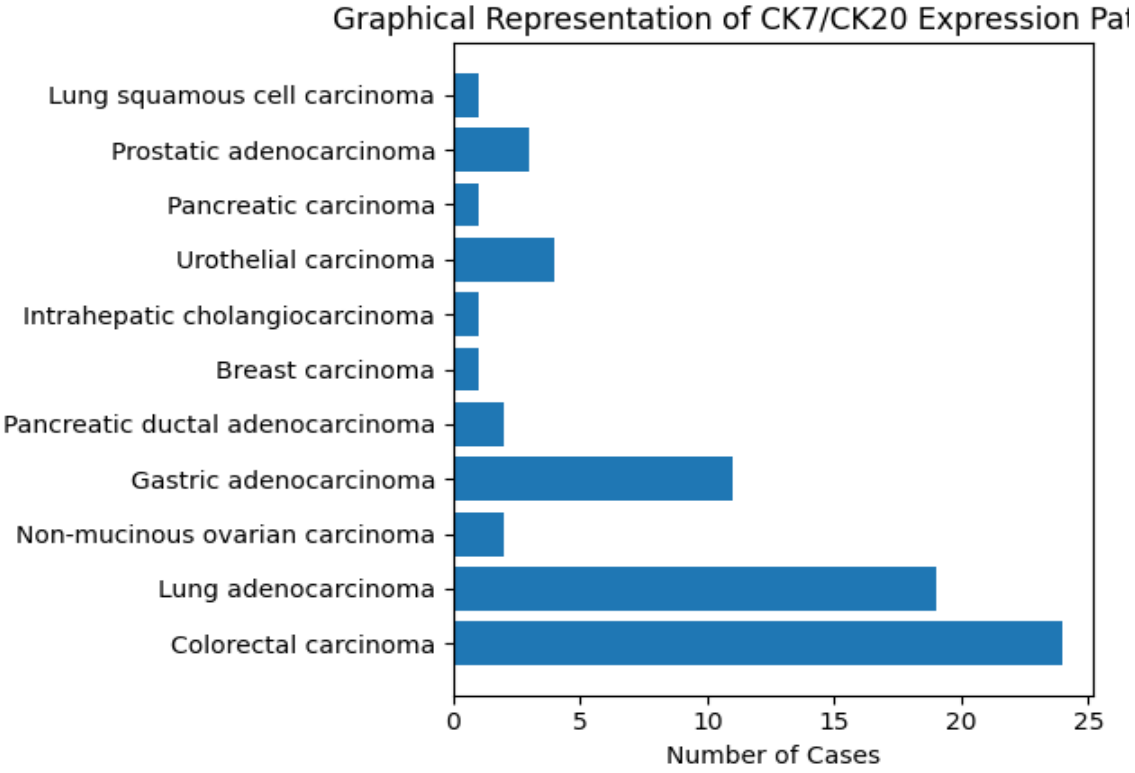


Table 4. Common expression patterns of CK-7 and CK-20

Common Expression Pattern	Tumour	Total Cases	Expressed Common Pattern
CK7− / CK20+	Colorectal carcinoma	24	24
CK7+ / CK20−	Lung adenocarcinoma	20	19
CK7+ / CK20−	Non-mucinous ovarian carcinoma	2	2
CK7+ / CK20−	Gastric adenocarcinoma	11	11
CK7+ / CK20−	Pancreatic ductal adenocarcinoma	2	2
CK7+ / CK20−	Breast carcinoma	2	1
CK7+ / CK20−	Intrahepatic cholangiocarcinoma	1	1
CK7+ / CK20+	Urothelial carcinoma	5	4
CK7+ / CK20+	Pancreatic carcinoma	3	1
CK7− / CK20−	Prostatic adenocarcinoma	5	3
CK7− / CK20−	Lung squamous cell carcinoma	1	1



DISCUSSION

The present study confirms that cytokeratin immunohistochemistry is indispensable in the diagnostic workup of epithelial malignancies. The age distribution in our cohort, with a peak in the sixth decade and a predominance of middle-aged and older adults, mirrors global cancer statistics and underscores the cumulative nature of carcinogenesis. Pan-CK, as expected, showed high sensitivity for epithelial differentiation and remains the ideal screening marker when carcinoma is suspected.

However, it lacked specificity for tumour type or site, necessitating the use of more discriminatory markers such as CK7, CK20 and CK5/6 in combination with organ-specific markers. The CK7/CK20 profile proved to be one of the most powerful diagnostic tools. The CK7−/CK20+ pattern was highly specific for colorectal carcinoma, whereas the

CK7+/CK20− profile strongly favoured primaries from the lung, breast, ovary and endometrium. The CK7+/CK20+ pattern was helpful in suggesting urothelial or pancreatobiliary origin, particularly when combined with markers such as GATA3, CA19-9 and CDX2. The CK7−/CK20− pattern was typical of prostatic adenocarcinoma and conventional hepatocellular carcinoma and could be further substantiated with PSA, NKX3.1 and HepPar-1/arginase-1 respectively. CK5/6 emerged as an excellent marker of squamous and basal differentiation. Its strong expression in esophageal, cervical and renal squamous cell carcinomas, in addition to mucoepidermoid carcinoma, is in keeping with the literature and supports its use in distinguishing squamous carcinoma and basal-like neoplasms from gland-forming adenocarcinomas. Its absence in prostatic adenocarcinoma and adenoid cystic carcinoma further reinforces its discriminatory role. In CUP cases, the integration of CK7/CK20 profiling with CK5/6 and a limited panel of organ-specific markers provided high diagnostic yield. The metastatic patterns inferred from IHC, such as lung primaries metastasising to brain, liver, bone and lymph nodes, and colorectal carcinoma spreading to liver and omentum, were concordant with known clinical behaviour. Overall, our findings emphasise that CK-based IHC is a robust, widely available and cost-effective strategy for

resolving challenging epithelial tumours. When interpreted within the proper clinical and morphologic context, CK profiles substantially narrow the differential diagnosis and guide appropriate site-specific therapy.

CONCLUSION

Cytokeratin immunohistochemistry using Pan-CK, CK7, CK20 and CK5/6 plays a pivotal role in the diagnosis and classification of epithelial malignancies. Pan-CK confirms epithelial lineage, whereas CK7 and CK20, particularly in combination, provide powerful clues to the site of origin in both primary and metastatic settings. CK5/6 is highly reliable for identifying squamous and basal differentiation. In carcinoma of unknown primary and in small or poorly differentiated biopsies, a tiered approach beginning with Pan-CK followed by CK7/CK20 and CK5/6, and supplemented by a small panel of organ-specific markers, enables assignment of a probable primary in most cases. Incorporation of cytokeratin profiling into routine diagnostic algorithms thus improves accuracy, aids prognostication and supports rational therapeutic decision-making.

REFERENCES

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality. CA Cancer J Clin. 2021;71(3):209-49.
2. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Appl Immunohistochem Mol Morphol. 2002;10(2):97-109.
3. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. Histochem Cell Biol. 2008;129(6):705-33.
4. Ordonez NG. Value of cytokeratin 7 and cytokeratin 20 in tumor diagnosis. Am J Surg Pathol. 1998;22(2):223-39.
5. Tot T. Cytokeratins 20 and 7 as biomarkers: An assessment of diagnostic utility. Eur J Cancer. 2002;38(6):758-63.
6. Wang NP, Zee S, Zarbo RJ, Bacchi CE, Gown AM. Coordinate expression of cytokeratins 7 and 20. Appl Immunohistochem. 1995;3(2):99-106.
7. Dabbs DJ. Diagnostic Immunohistochemistry. 5th ed. Philadelphia: Elsevier; 2020.
8. Rosai J, Ackerman LV. Rosai and Ackerman's Surgical Pathology. 12th ed. Philadelphia: Elsevier; 2021.
9. Hainsworth JD, Greco FA. Cancer of unknown primary: Current concepts. N Engl J Med. 1993;329(4):257-63.
10. Varadhachary GR, Raber MN. Cancer of unknown primary site. N Engl J Med. 2014;371(8):757-65.
11. Pelosi G, Fraggetta F, Pasini F, Maisonneuve P, Sonzogni A, Iannucci A, et al. CK7/CK20 patterns in lung carcinomas. Mod Pathol. 2001;14(6):554-61.
12. Rekhtman N. Neuroendocrine tumors: IHC algorithmic approach. Arch Pathol Lab Med. 2014;138(6):852-66.
13. Truong LD, Shen SS. Immunohistochemical diagnosis of renal neoplasms. Arch Pathol Lab Med. 2011;135(1):92-109.

14. Lau SK, Prakash S, Geller SA, Alsabeh R. Cytokeratin profile of hepatocellular carcinoma. Appl Immunohistochem Mol Morphol. 2002;10(3):266-74.
15. Kummar S, Fogarasi M, Canova A, Mota A, Levine EA. CK7 and CK20 staining in lung and colorectal tumors. Pathol Oncol Res. 2002;8(3):175-9.
16. Harbaum L, Pollheimer MJ, Kornprat P, Lindtner RA, Schlemmer A, Langner C. CK7/CK20 in colorectal carcinoma: Diagnostic relevance. Virchows Arch. 2011;459(2):121-30.
17. Yanai H, Wani Y, Ohta M, Hirano A, Yoshida H, Tada T, et al. CK5/6 in differentiating squamous vs urothelial carcinoma. Histopathology. 2001;38(2):150-8.
18. Miettinen M, Sarlomo-Rikala M. CK5/6 and mesothelioma differentiation. Am J Surg Pathol. 1998;22(3):293-9.
19. Bishop JA, Sharma R, Westra WH. Pan-CK utility in poorly differentiated carcinomas. Head Neck Pathol. 2011;5(1):36-40.
20. McCluggage WG. Immunohistochemistry in the evaluation of gynecologic tumors. J Clin Pathol. 2008;61(12):1225-35.