Supplementary data

Title: Diagnostic Yield and Cost of Morphology-Guided Immunohistochemistry in Liver Tumors

Running title: Morphology-guided IHC in liver tumors

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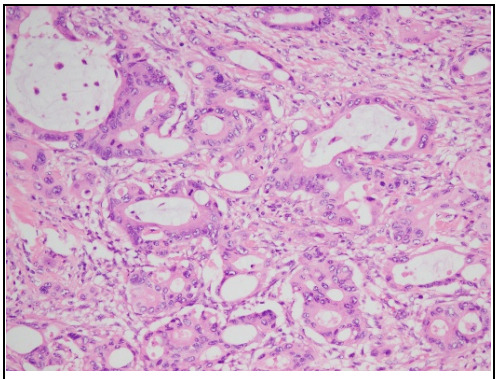
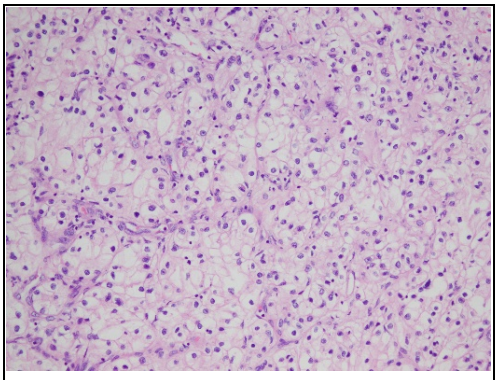
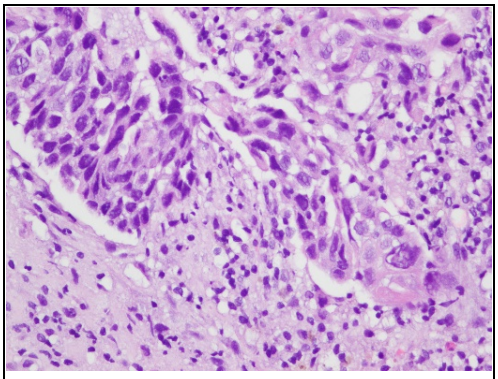
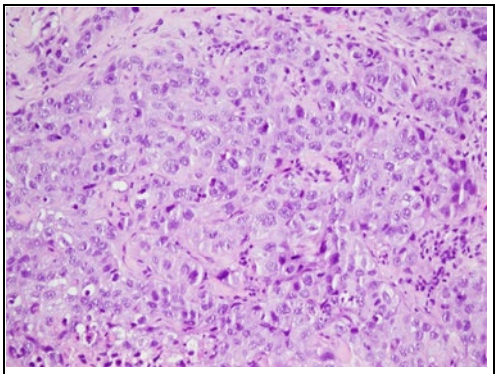
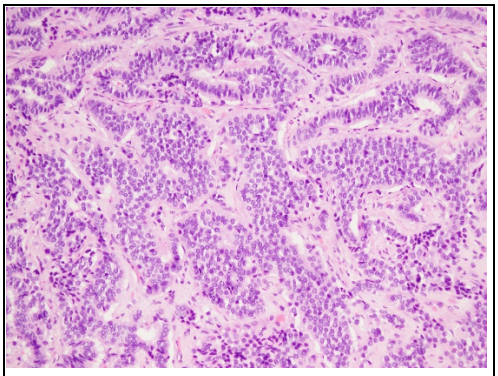
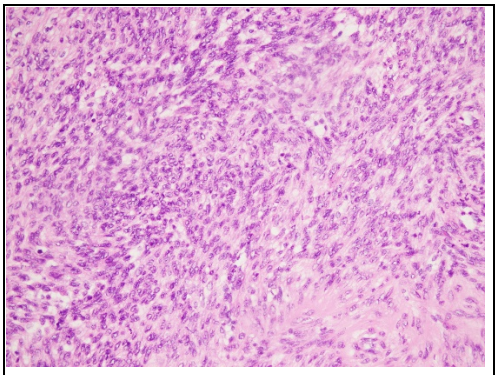
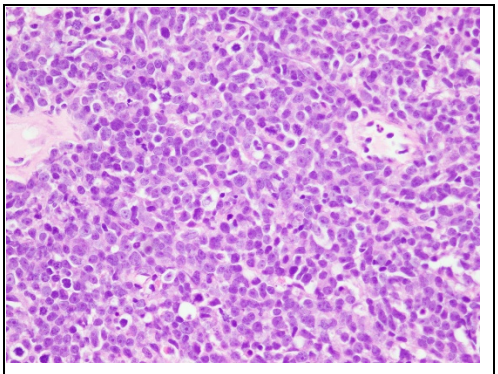
Supplementary Table S1. Morphology-based triage categories for liver tumors and typical confirmatory immunohistochemical markers used in this study

Triage category	Key morphologic features	Histomorphology	Typical confirmatory immunohistochemistry*
Adenocarcinoma	Gland formation and/or intracellular or extracellular mucin		CK7/CK20 patterning for initial triage; site-directed markers such as CDX2 (intestinal), TTF-1 and/or Napsin A (lung/thyroid), PAX8 (renal, gynecologic, thyroid), and GATA3 with or without ER/PR (breast/urothelial)
Carcinoma, not otherwise specified (non-neuroendocrine)	Carcinoma lacking specific glandular or squamous differentiation on initial review		Broad-spectrum cytokeratins; targeted escalation guided by morphology, CK7/CK20 profile, site-directed markers, and clinicoradiologic information
Squamous cell carcinoma	Squamous differentiation with keratinization or intercellular bridges		p40 and/or p63, CK5/6

Triage category	Key morphologic features	Histomorphology	Typical confirmatory immunohistochemistry*
Poorly differentiated carcinoma (non-neuroendocrine)	Anaplastic or undifferentiated sheets lacking lineage-specific architecture		Broad-spectrum cytokeratins; targeted site-directed markers based on morphologic and clinical context (e.g., TTF-1/Napsin A, PAX8, GATA3, CDX2)
Neuroendocrine neoplasm	Organoid, nested, trabecular, or rosette-like architecture; salt-and-pepper chromatin		INSM1, chromogranin A, synaptophysin, with or without CD56; Ki-67 used for grading when applicable
Sarcoma / gastrointestinal stromal tumor (GIST)	Spindle, epithelioid, or pleomorphic mesenchymal morphology		c-KIT (CD117) and/or DOG1 for GIST; lineage-specific markers (e.g., SMA, desmin, S100) for other sarcomas
Hematolymphoid malignancy	Diffuse sheets or sinusoidal infiltration with hematolymphoid cytology		CD45 (LCA) followed by lineage-specific markers (e.g., CD20, CD3, PAX5)

Footnotes:

*Markers listed represent typical confirmatory stains selected in a context-dependent, morphology-guided manner and do not constitute fixed immunohistochemistry panels.

This table summarizes the diagnostic triage framework applied in the present study and is not intended as a clinical guideline.

Primary hepatocellular tumors were evaluated primarily using HepPar-1, Arginase-1, Glypican-3, with or without HSP70, and are reported separately under primary liver cancers.

Hematolymphoid and mesenchymal tumors are shown for completeness but were not included in the primary-versus-metastatic epithelial carcinoma comparisons.

Supplementary Table S2. Morphologic categories among metastatic liver tumors (N = 135)

Morphologic category	Cases, n	%
Adenocarcinoma	75	55.6
Poorly differentiated carcinoma (non-neuroendocrine)	26	19.3
Neuroendocrine neoplasm, total	10	7.4
• Small cell carcinoma	4	—
• Neuroendocrine carcinoma (non-small cell)	3	—
• Neuroendocrine tumor (NET, G1-G2)	3	—
Squamous cell carcinoma	2	1.5
Sarcoma / gastrointestinal stromal tumor, total	11	8.1
• Gastrointestinal stromal tumor (GIST)	9	—
• Leiomyosarcoma	1	—
• Solitary fibrous tumor	1	—
Hematologic malignancy, total	8	5.9
• Diffuse large B-cell lymphoma	6	—
• Burkitt lymphoma	1	—
• Acute myeloid leukemia	1	—
Other epithelial malignancies	3	2.2
• Clear cell renal cell carcinoma	2	—
• Thymoma, type AB	1	—
Total	135	100.0

Footnotes:

Percentages are calculated using the total number of metastatic liver tumors (N = 135).

Subcategories are shown for descriptive purposes; percentages are not calculated for very small subgroups.
Morphologic classification was based on routine histopathologic evaluation supported by targeted immunohistochemistry.

Supplementary Table S3. Immunohistochemistry utilization, laboratory cost, and key marker usage by primary liver cancer subtype (N = 144)

Primary liver cancer subtype	Cases, n	Biopsy, n	Resection, n	IHC stains per case, median (IQR)	IHC cost per case, USD (2023), median (IQR)	Key immunohistochemical markers used, n (%)
Hepatocellular carcinoma	116	92	24	2 (2-3)	29.90 (21.73-45.97)	HepPar-1, 94 (81.0); Glypican-3, 62 (53.4); CD34, 48 (41.4)
Intrahepatic cholangiocarcinoma	24	19	5	4 (3-5)	56.33 (43.44-63.88)	CK7, 22 (91.7); CK20, 18 (75.0); CDX2, 7 (29.2)
Combined hepatocellular-cholangiocarcinoma†	2	2	0	5 (—)	63.88 (—)	HepPar-1, 2 (100); CK7, 2 (100); Glypican-3, 2 (100)
Other primary liver cancers†	2	2	0	4 (—)	56.33 (—)	Lineage-appropriate markers (case-specific)
Total	144	115	29	—	—	—

Footnotes:

Values are presented as median (interquartile range, IQR) unless otherwise stated.

Marker usage is shown as number (percentage) of cases within each subtype receiving that stain; listed markers represent the most frequently used confirmatory stains.

Costs represent laboratory charges calculated using 2023 unit prices and are reported in U.S. dollars (USD).

Biopsy specimens included core needle biopsies and wedge biopsies.

†Very small subgroups (n < 5) yield unstable estimates and should be interpreted with caution.

Supplementary Table S4. Immunohistochemistry utilization, laboratory cost, and rate of cancer of unknown primary (CUP) according to metastatic histologic pattern†

Histologic pattern	Cases, n	Biopsy, n	Resection, n	IHC stains per case, median (IQR)	IHC cost per case, USD (2023), median (IQR)	CUP, n/N (%)
Adenocarcinoma	75	56	19	3 (2-4)	31.80 (21.73-45.97)	6/75 (8.0)
Poorly differentiated carcinoma (non-neuroendocrine)	26	20	6	8 (6-10)	103.86 (77.27-127.19)	7/26 (26.9)
Neuroendocrine neoplasm	10	8	2	7 (6-10)	84.68 (64.40-119.70)	4/10 (40.0)
Squamous cell carcinoma††	2	1	1	2 (2-2)	26.75 (26.13-26.75)	0/2 (0.0)
Carcinoma, not otherwise specified††	3	2	1	5 (4-5)	56.33 (43.44-63.88)	0/3 (0.0)
Sarcoma / gastrointestinal stromal tumor	11	8	3	4 (1-7)	49.14 (17.62-88.12)	3/11 (27.3)
Total (excluding hematologic malignancies)	127	95	32	—	—	—

Footnotes:

Values are presented as median (interquartile range, IQR) unless otherwise stated.

Cancer of unknown primary (CUP) was defined as a metastatic liver tumor in which the most likely primary site could not be determined at the time of pathology sign-out despite targeted immunohistochemistry and review of available clinicoradiologic information.

Costs represent laboratory charges calculated using 2023 unit prices and are reported in U.S. dollars (USD).

Biopsy specimens included core needle biopsies and wedge biopsies.

†Hematologic malignancies were excluded from this analysis because diagnostic workflows and

immunohistochemistry utilization differ substantially from those of epithelial tumors.

††Very small subgroups (n < 5) yield unstable estimates and should be interpreted with caution.