Bladder Tumors: Immunohistochemistry

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Cornell University

November 9, 2013

Methodist The Methodist Hospital System

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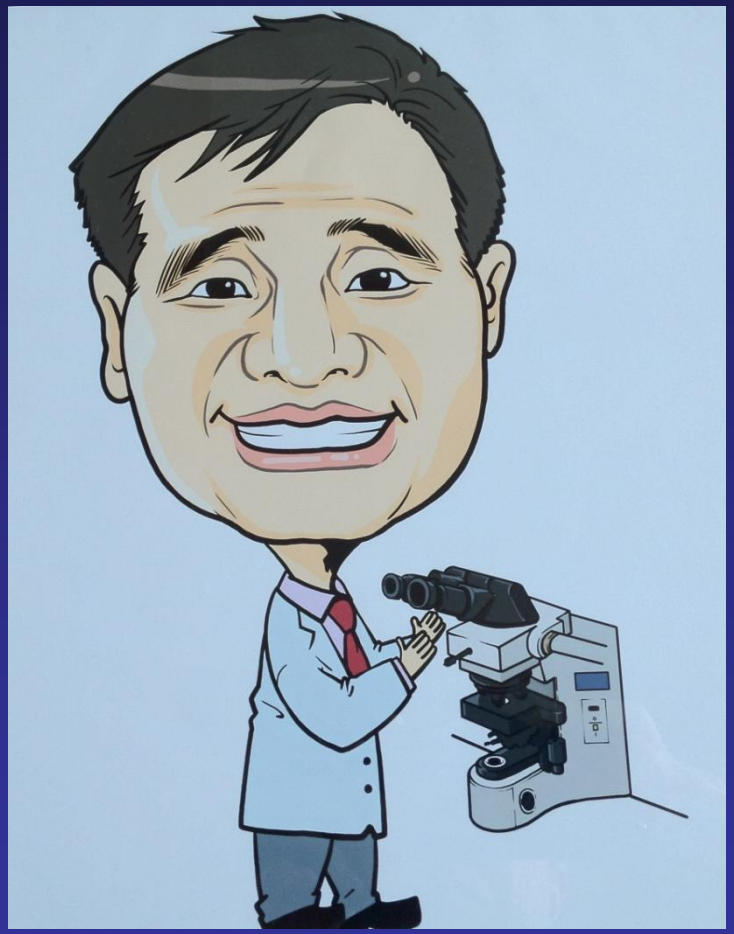
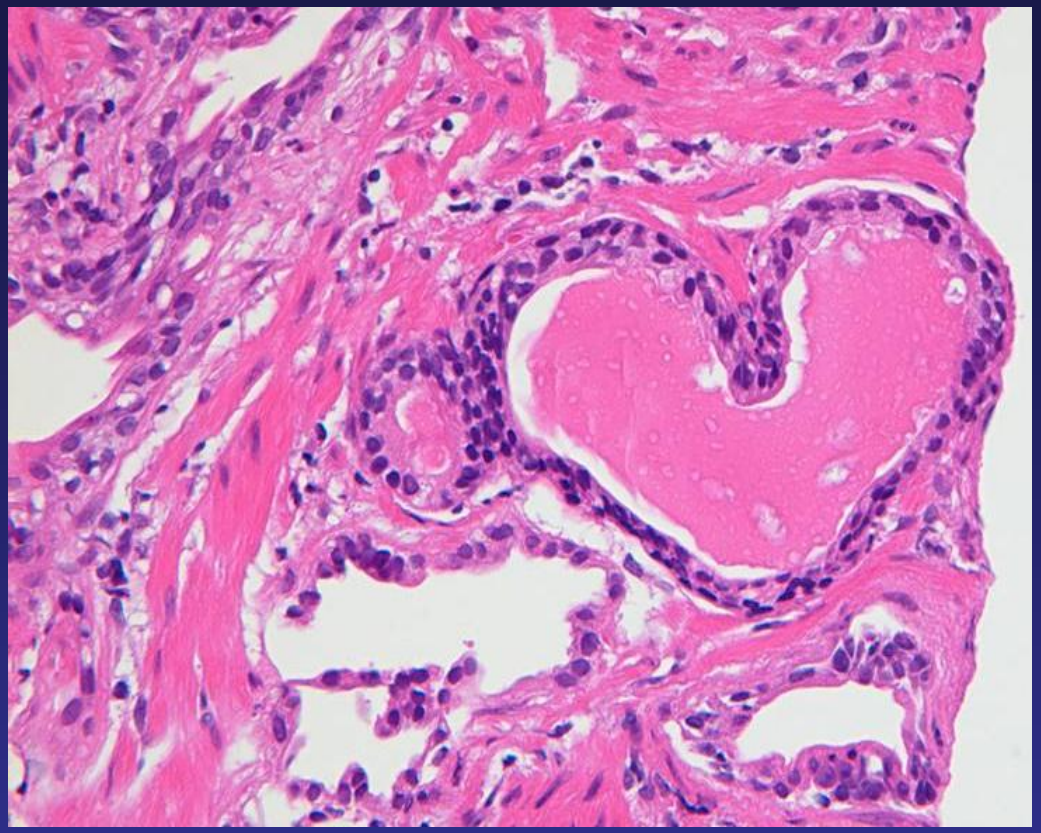
SEVERANCE HOSPITAL
YONSEI UNIVERSITY HEALTH SYSTEM





Do your BeST!

November 9, 2013

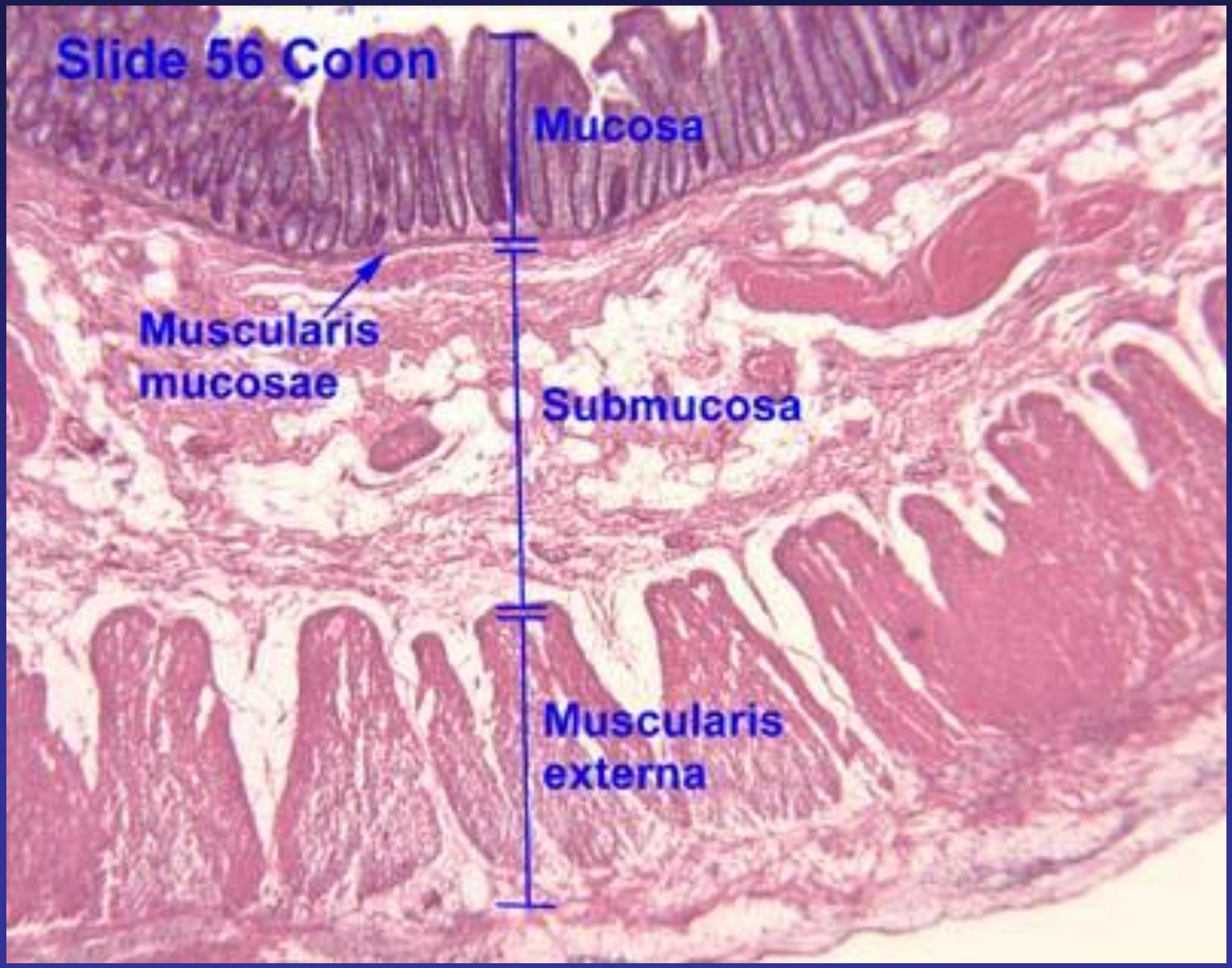
TOPICS (BeST)

- **Basic:** Normal histology & anatomy
- **Study (effort): Immunohistochemistry**
  - ✓ Immunohistochemistry in flat lesions
  - ✓ Diagnostic utility in invasive cancers
  - ✓ DD between urothelial vs. non-urothelial
- **Think:** Prognostic factors
- **Enjoy**

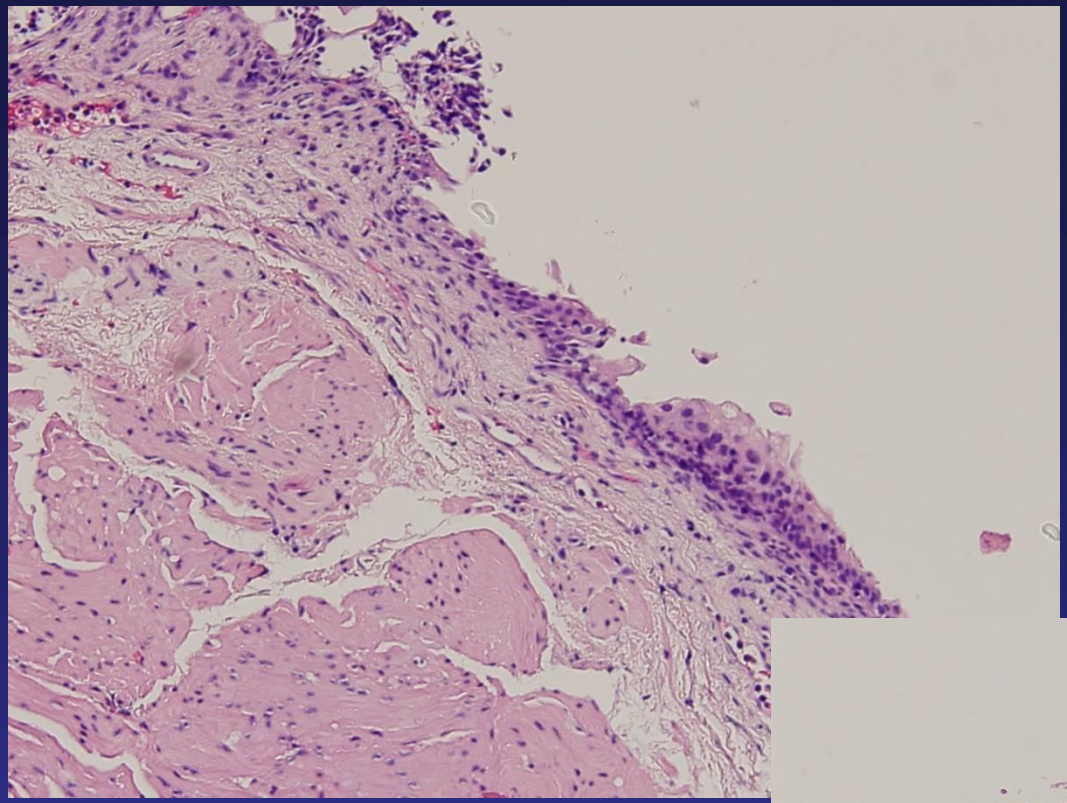
1. Basic

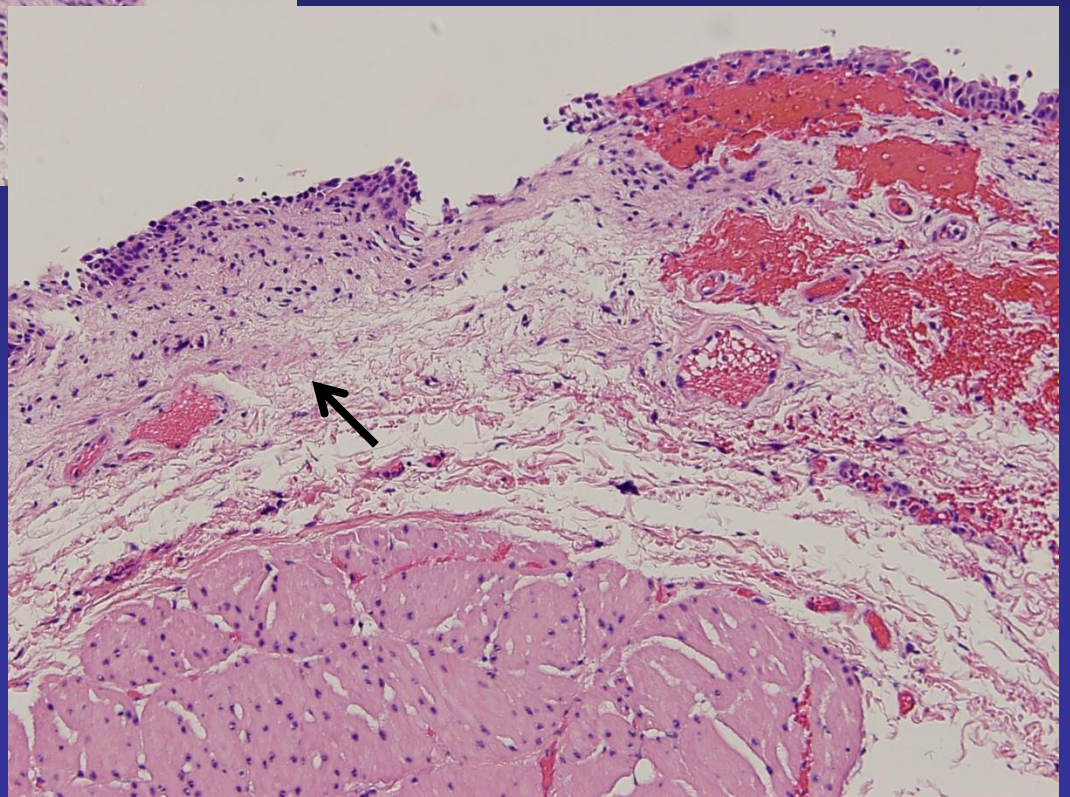
- Mucosa
- Submucosa, lamina propria vs. suburothelial tissue
- Proper muscle layer
- Perivesical fat

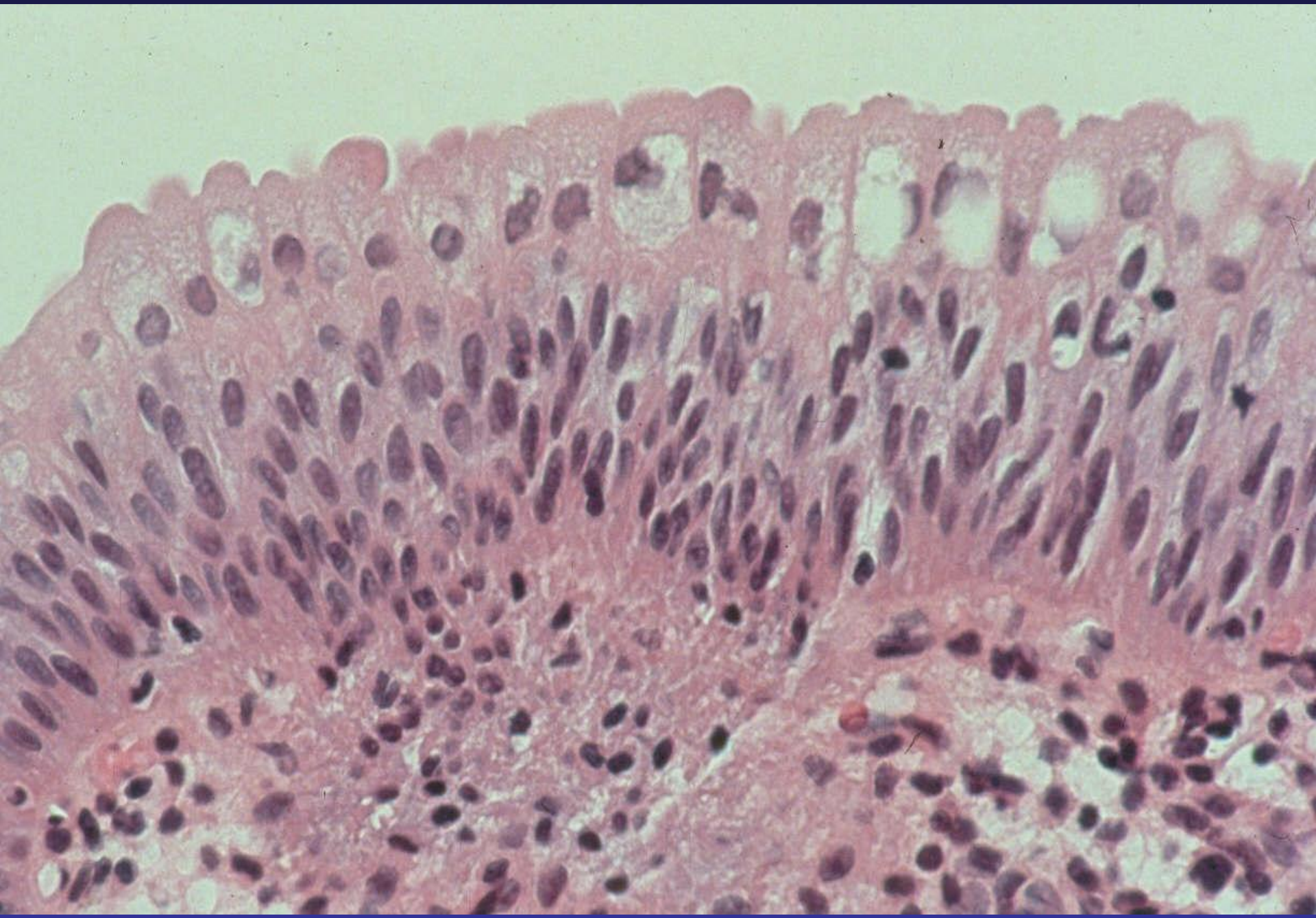
Colon

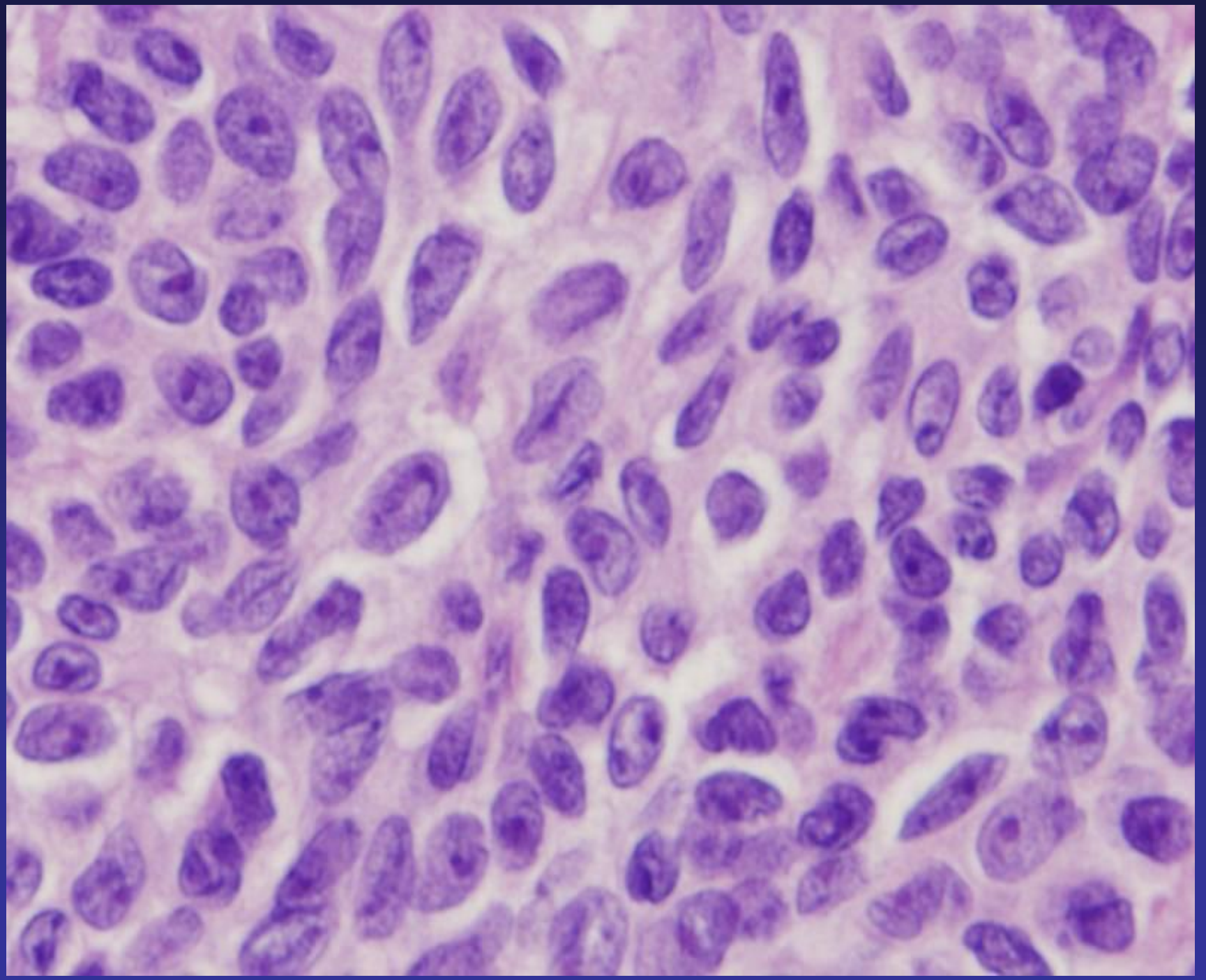

Slide 56 Colon
Mucosa
Muscularis mucosae
Submucosa
Muscularis externa

Bladder

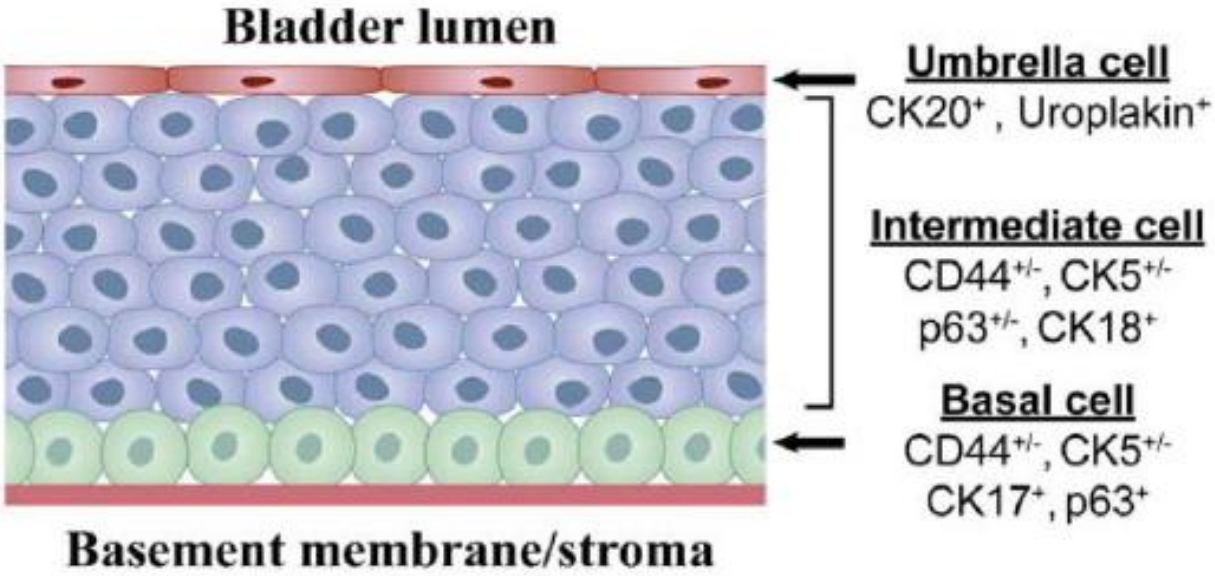






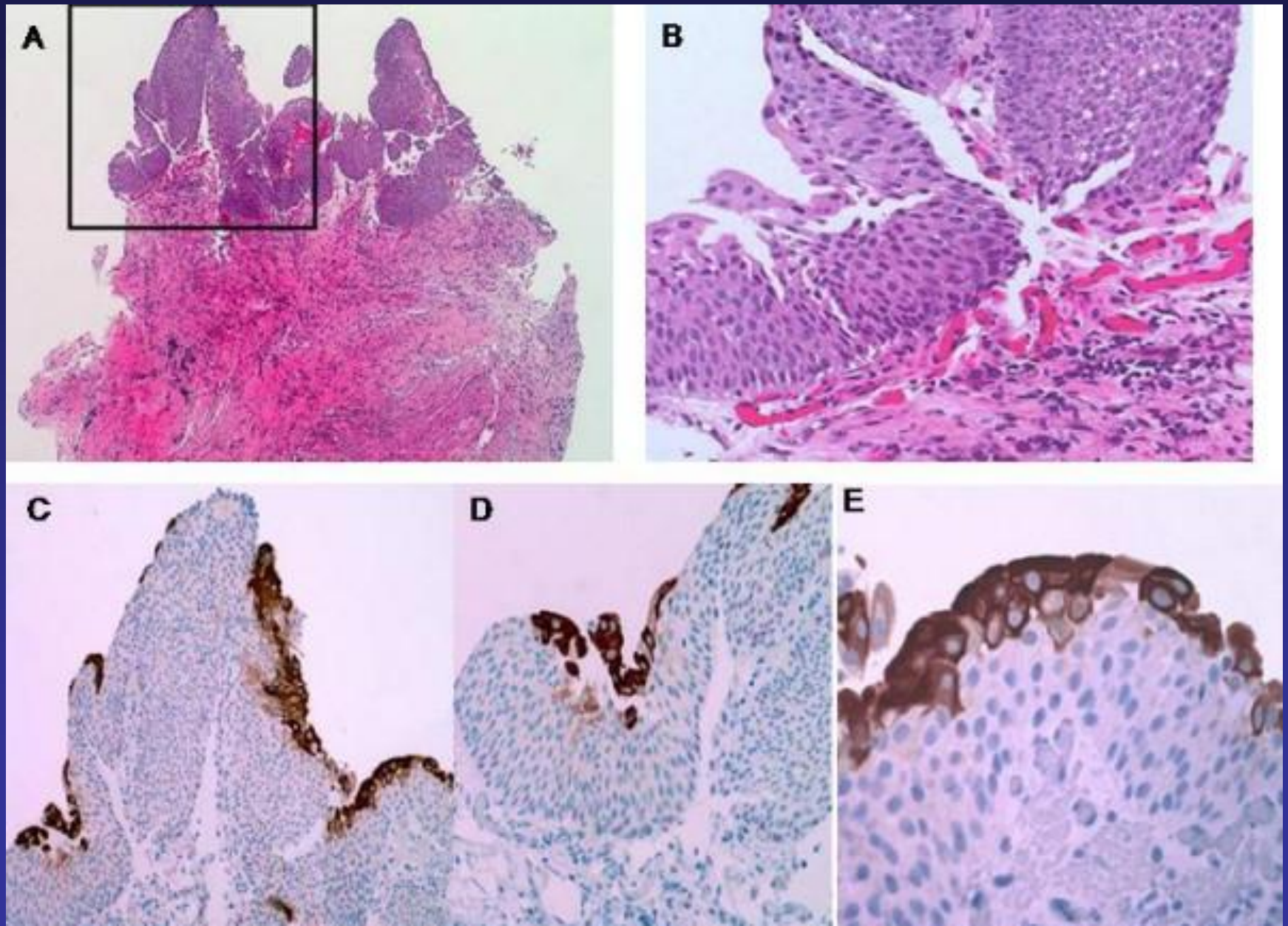


Urothelium

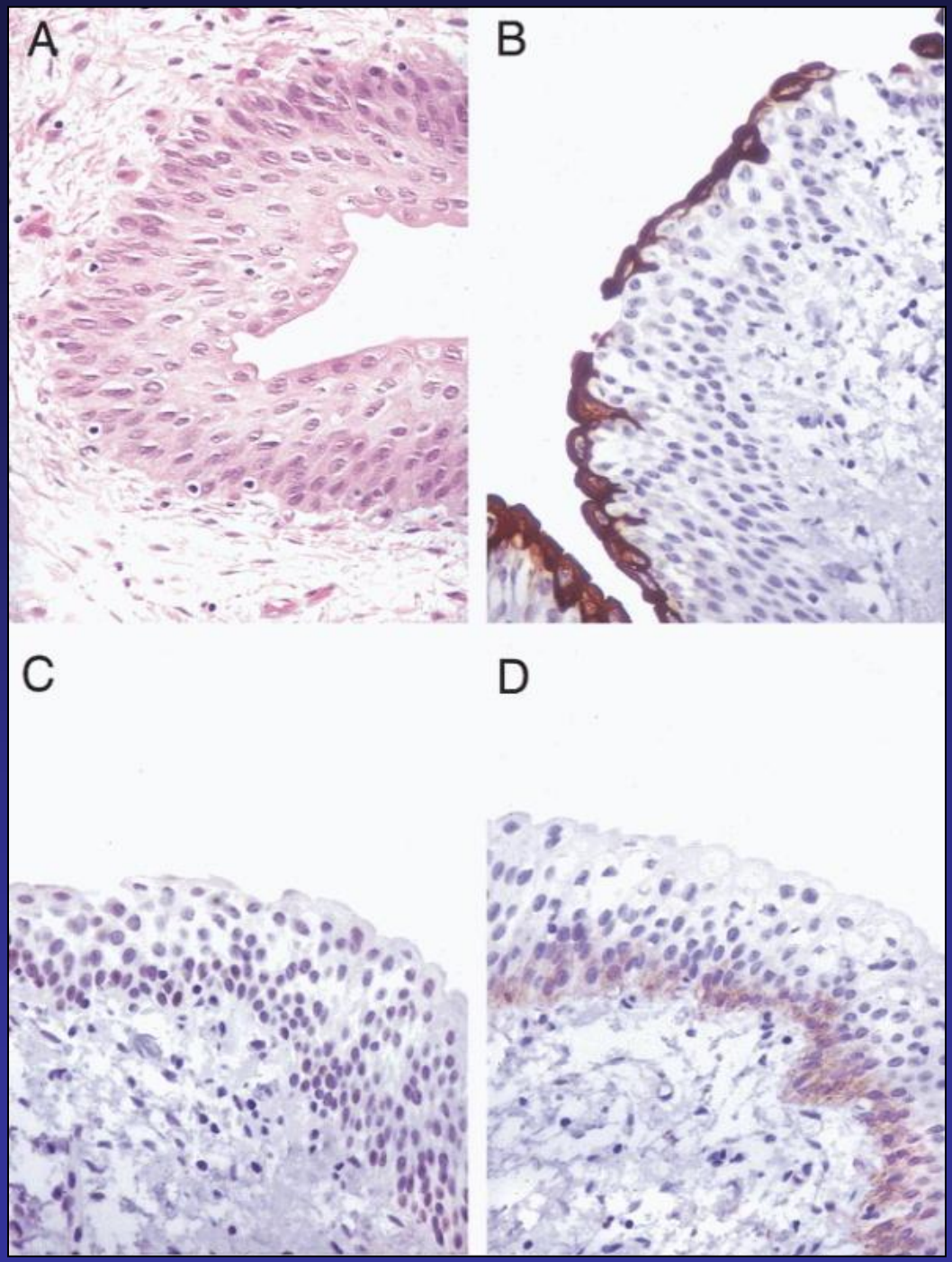


CD44: intermediate and basal cells +
Reactive atypia: diffuse +
Dysplasia and CIS: loss

Normal/Reactive Urothelium



CK20



p53

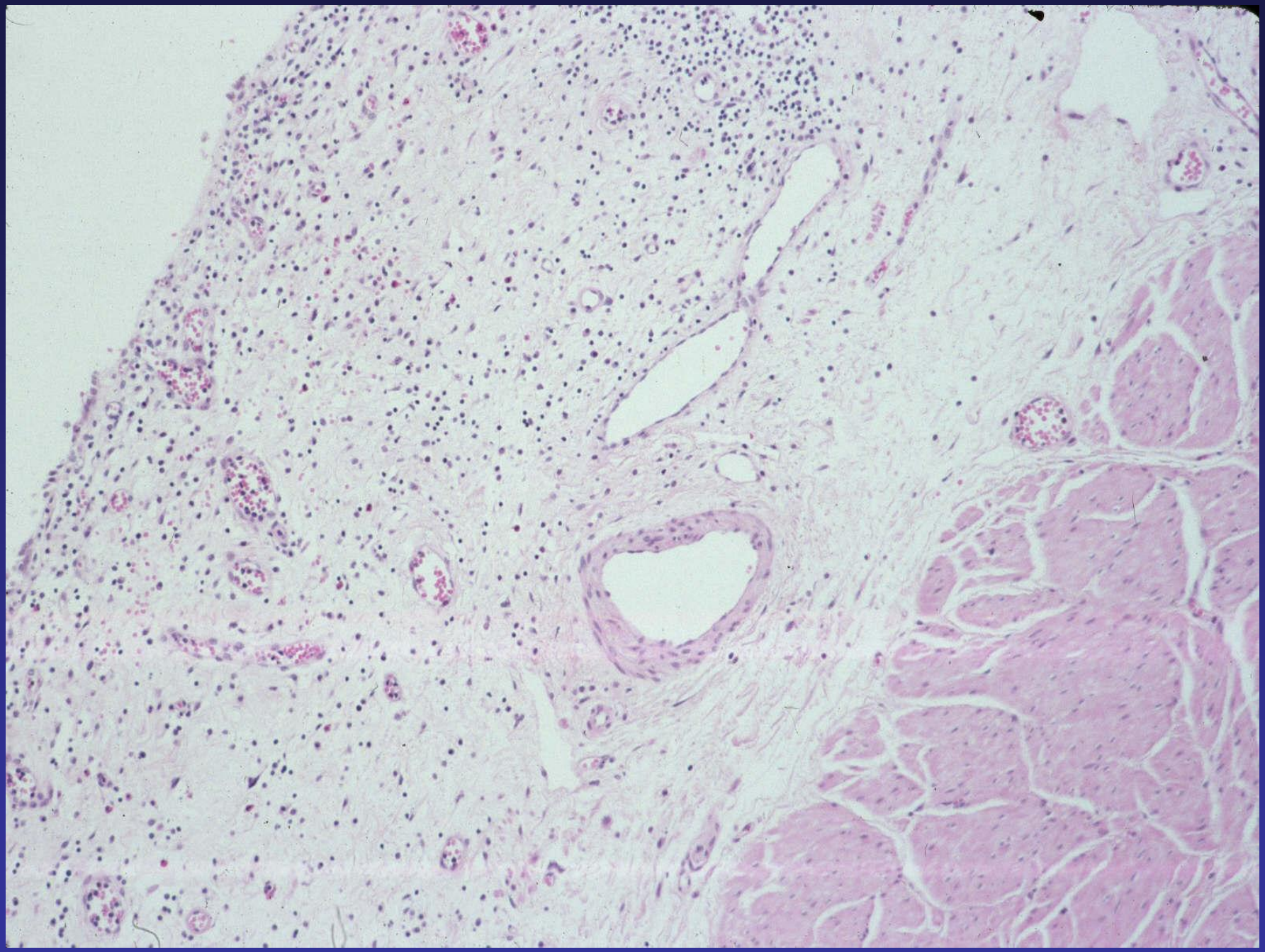
CD44

AJSP
25:1074, 2001

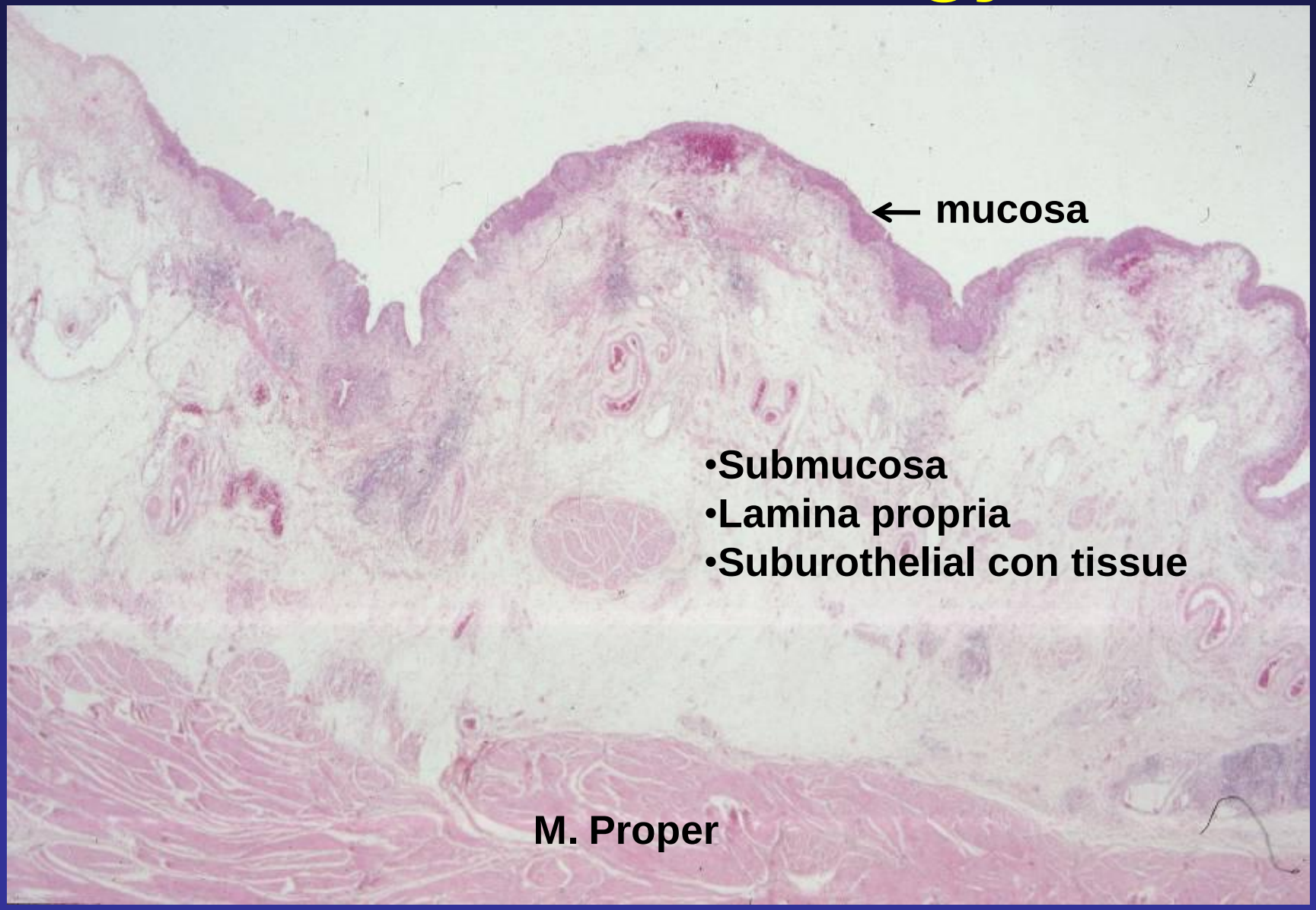
Normal Urothelium

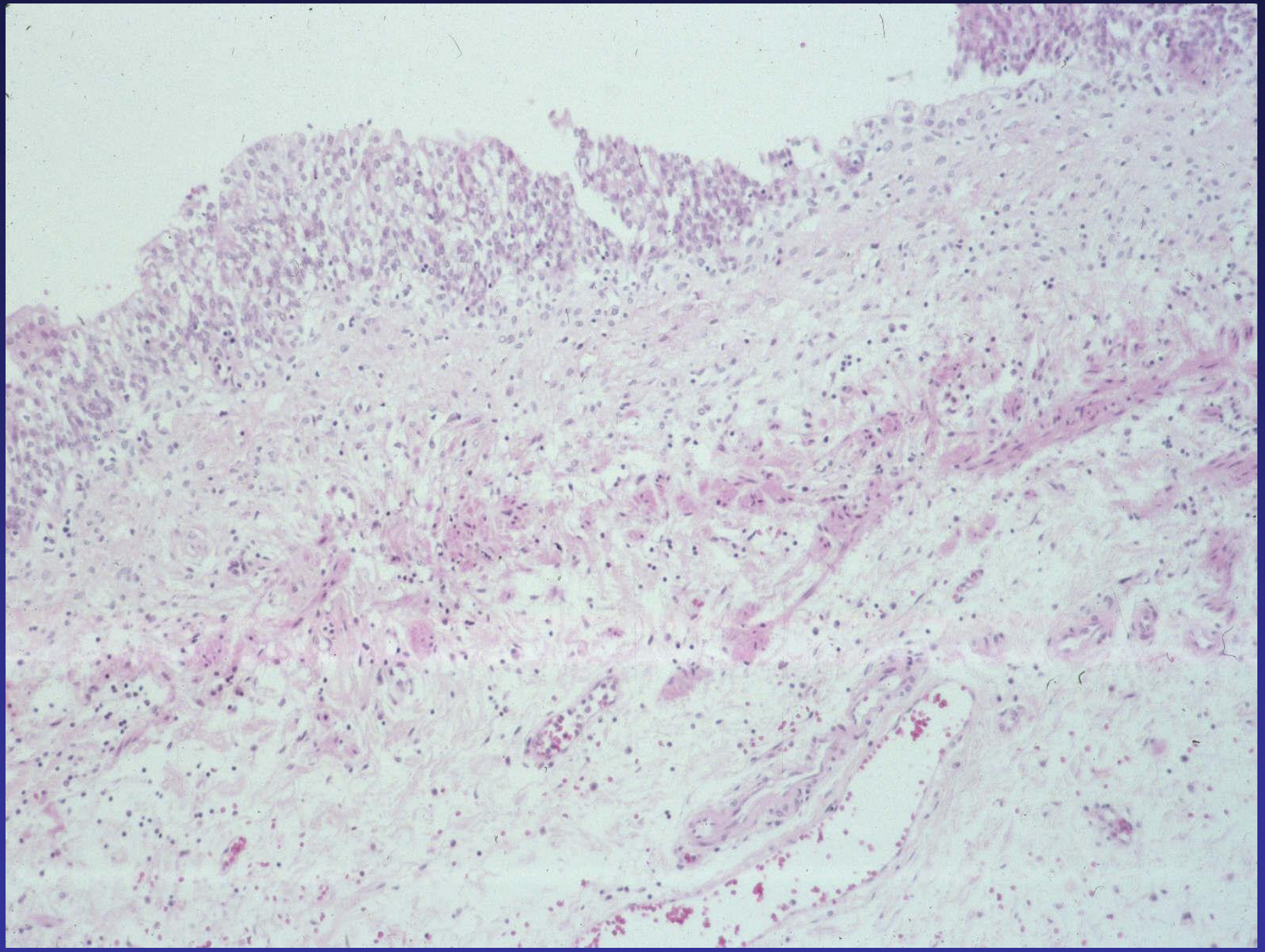
	CK20	CD44	P53
• Normal	UC only	Basal cell	-/+
• Reactive	“	Full Thick	-/+
• CIS	Expand	No stain except for basal cells	+++

AJSP 2001 Aug;25(8):1074-8.



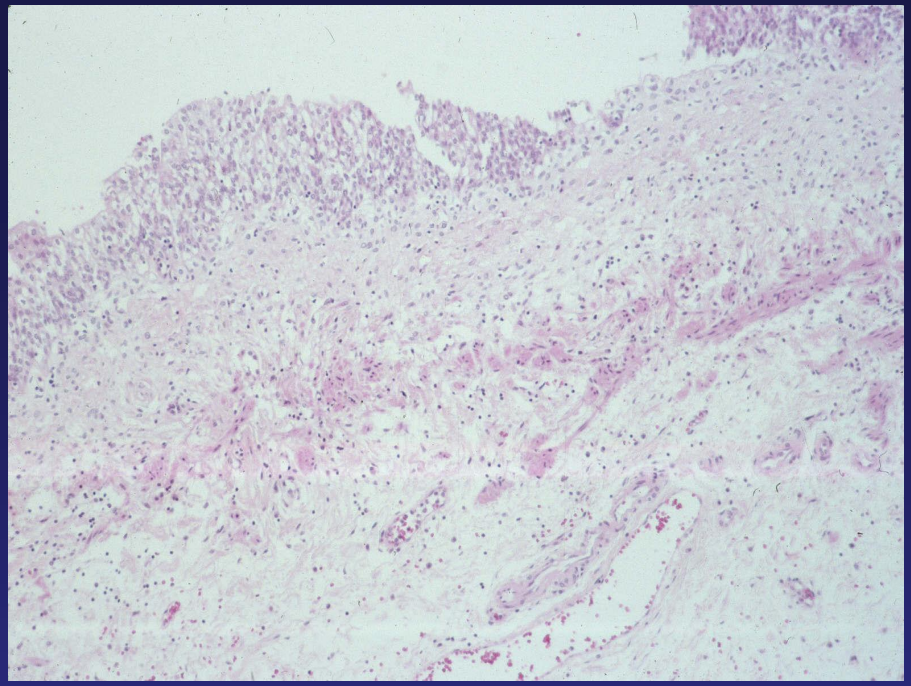
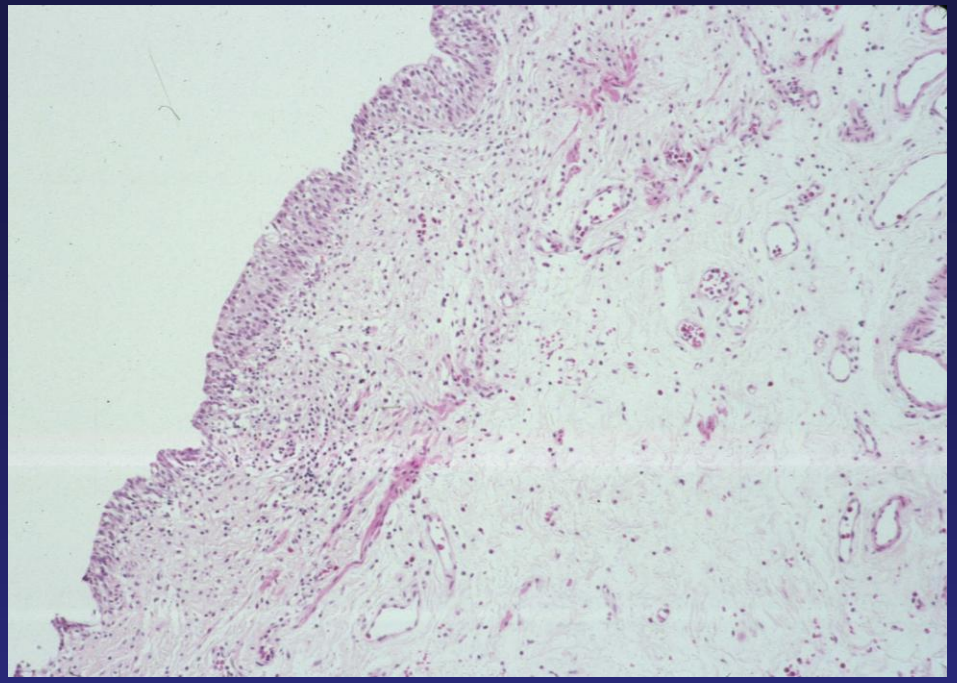
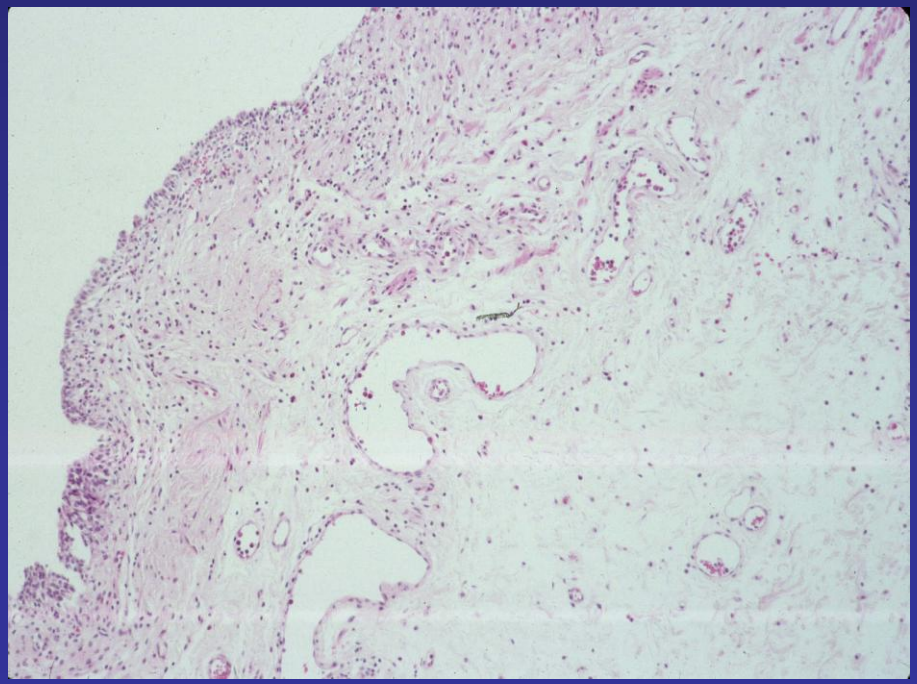
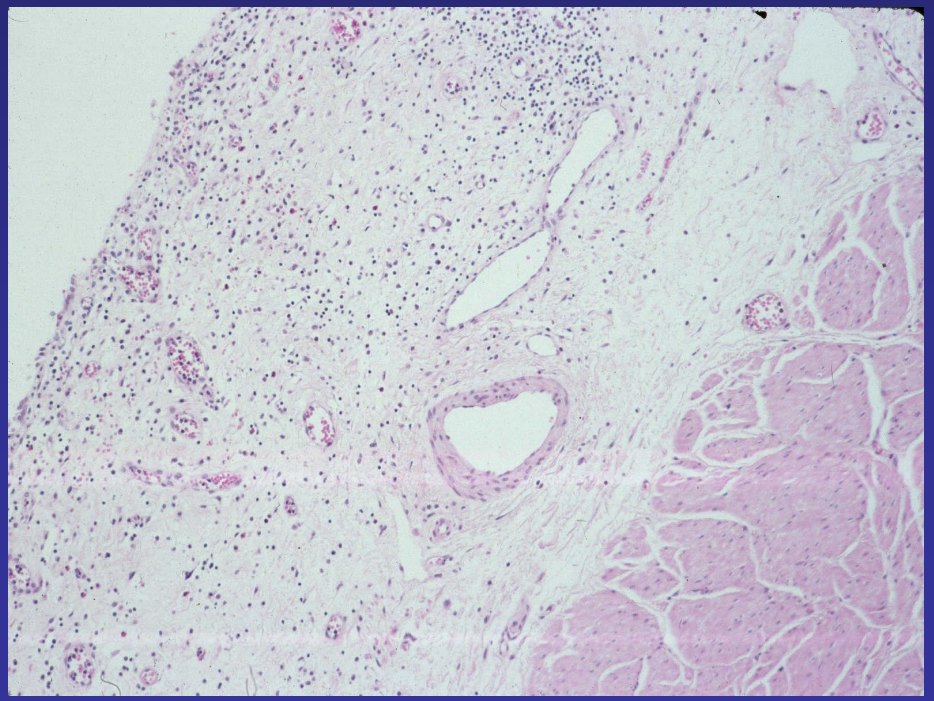
Normal Histology



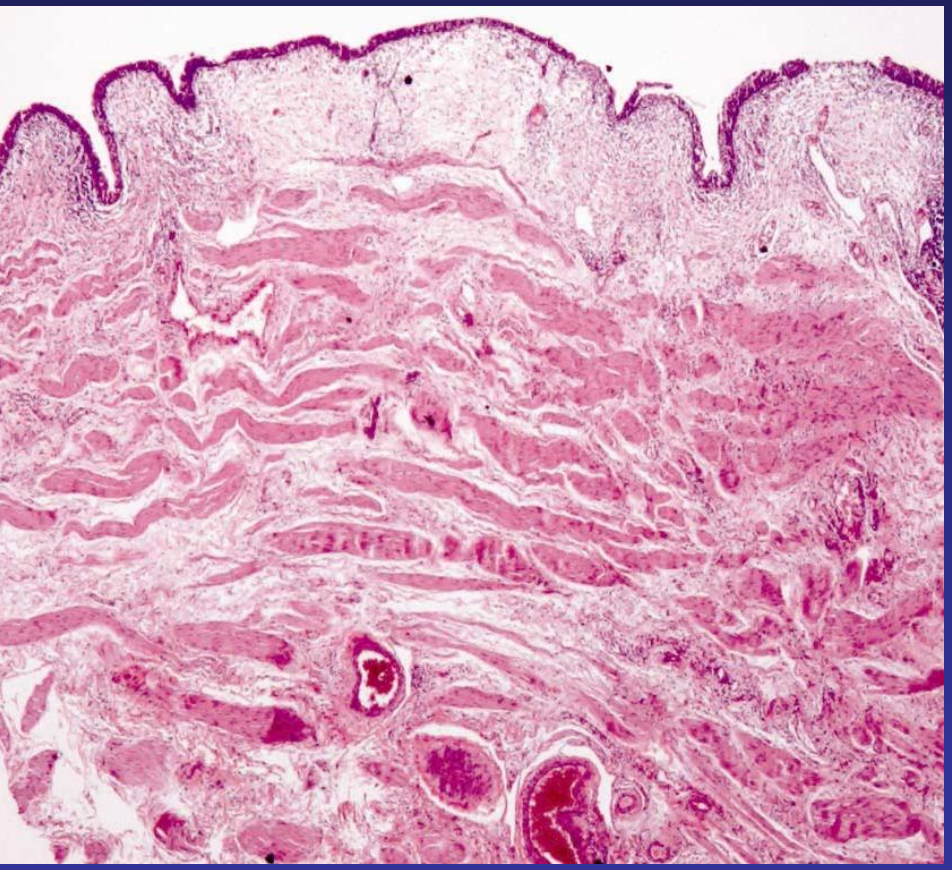


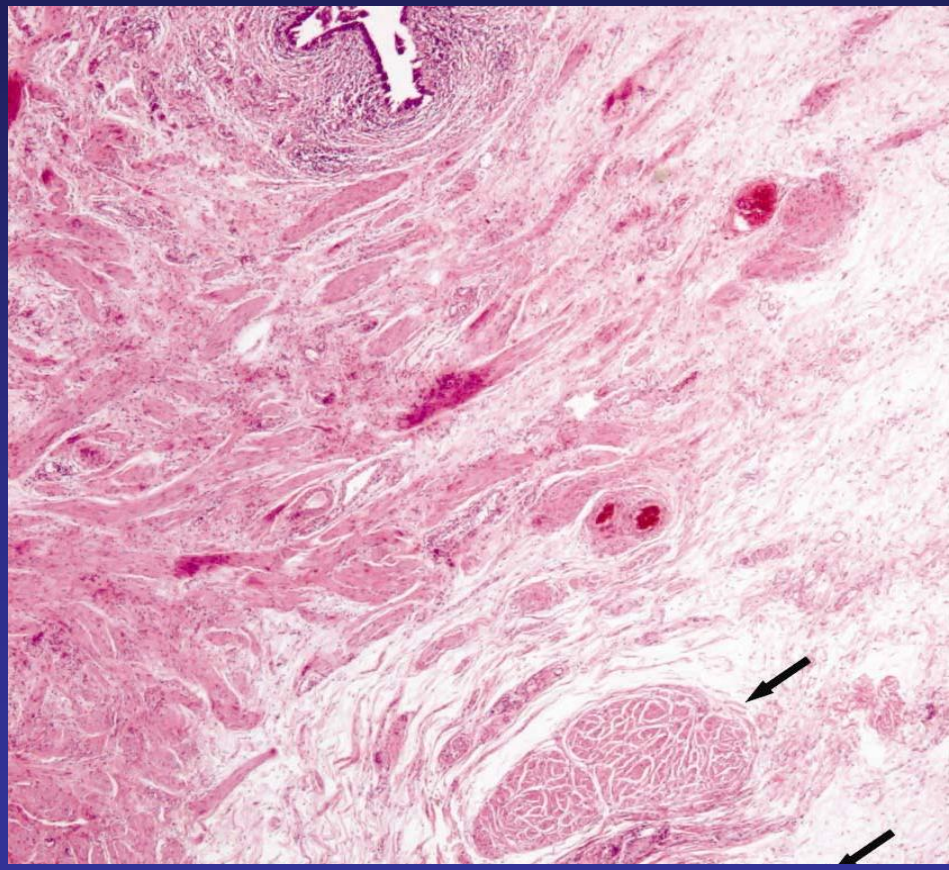
Muscularis Mucosae of Bladder

- Three patterns of smooth muscle fibers
  - Continuous layer (3%)
  - Interrupted (discontinuous) (20%)
  - Scattered fibers without obvious layer (71%)
  - No muscle in lamina propria (6%)

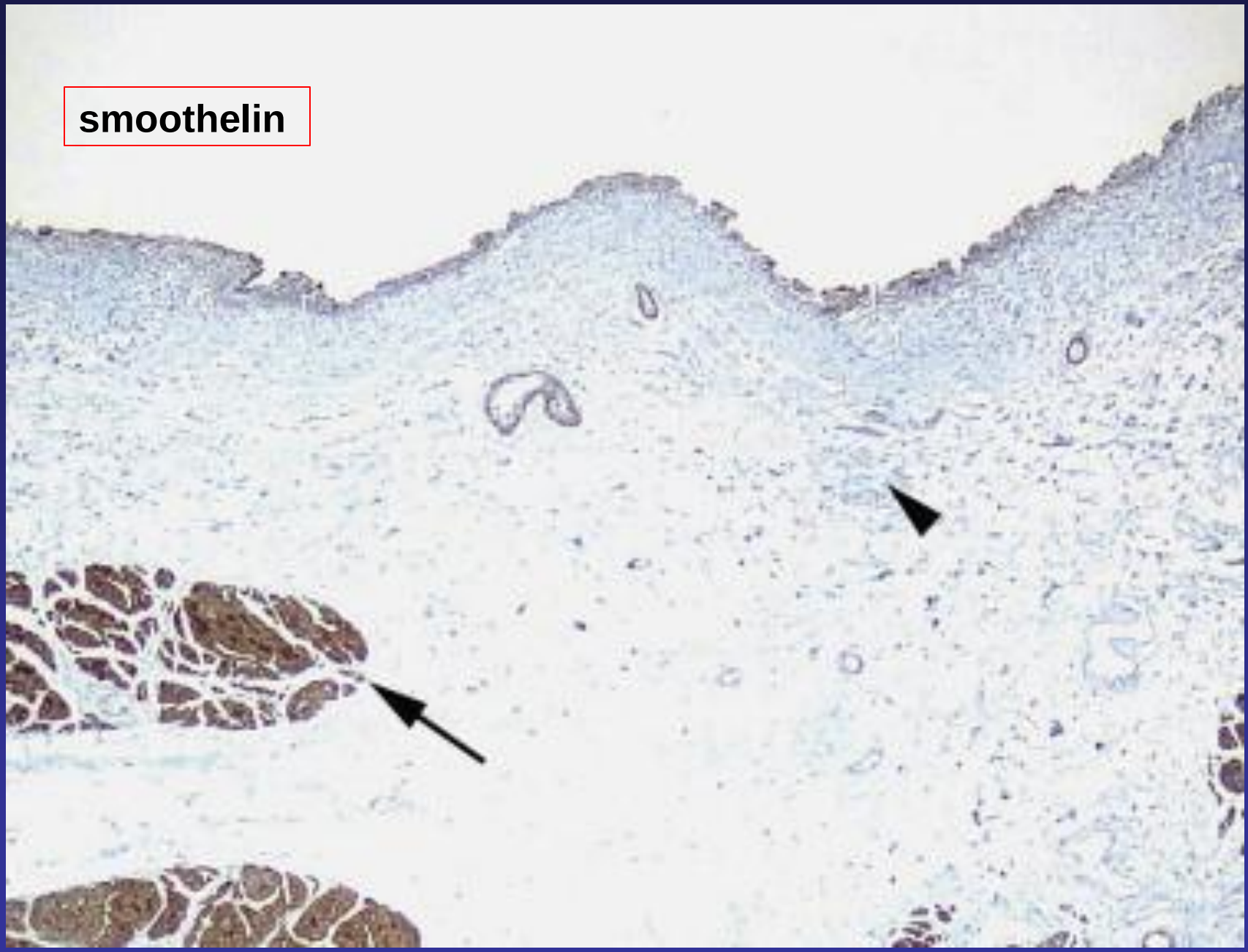





HYPERPLASTIC MUCULARIS MUCOSAE OF URINARY BLADDER

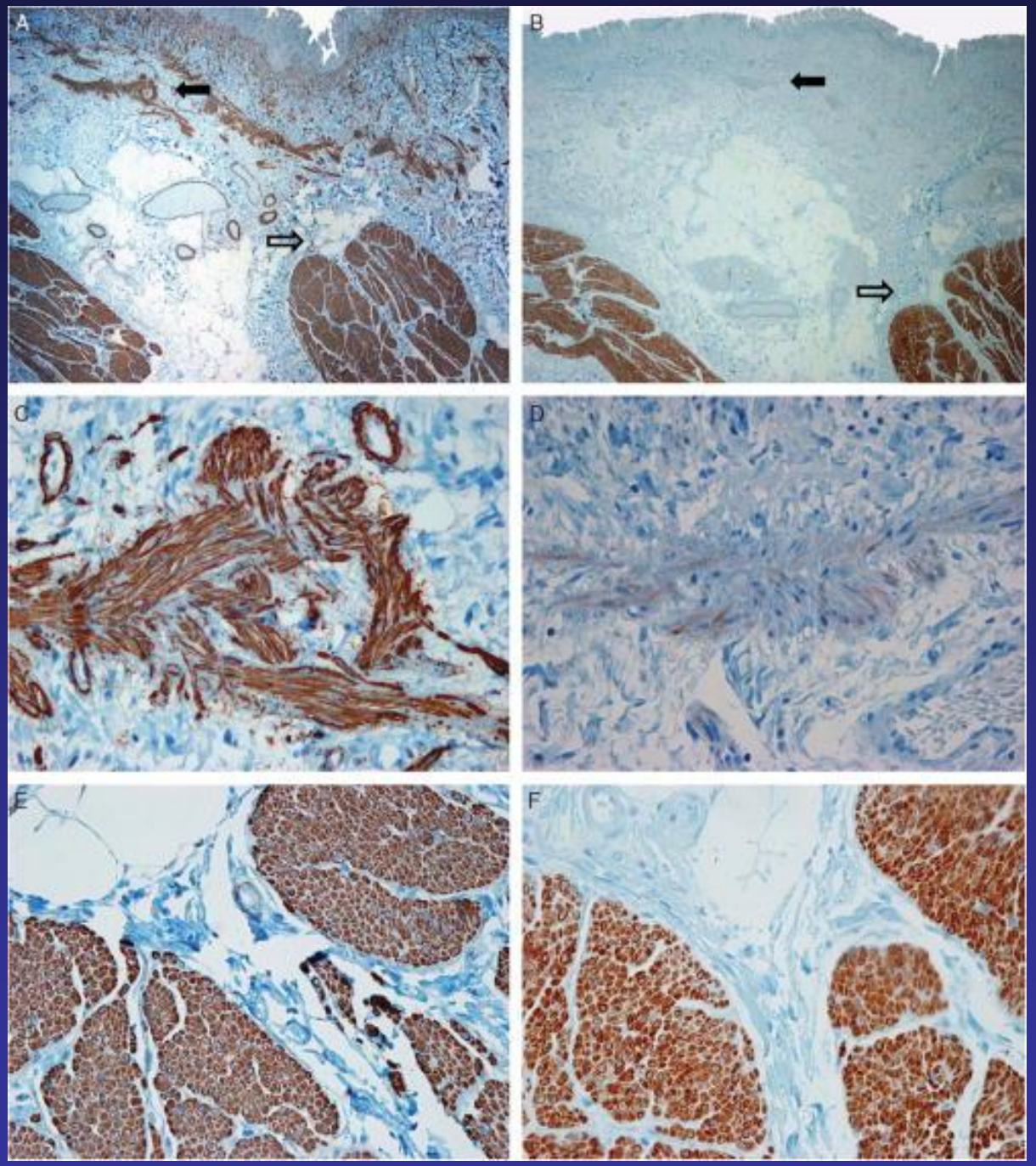




Vakar-Lopez F, Shen SS, Ro JY. Annals Diag Pathol 2007 11(6):395-401


smoothelin

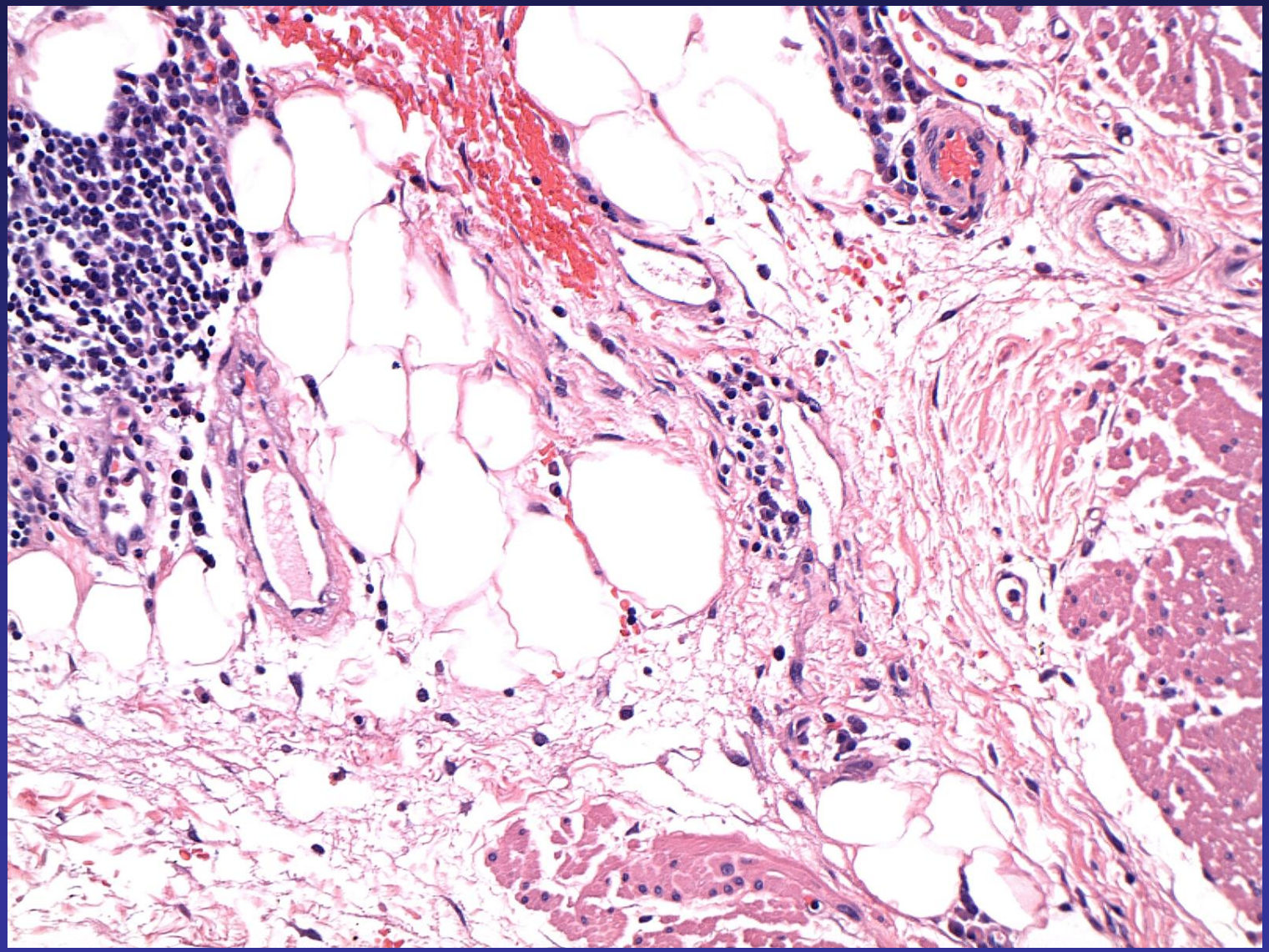
SMA



Smoothelin

AJSP 33:91-98, 2009

FAT IN SUBMUCOSA AND MUSCULARI PROPRIA

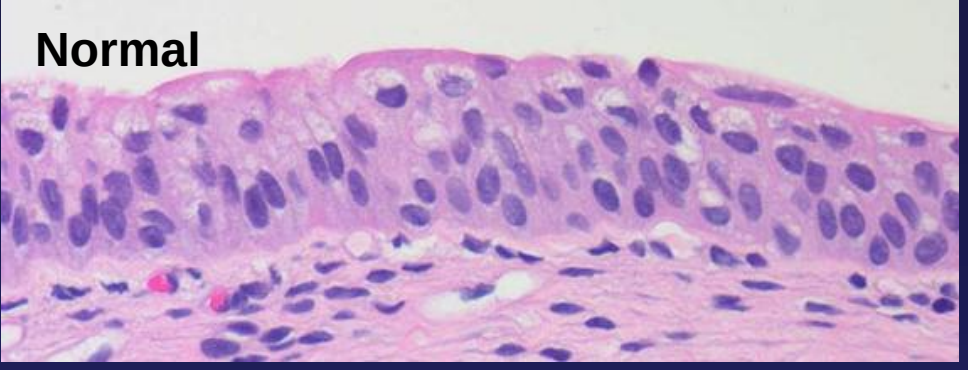


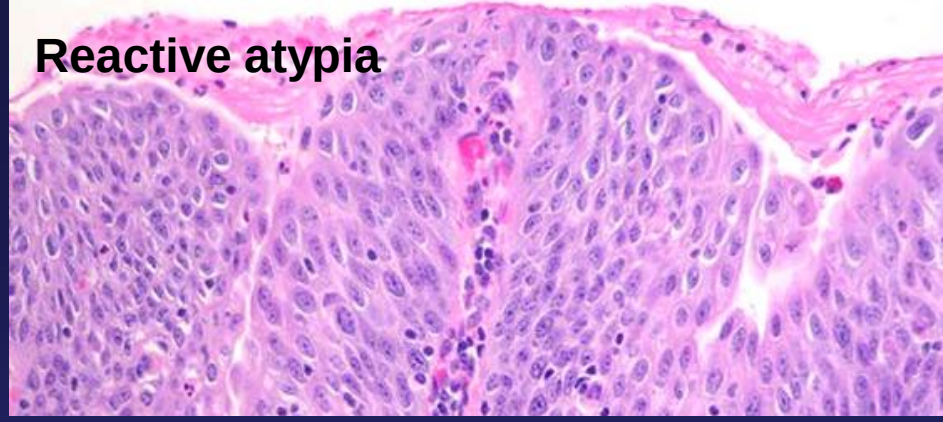
2. (effort) Study

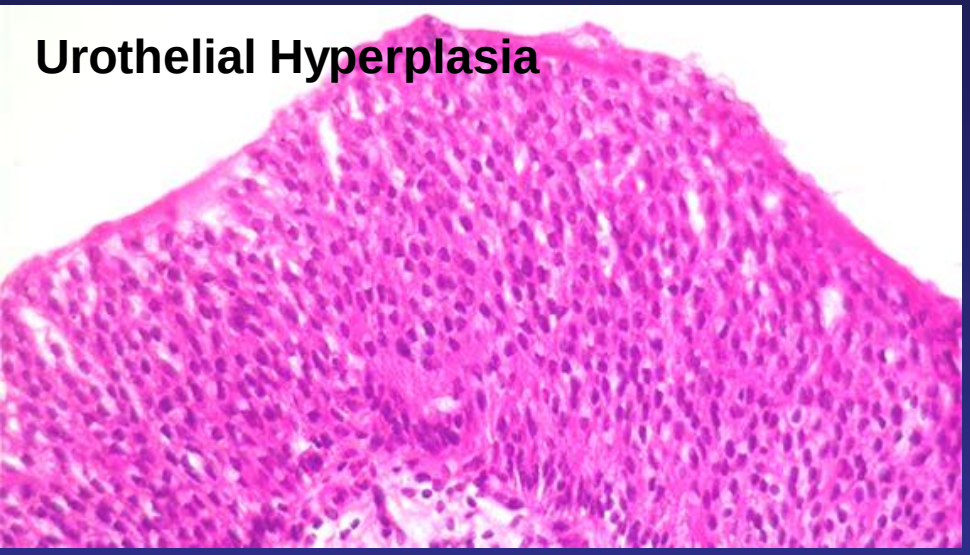
- Immuno in flat lesions
- Immuno for invasive ca.
- Immuno between urothelial vs. non-urothelial

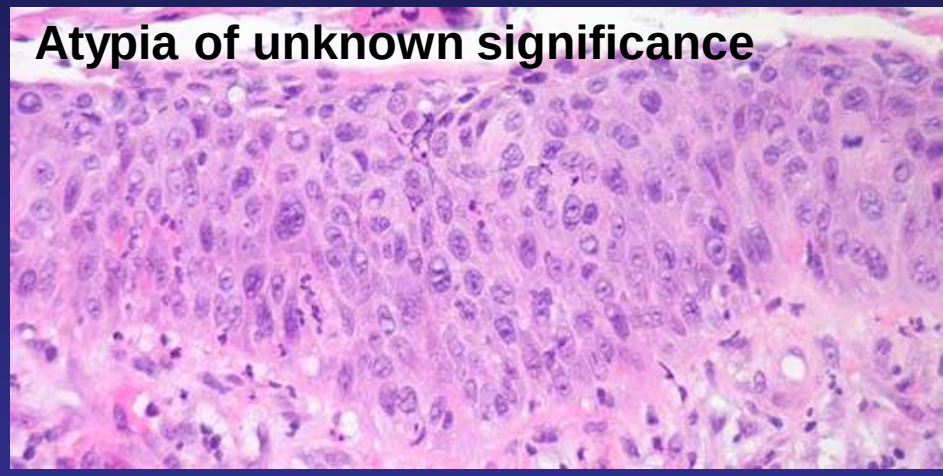
FLAT LESIONS WITH ATYPIA (2004 WHO/ISUP Consensus)

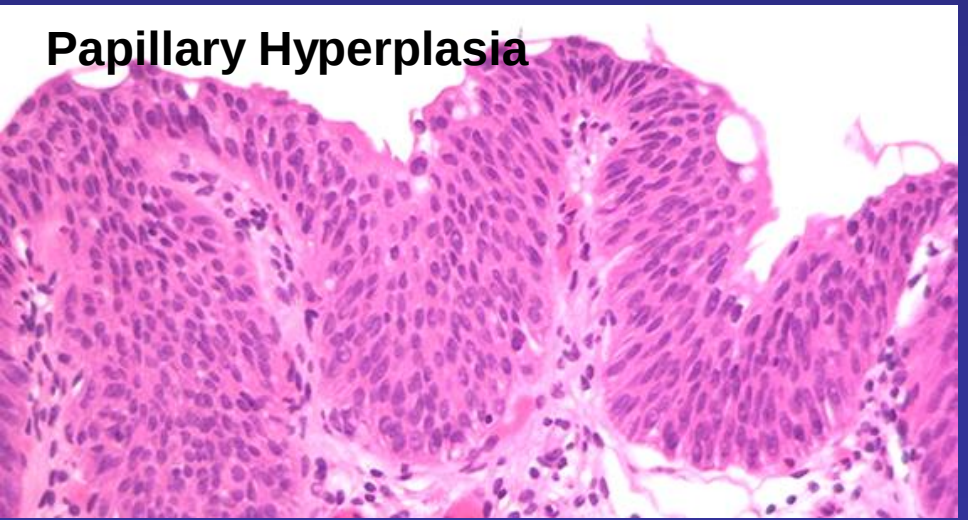
- Reactive atypia
- Atypia of unknown significance
- Dysplasia (low grade intra-urothelial neoplasia)
- Carcinoma in-situ (high grade intra-urothelial neoplasia)

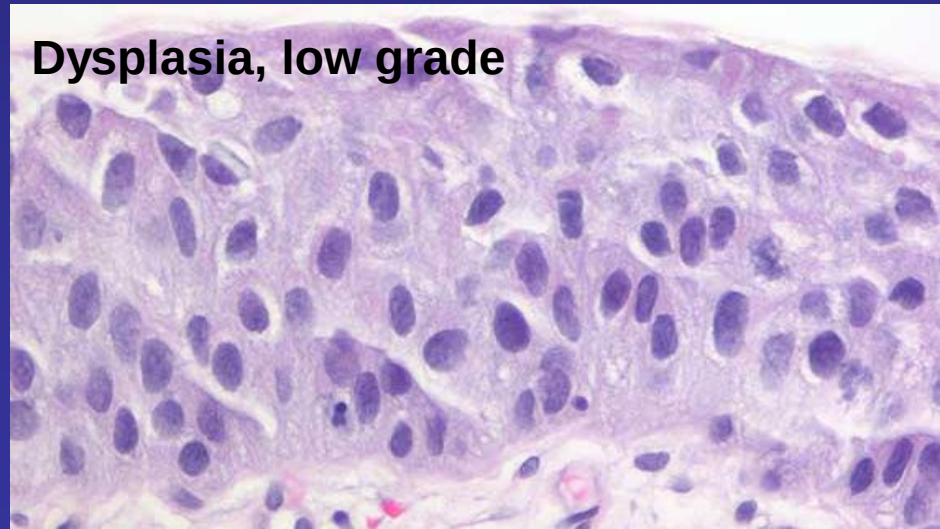

Normal

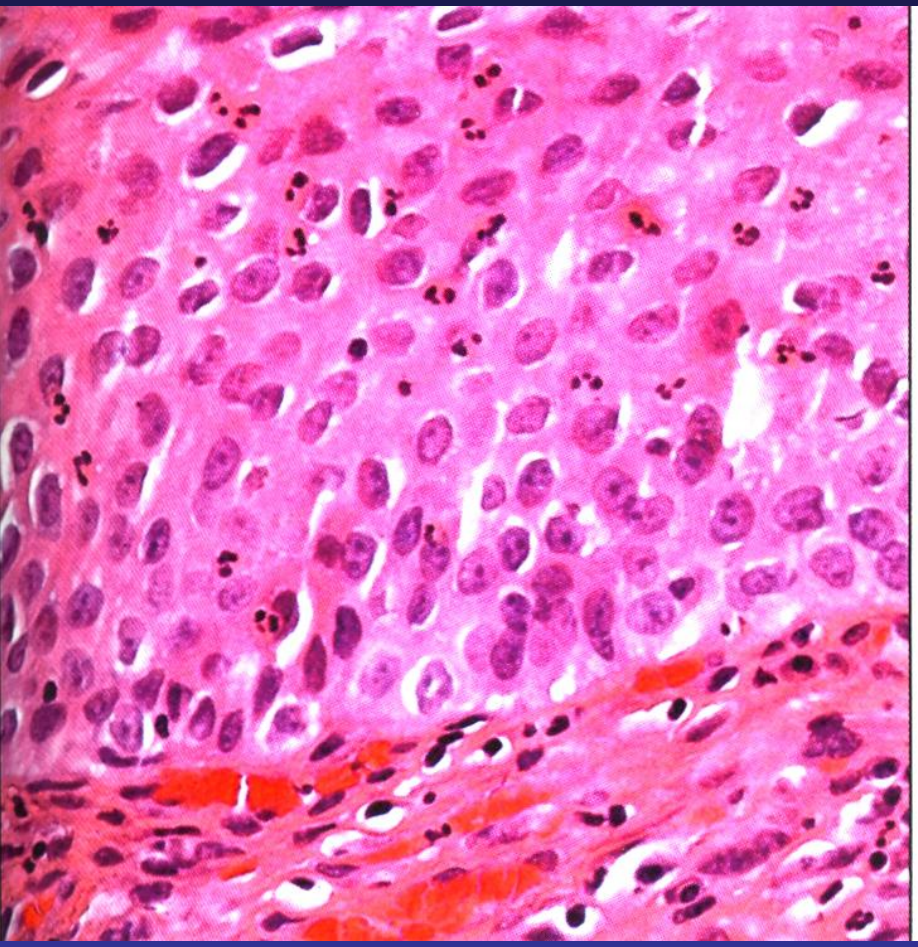

Reactive atypia

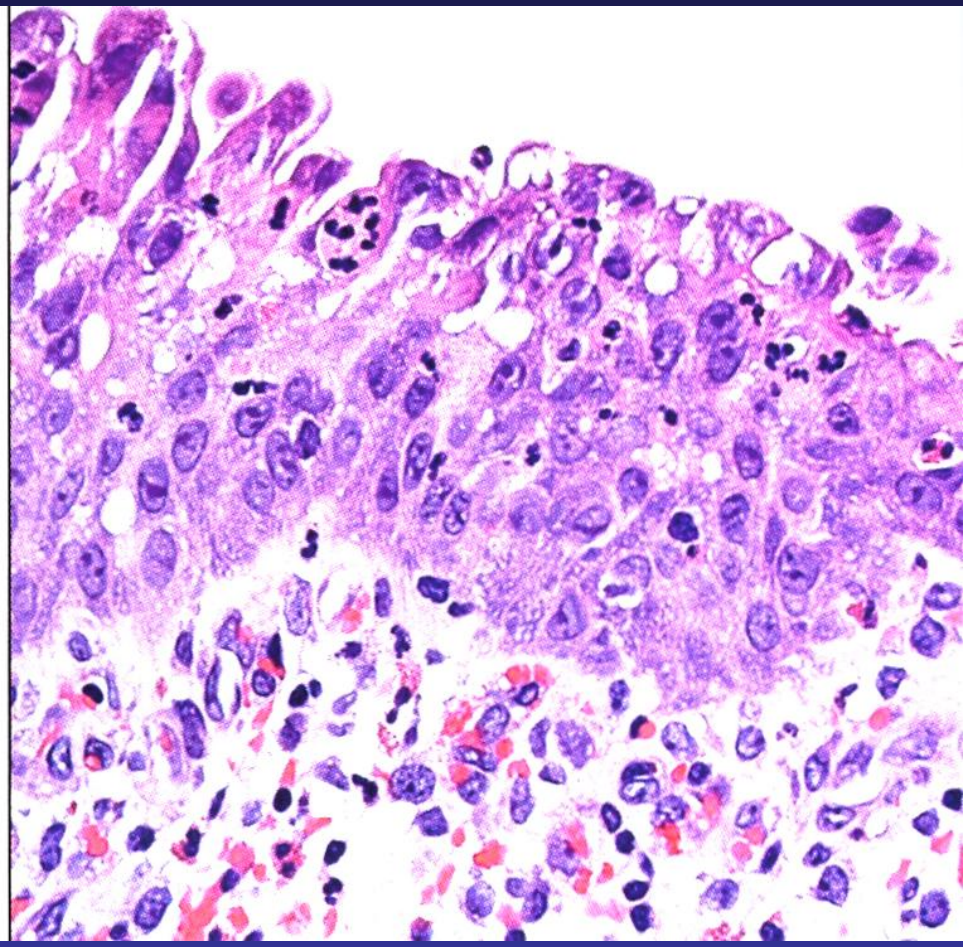

Urothelial Hyperplasia

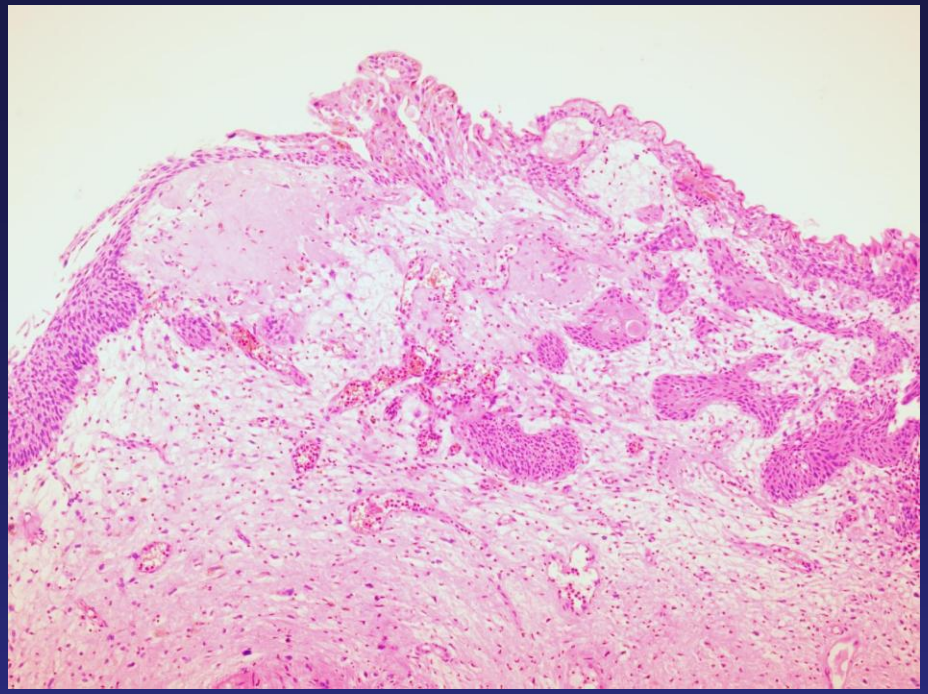
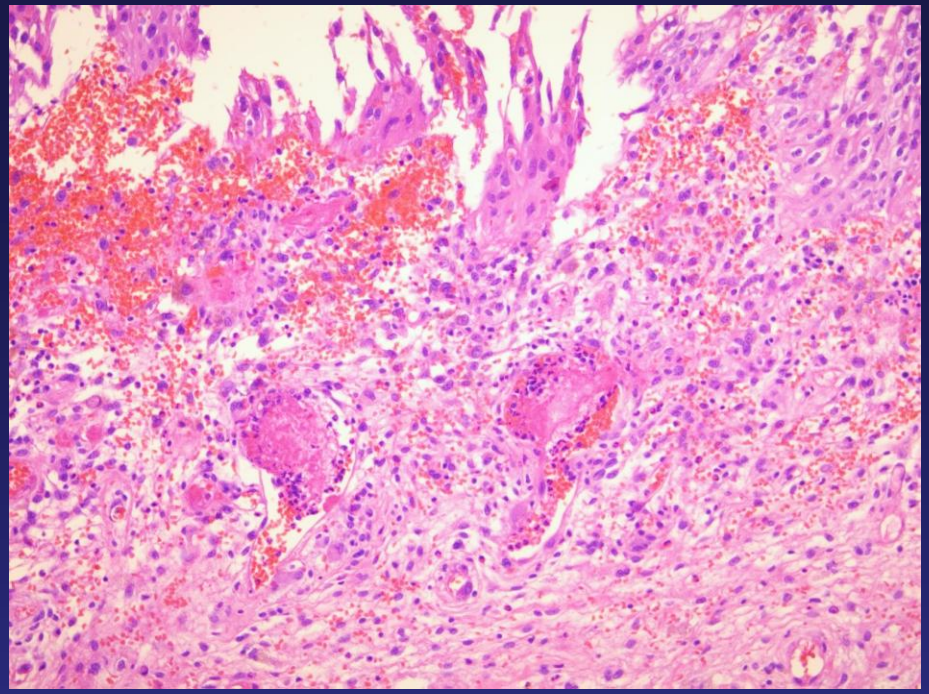
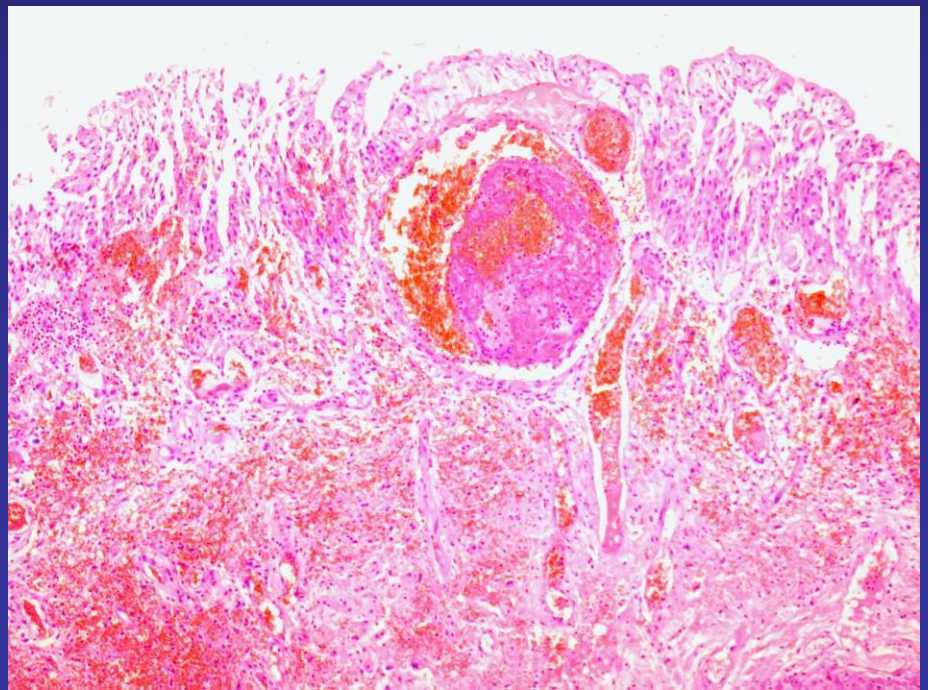
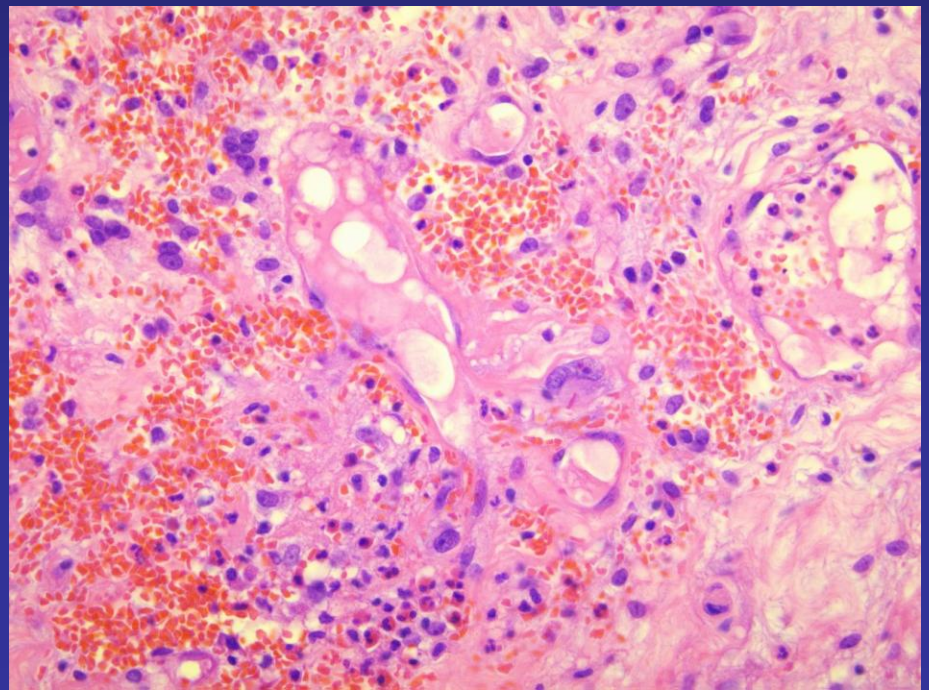

Atypia of unknown significance


Papillary Hyperplasia

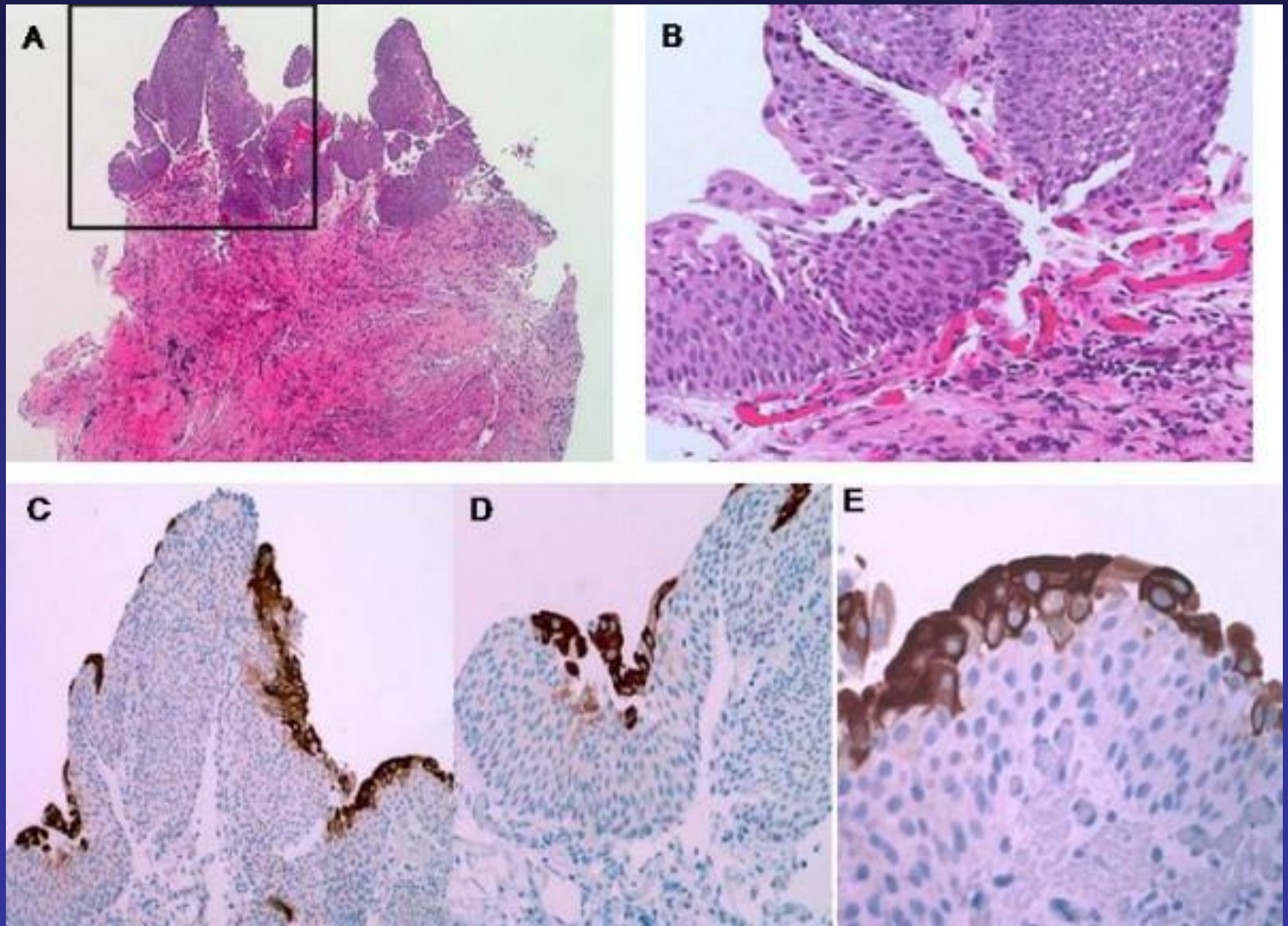

Dysplasia, low grade

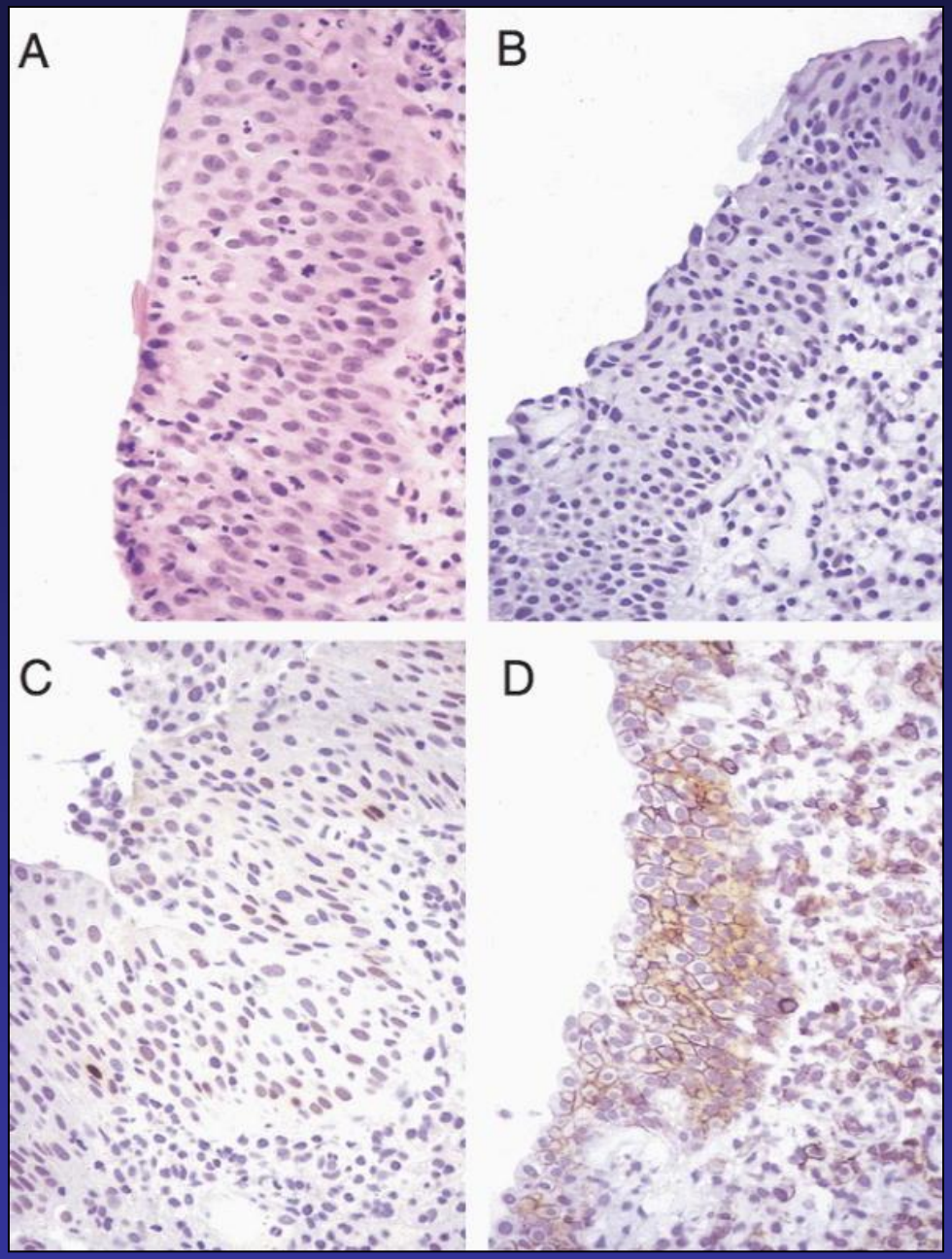




Reactive urothelium and CK20





CK20

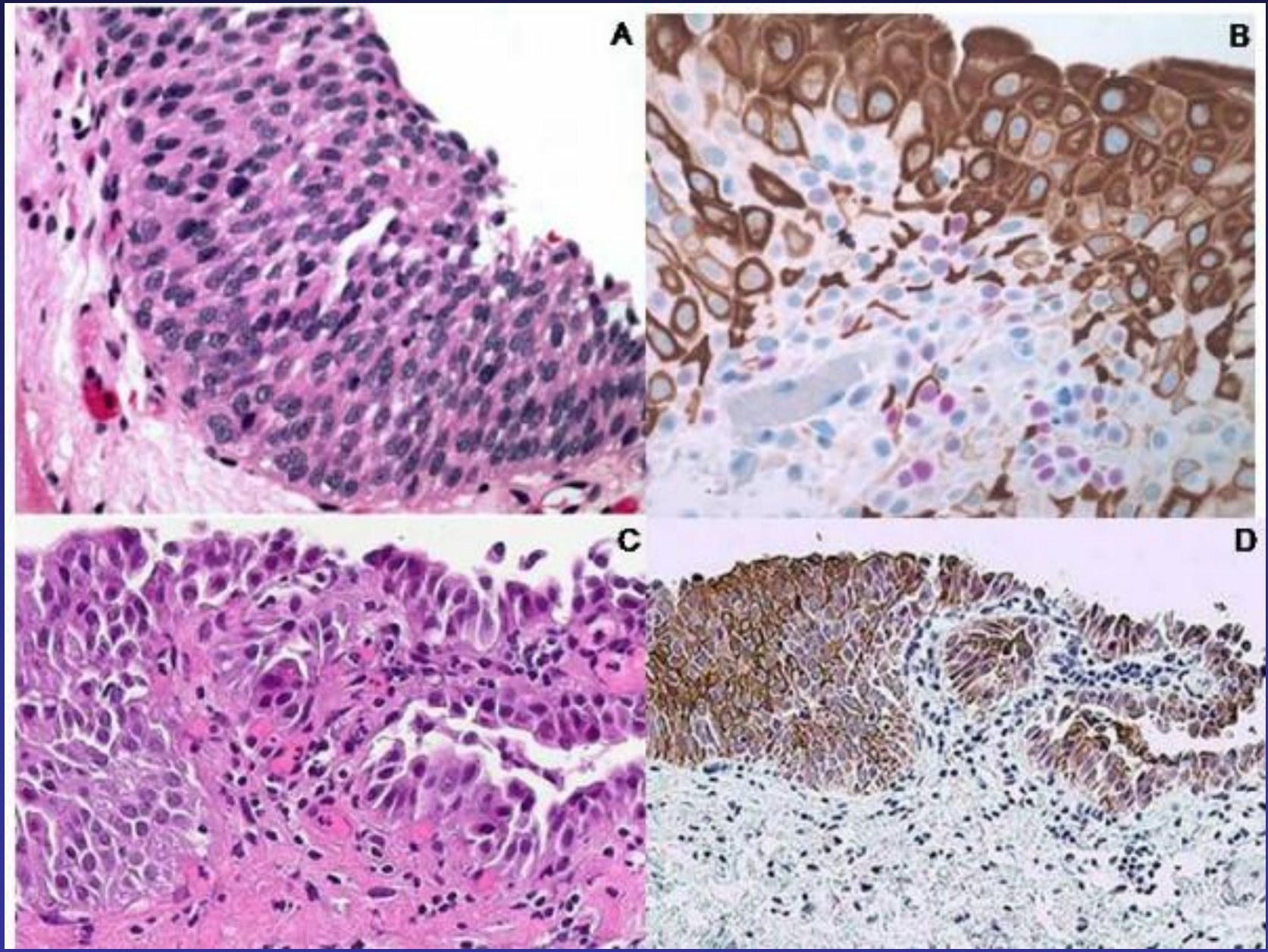
p53

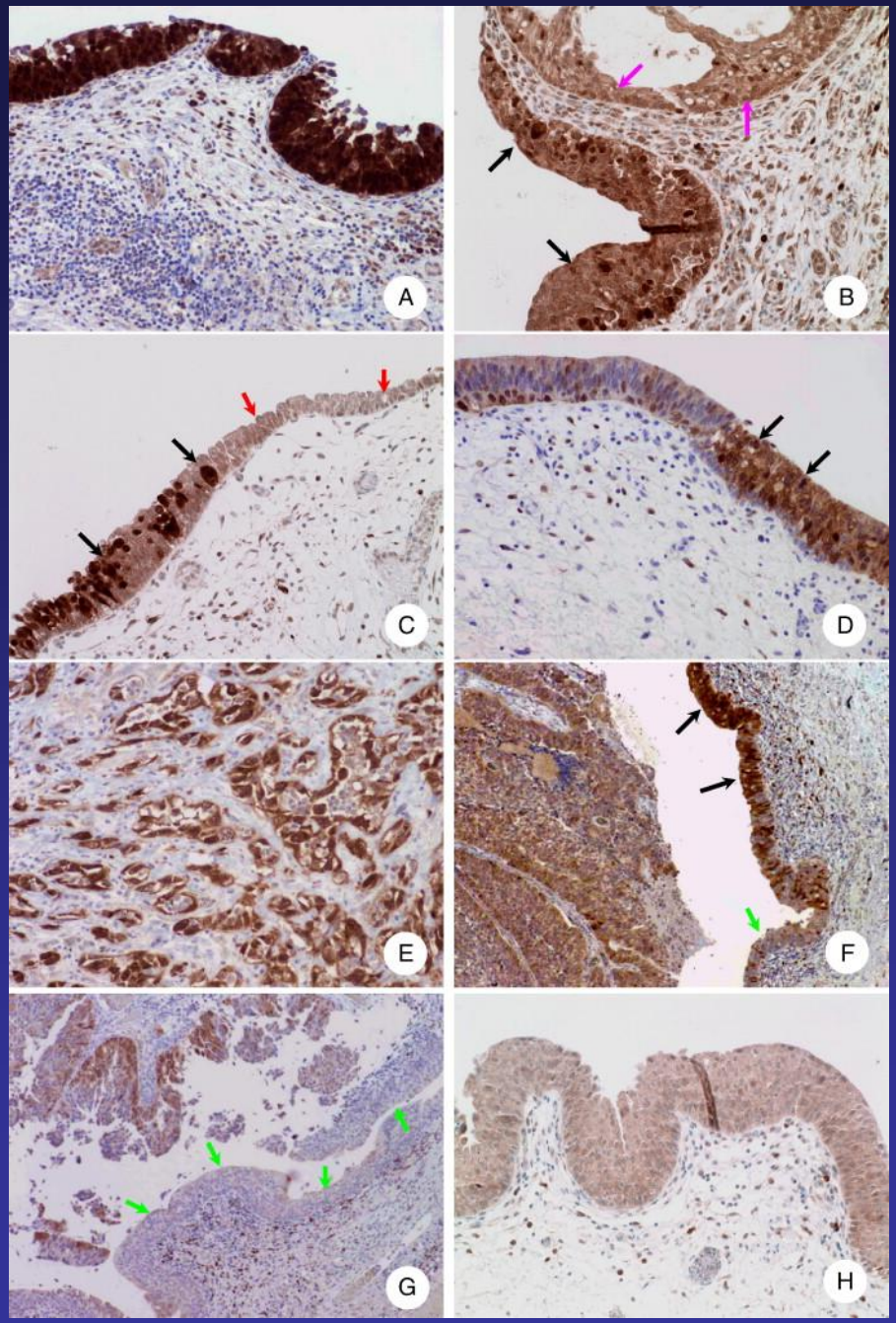
CD44

AJSP 25:1074, 2001

Reactive urothelial atypia

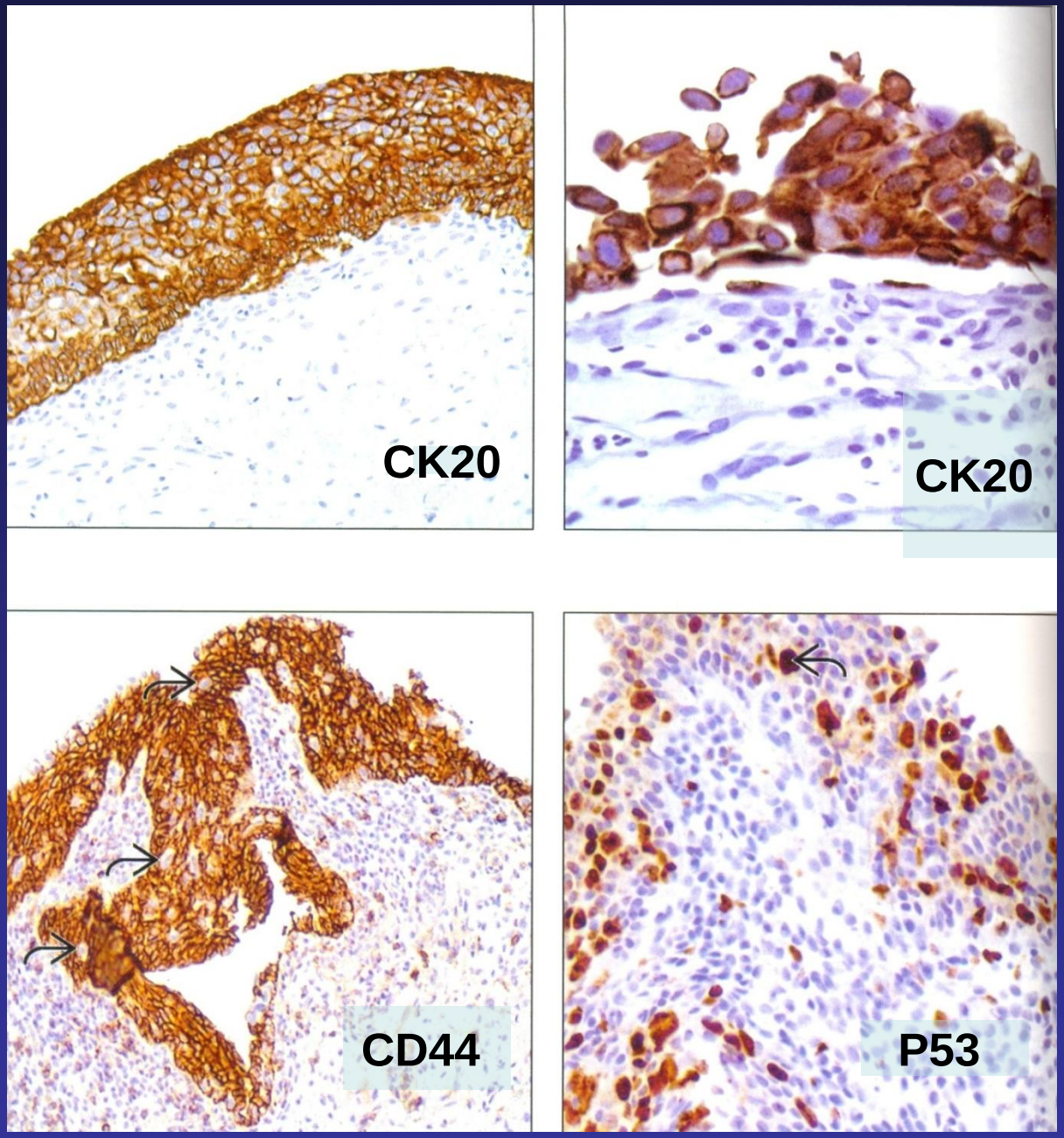
Dysplasia and CK20





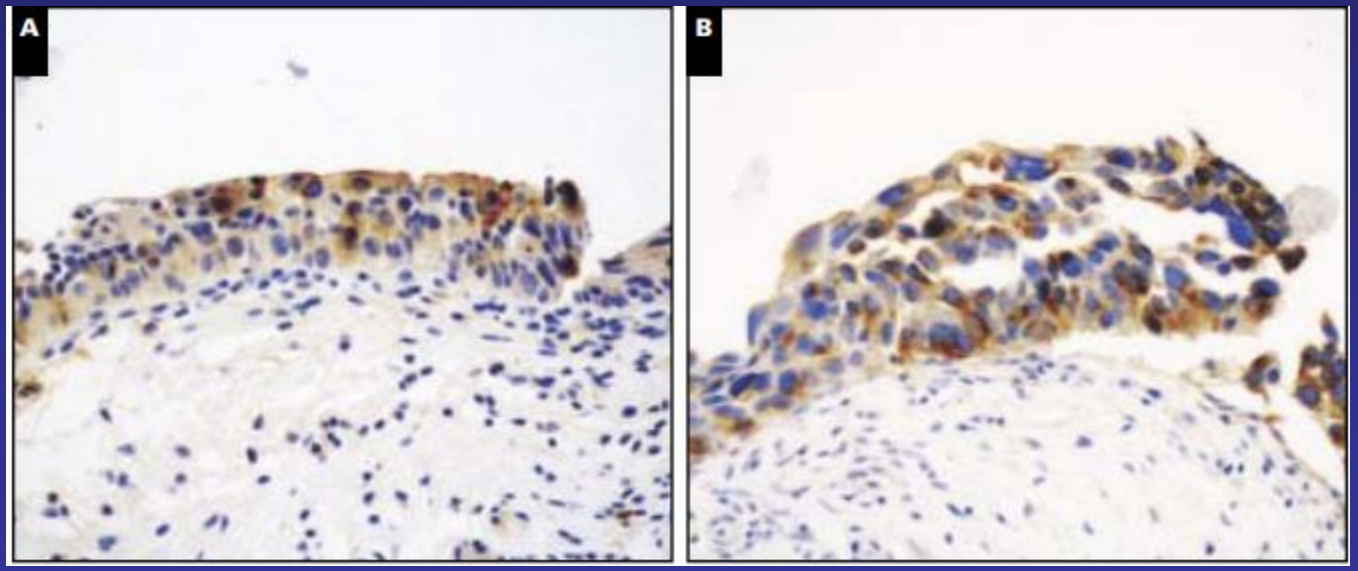
P16 immunoreactivity

Yin et al:
Hum Pathol
39:527-35, 2008



Oliva E, et al: Hum Pathol 2012 Nov 27. pii: S0046-8177(12)00312-7.

CD10 in Urothelial cells



Am J Clin Pathol 2005;124:371-379

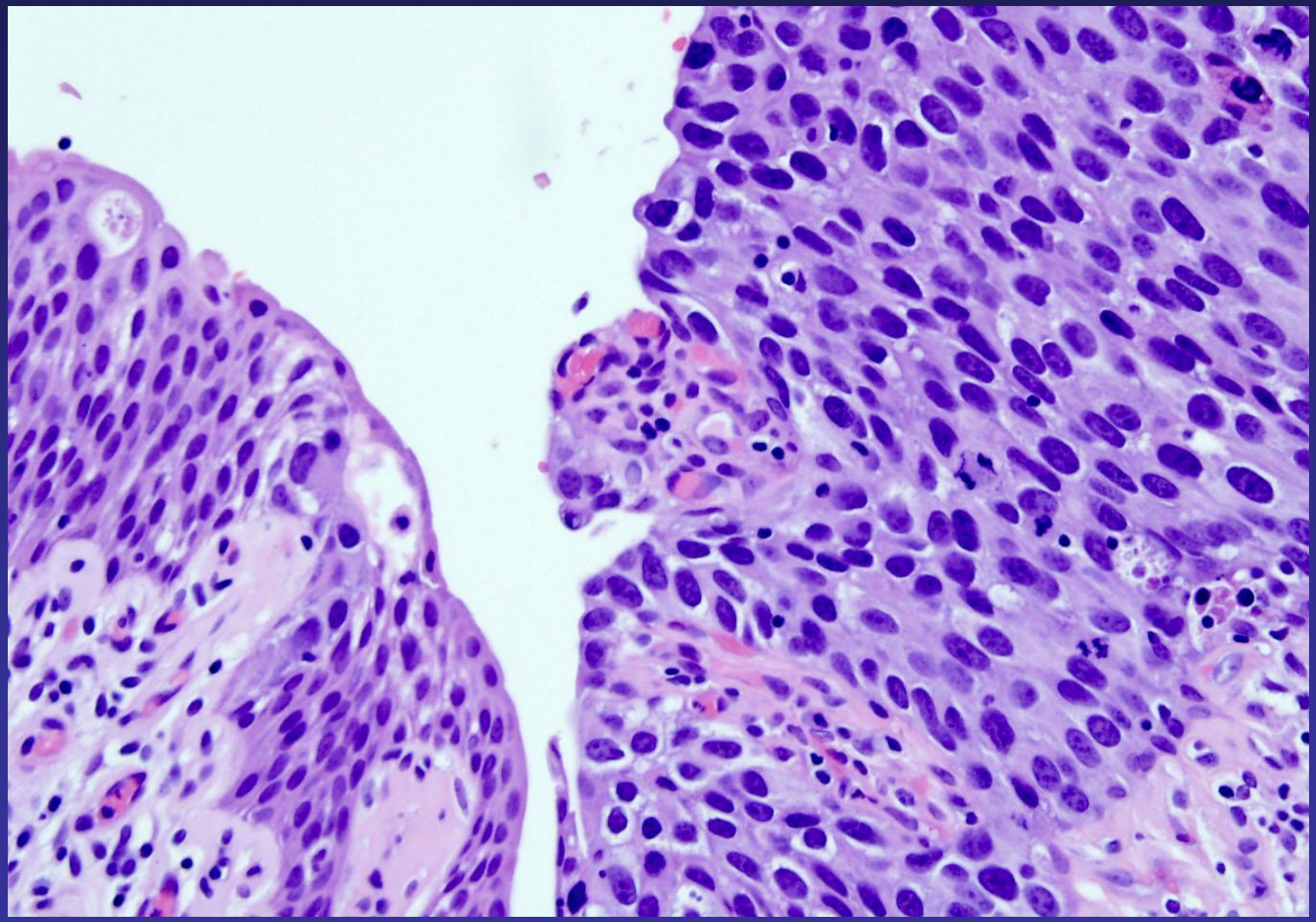
Urothelial Flat Lesions Reproducibility Study

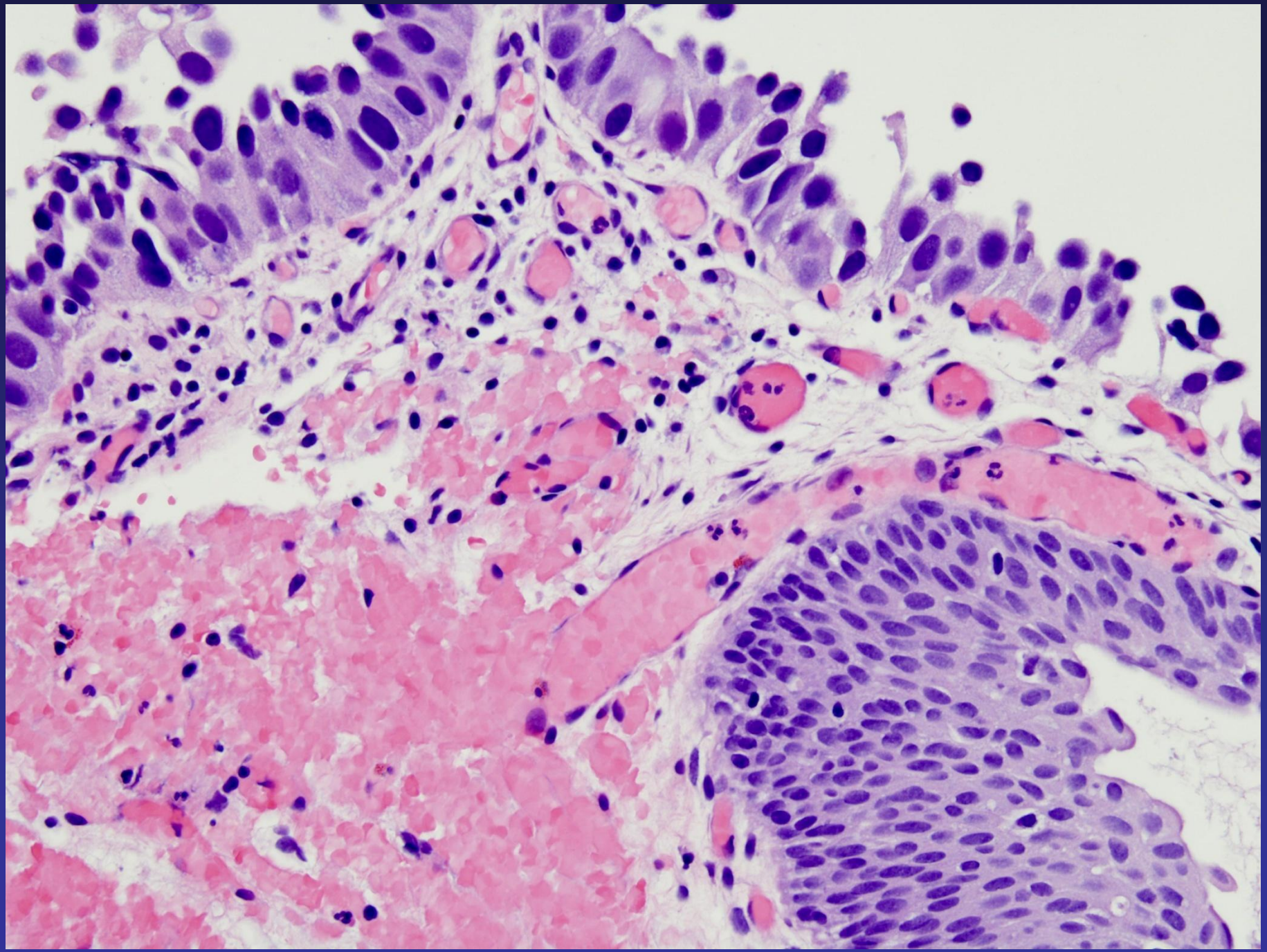
Category	Kappa	Agreement
Normal	0.484	Good
Reactive	0.361	Fair
Atypia ?	0.317	Fair
LGD	0.174	Poor
HGD/CIS	0.653	Excellent
Overall	0.453	Good

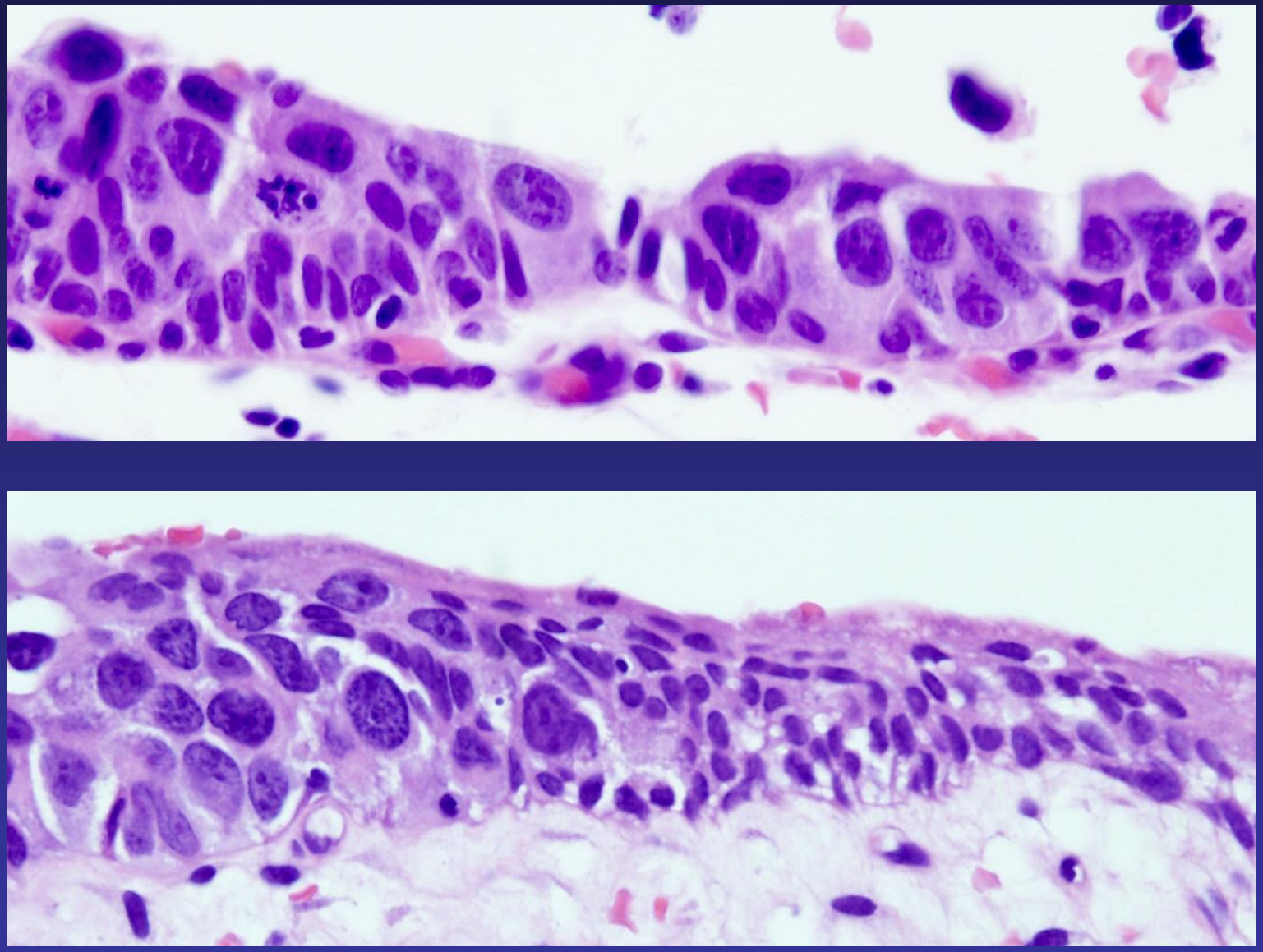
Amin M et al, Modern Pathol, 1997

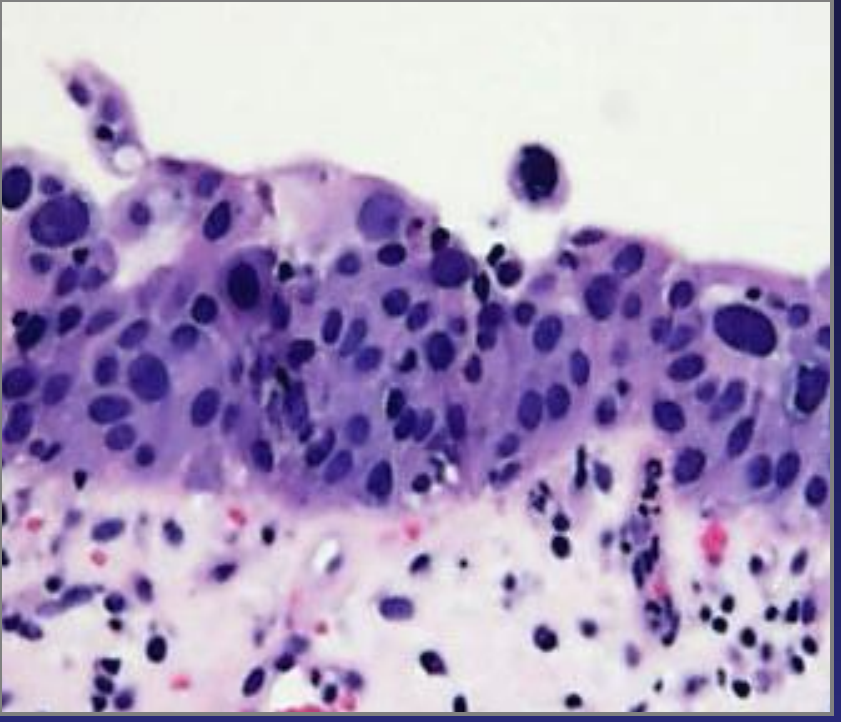
Carcinoma in-Situ

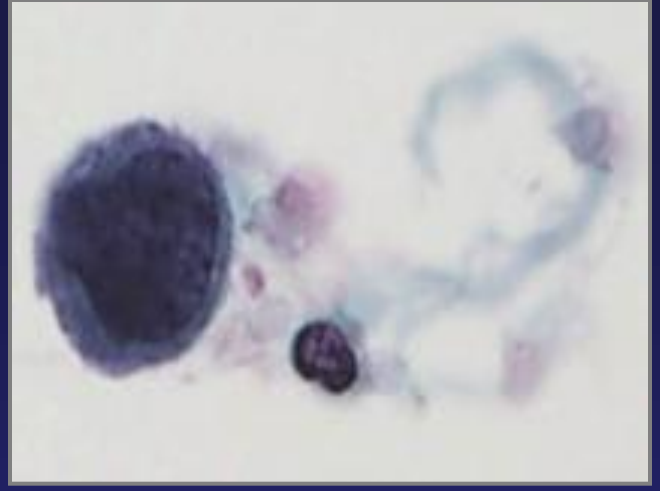
CIS can be best seen by comparison to adjacent normal urothelium



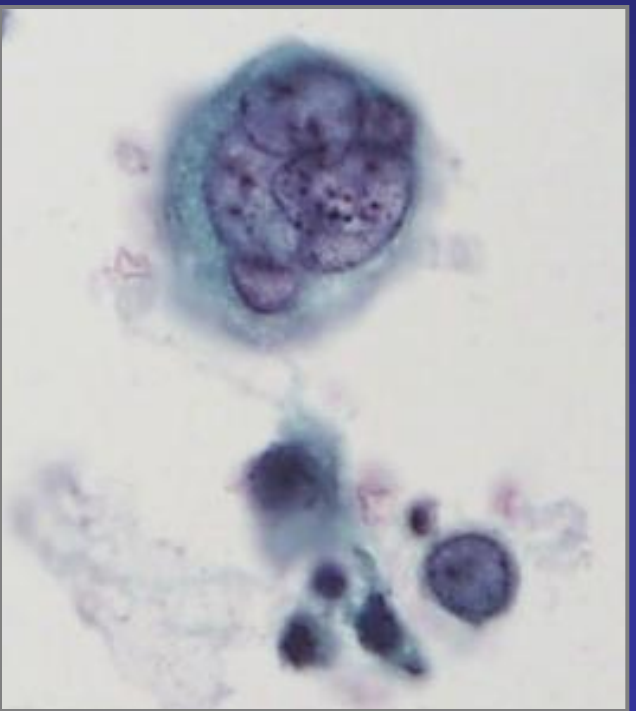


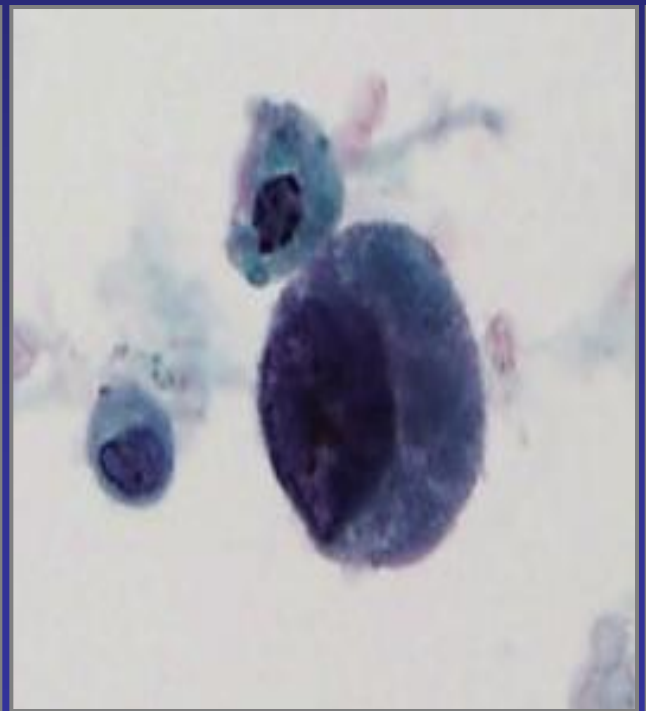


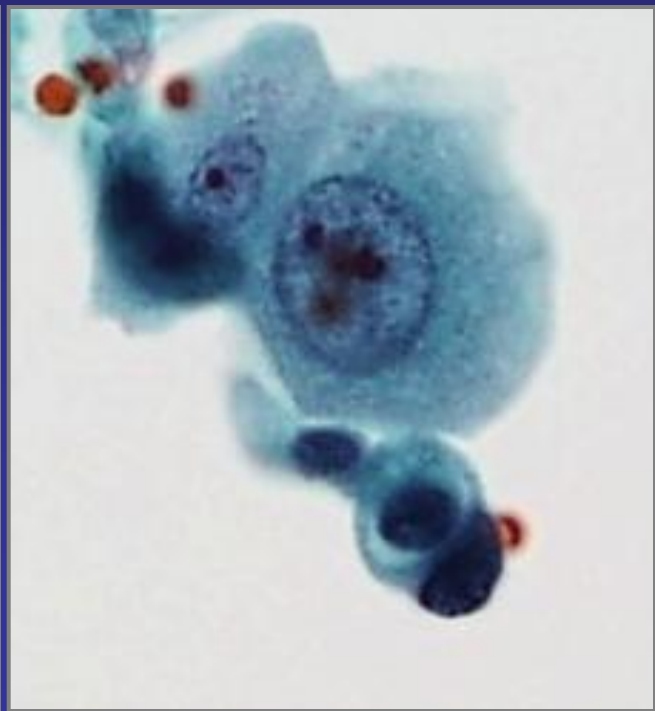




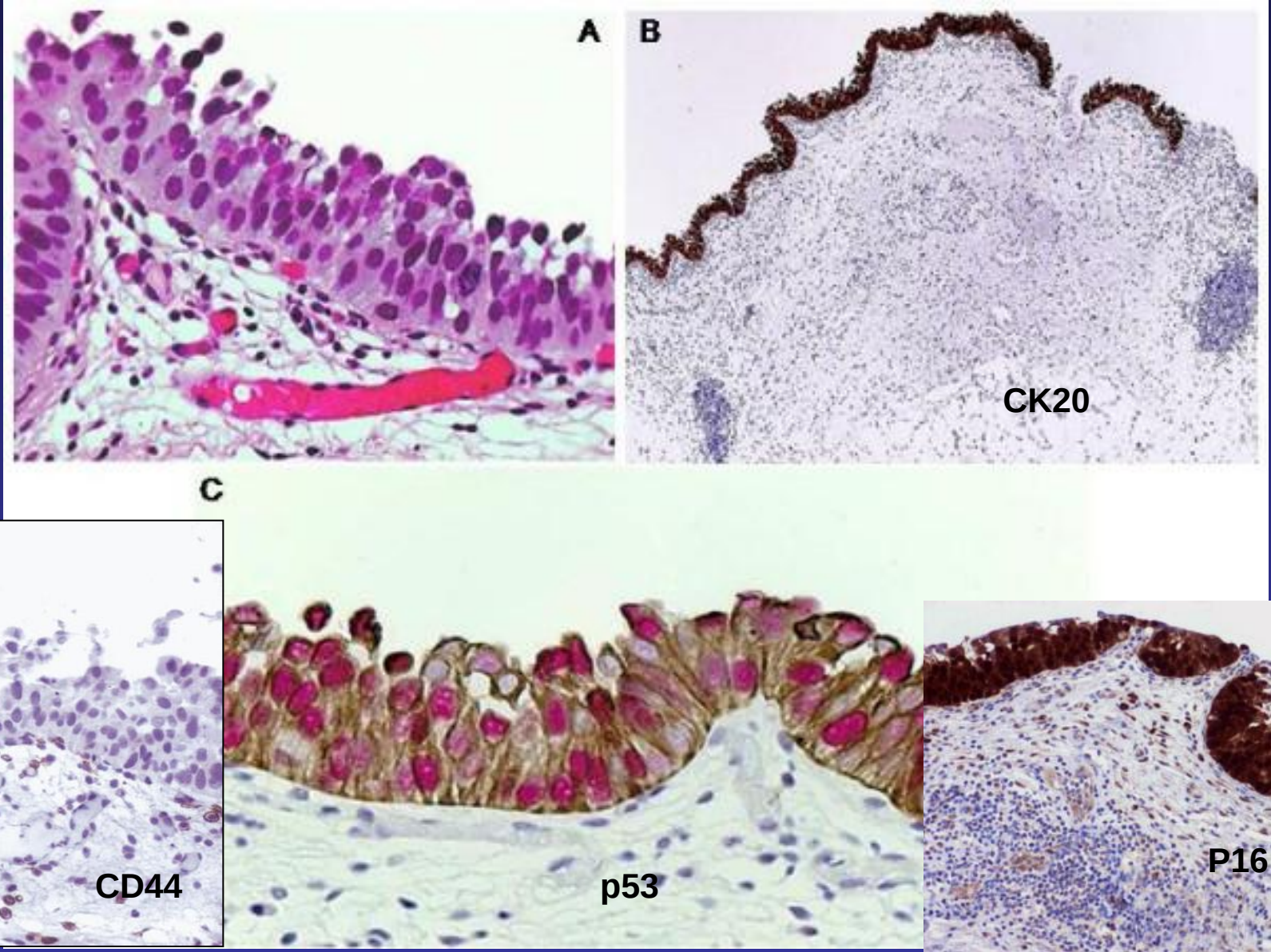
UROTHELIAL CARCINOMA IN SITU

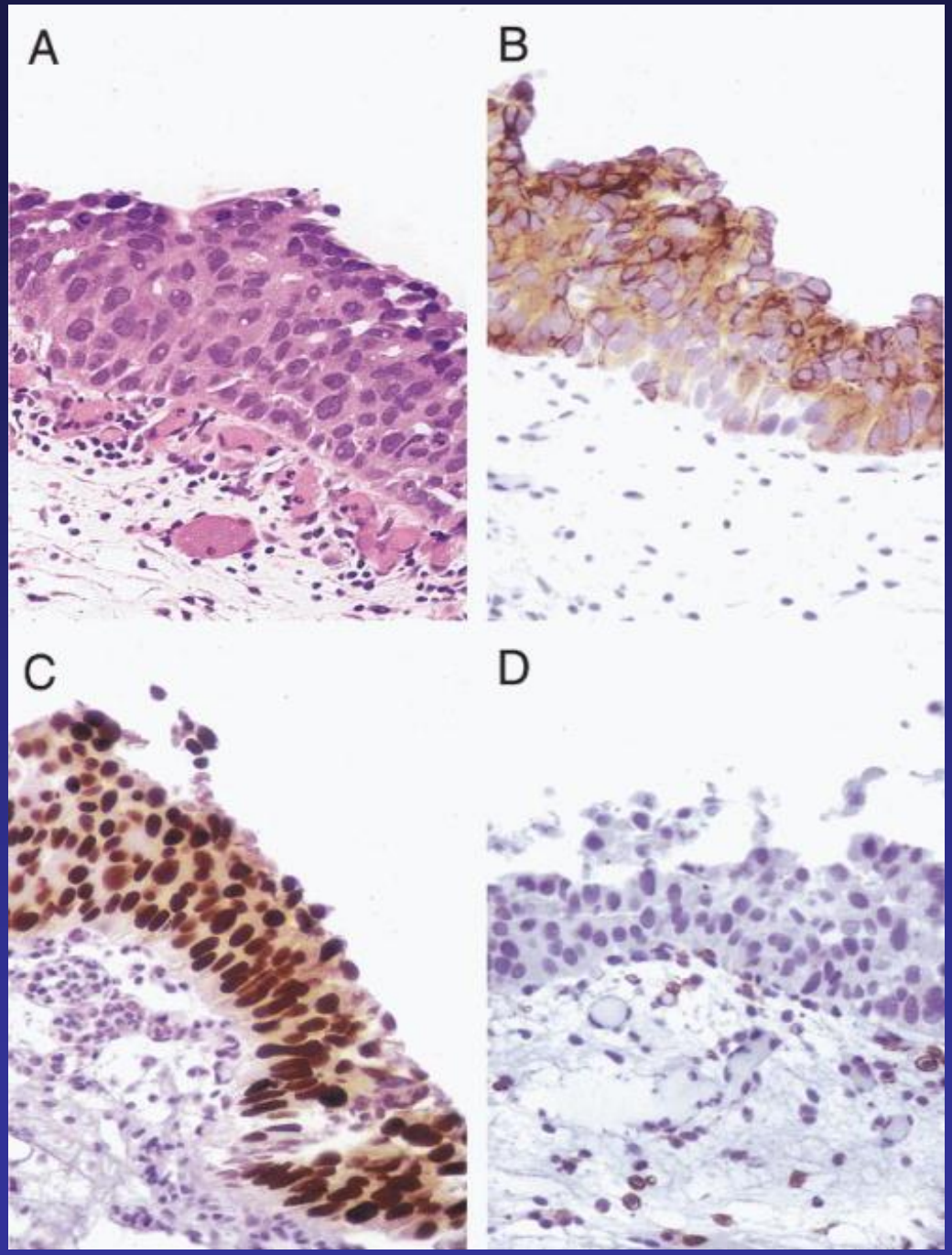






CK20, CD44, p53 and P16 in CIS



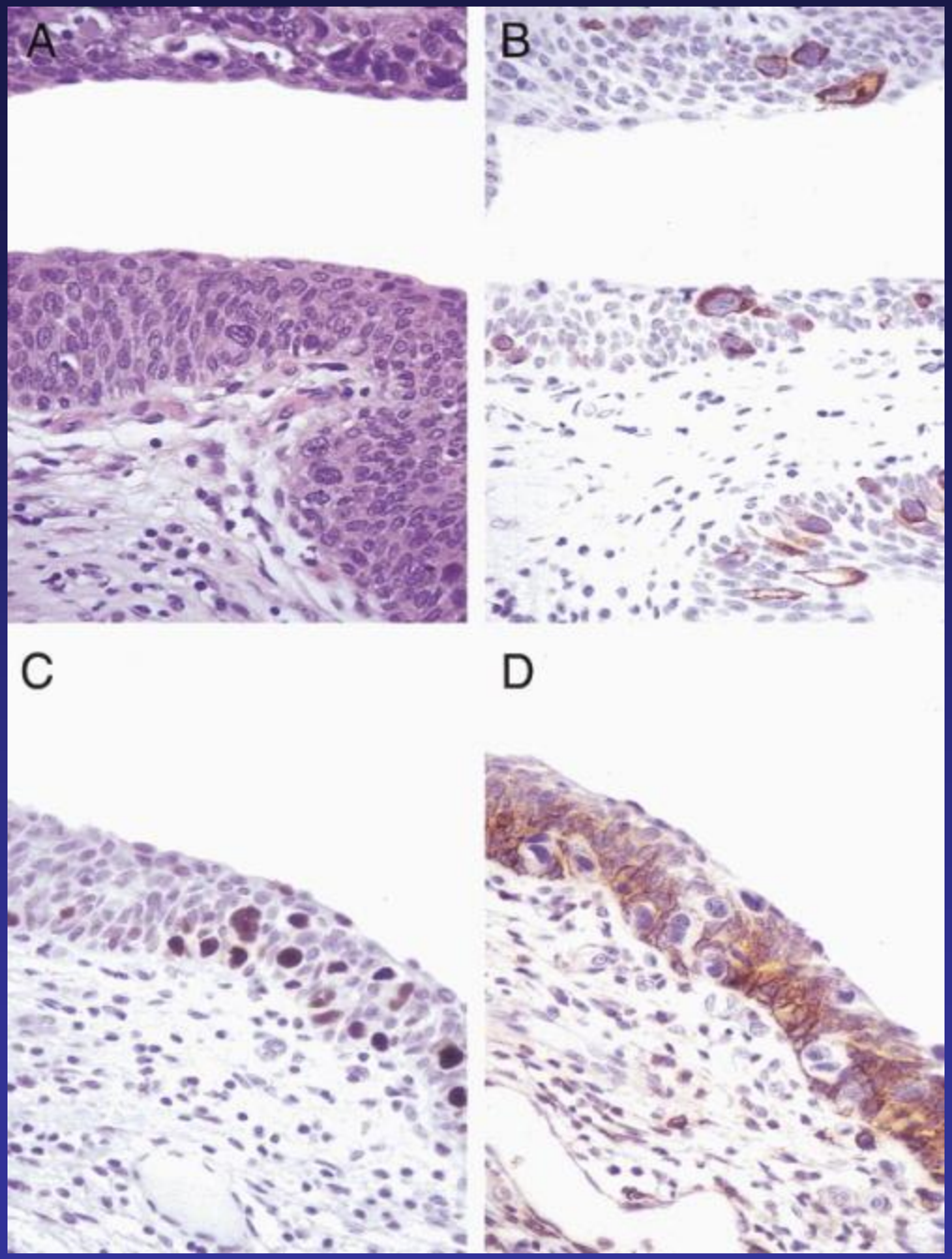


CK20

p53

CD44

AJSP 25:1074, 2001



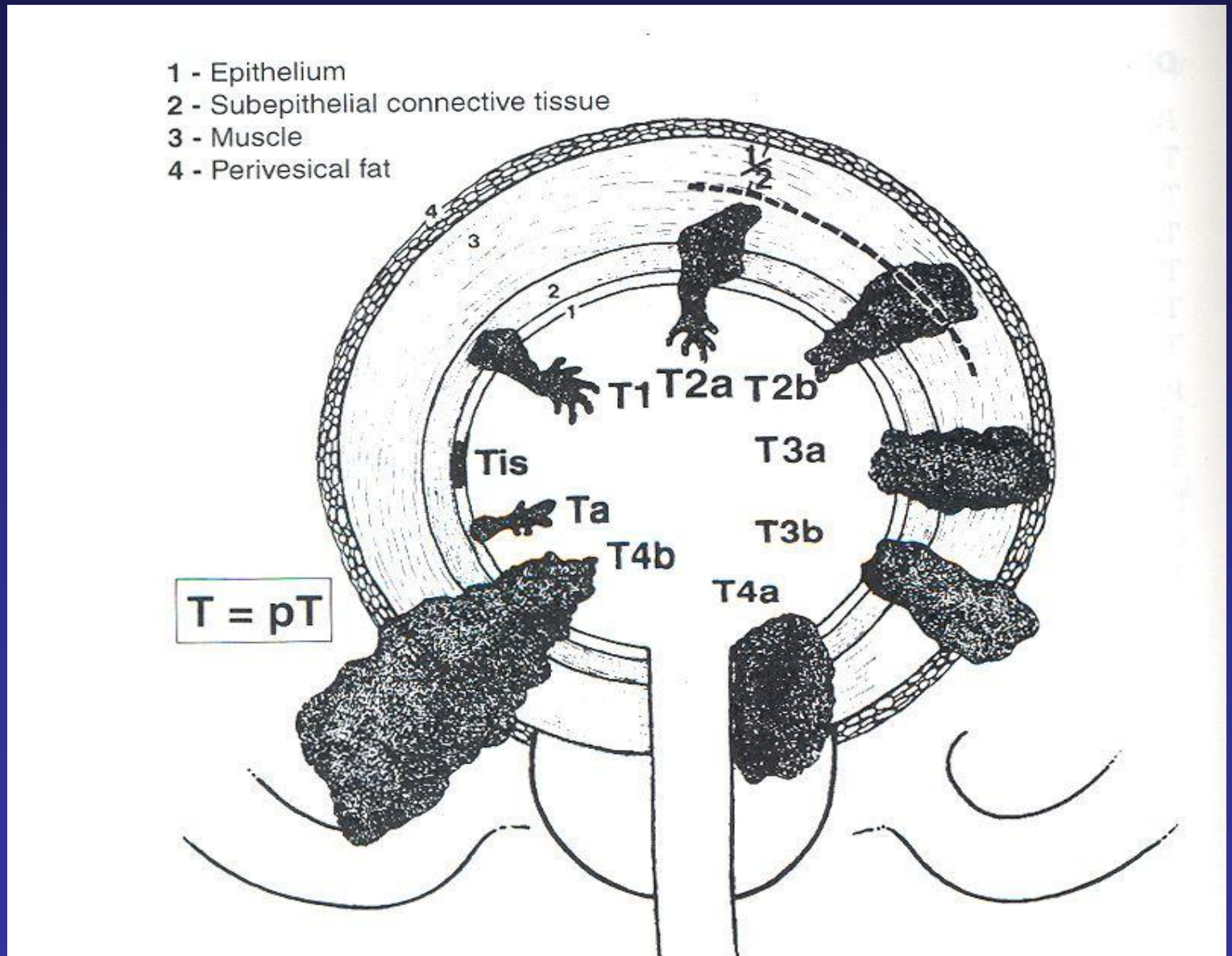
Pagetoid CIS

CK20

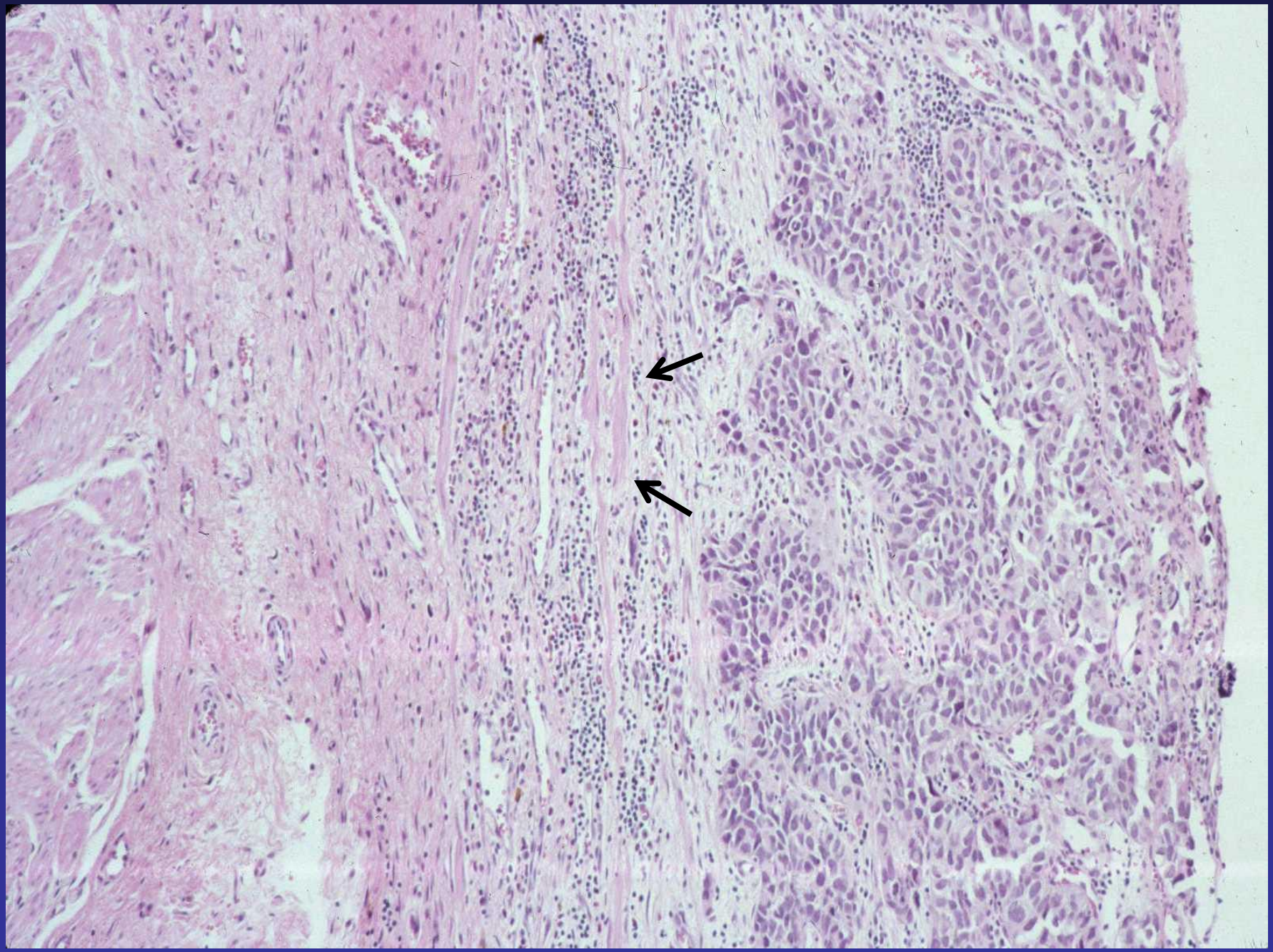
P53 and CD44

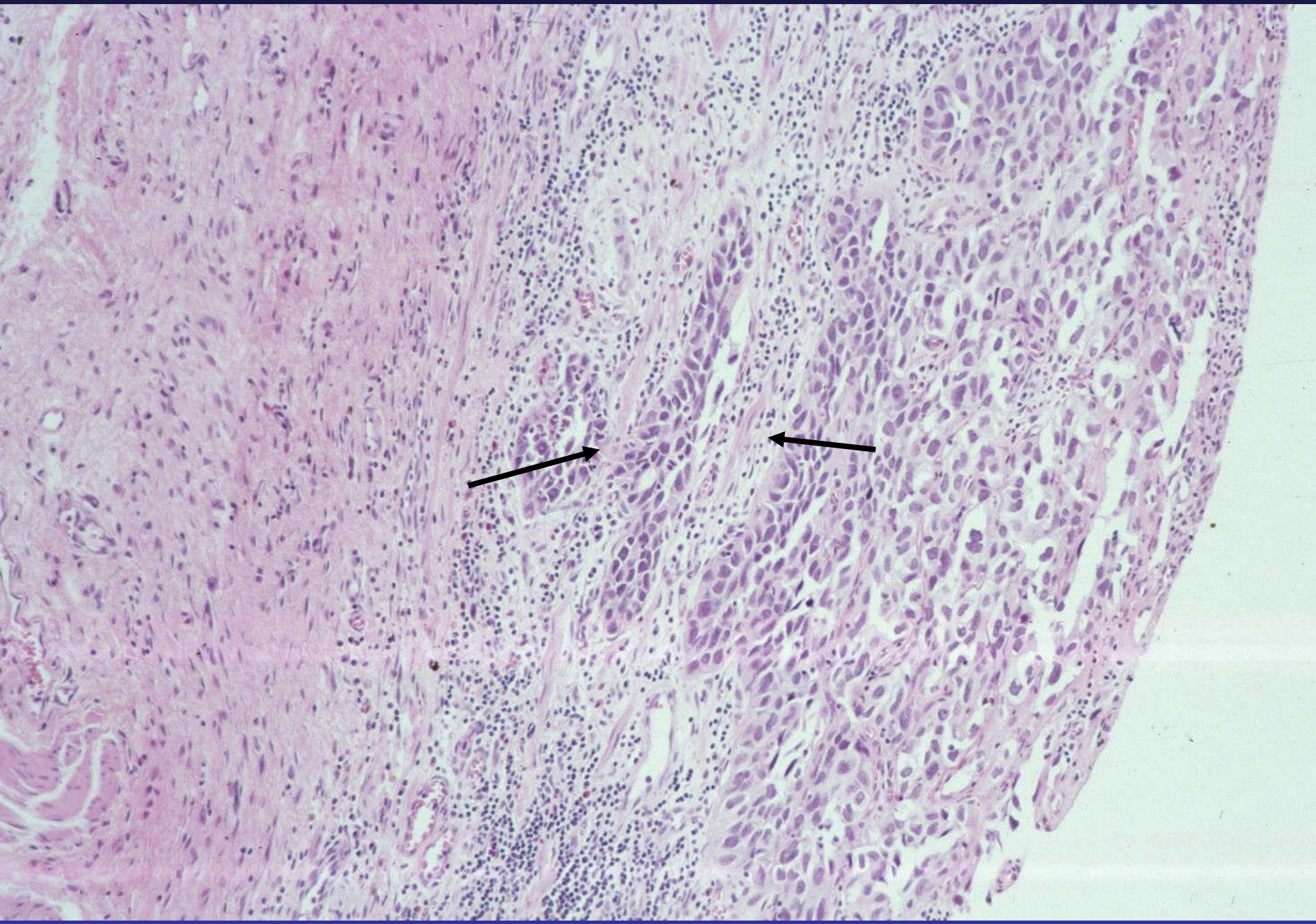
AJSP 25:1074, 2001

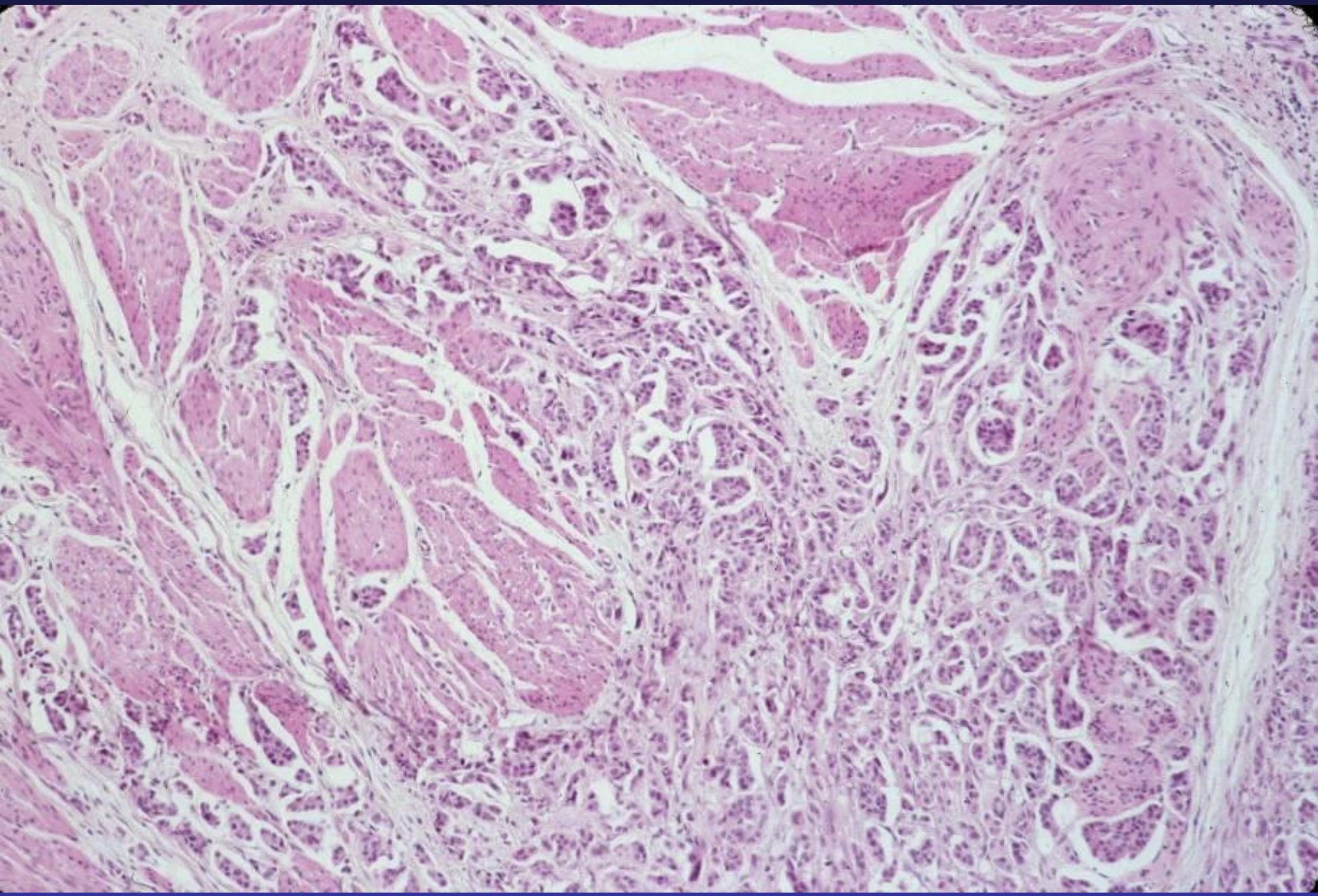
T STAGES OF BLADDER CANCER

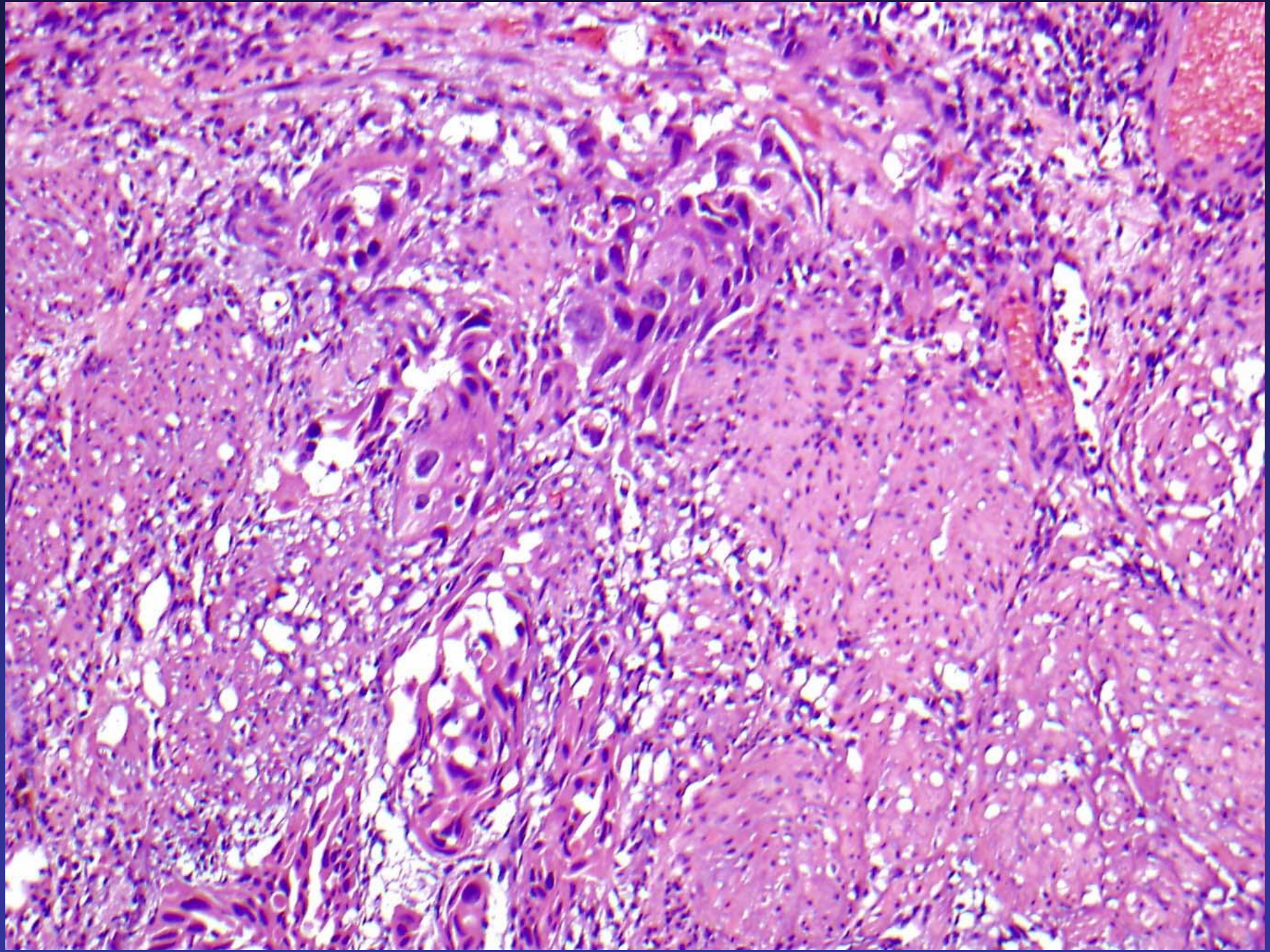


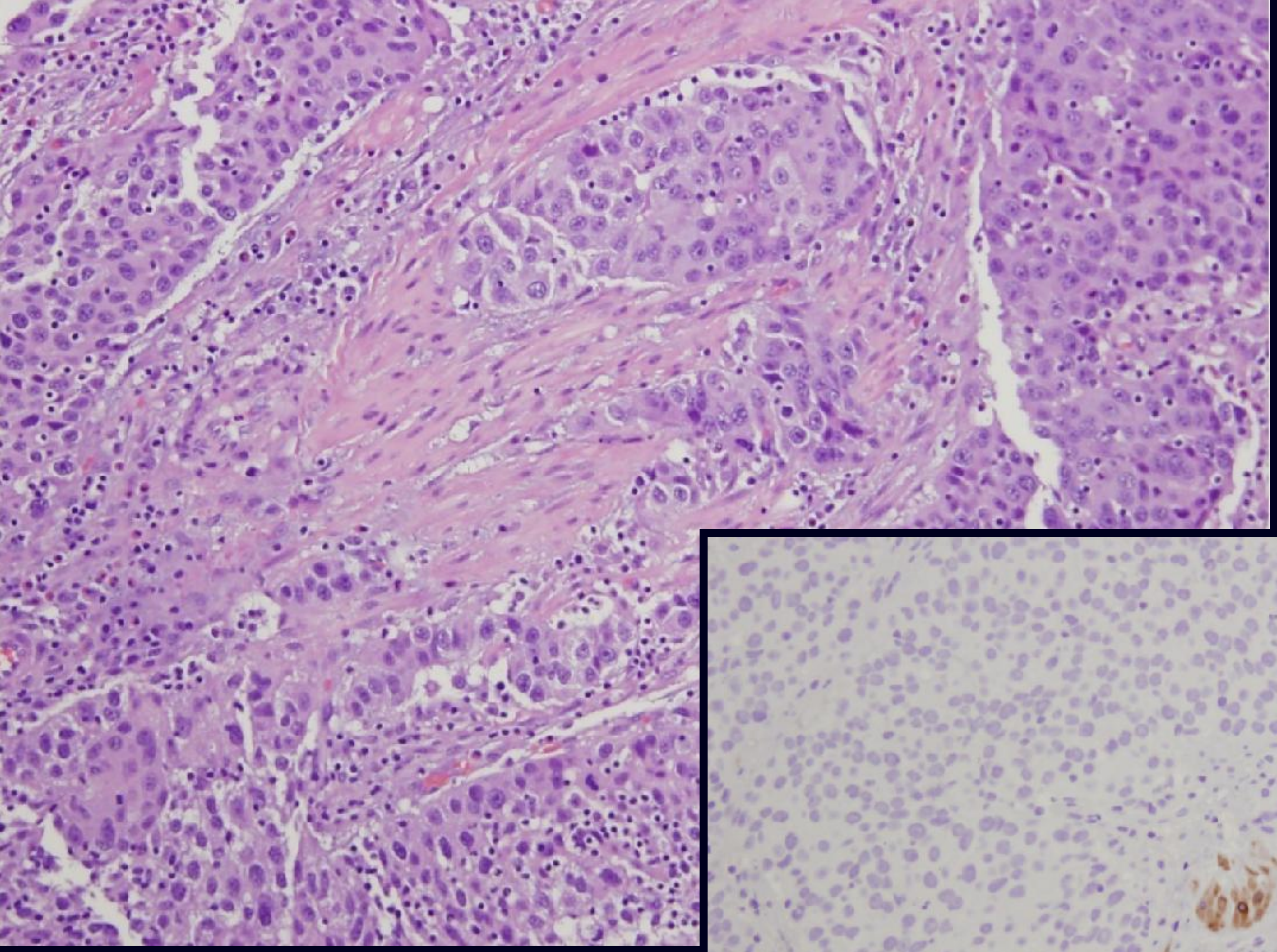
Lamina Propria vs. Muscle Proper Invasion

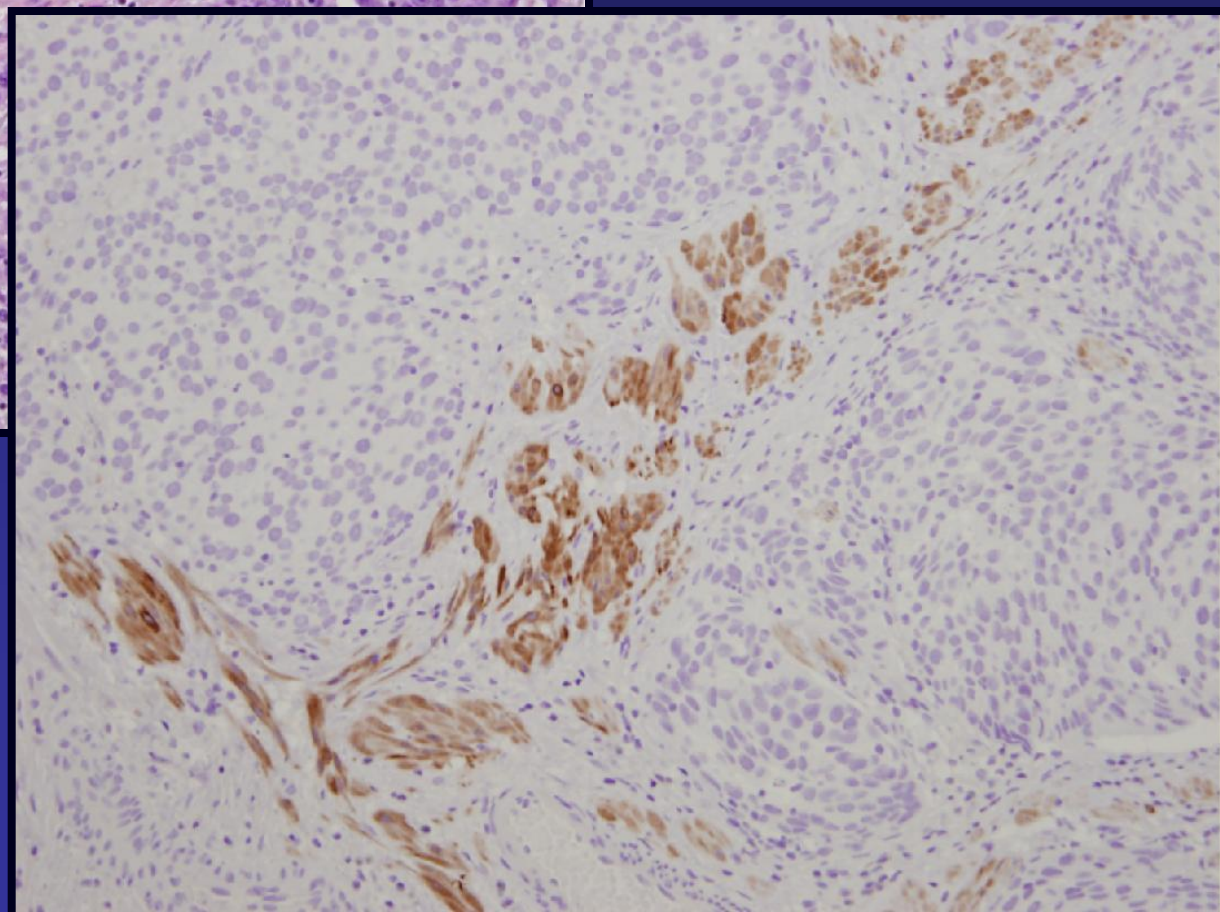




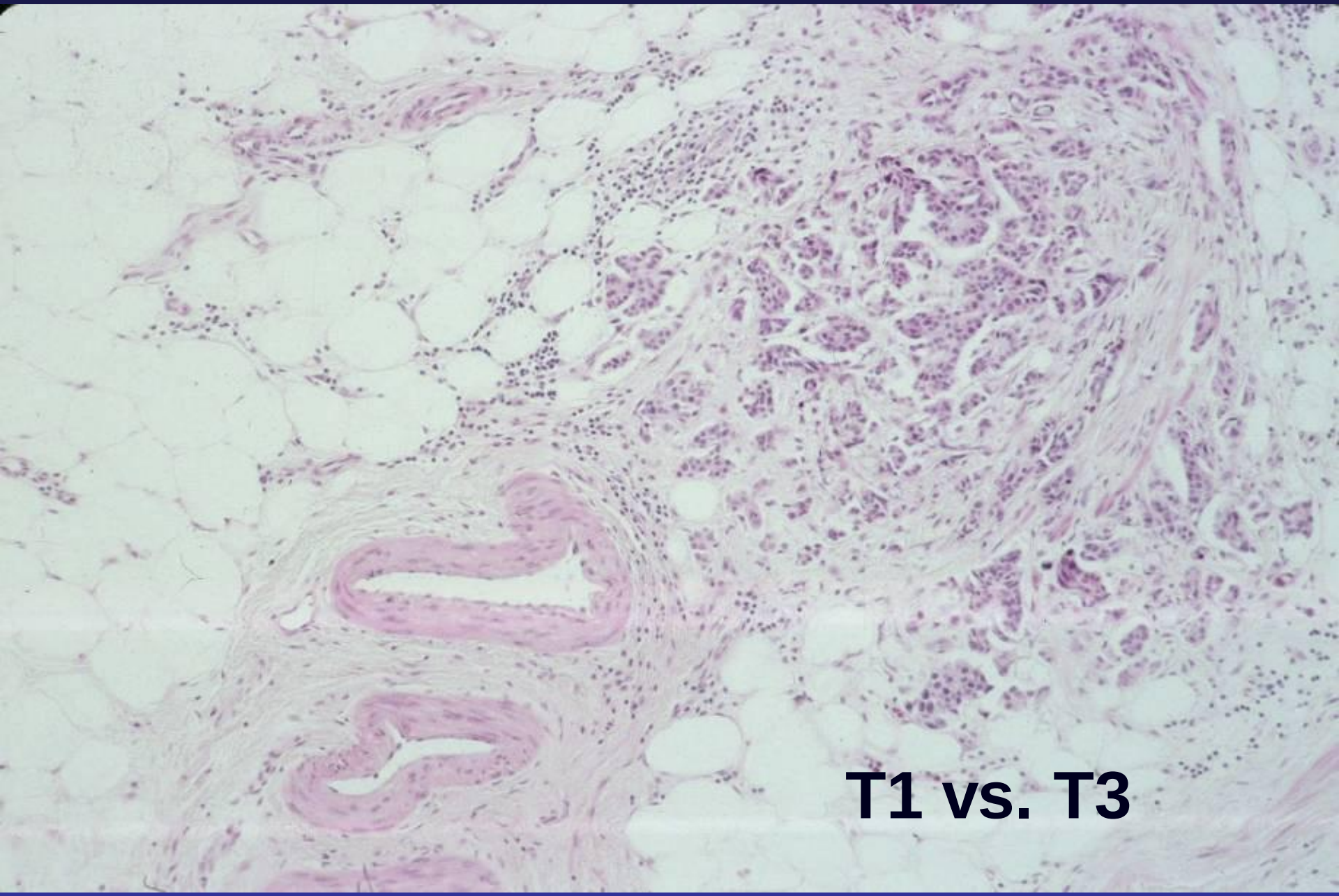


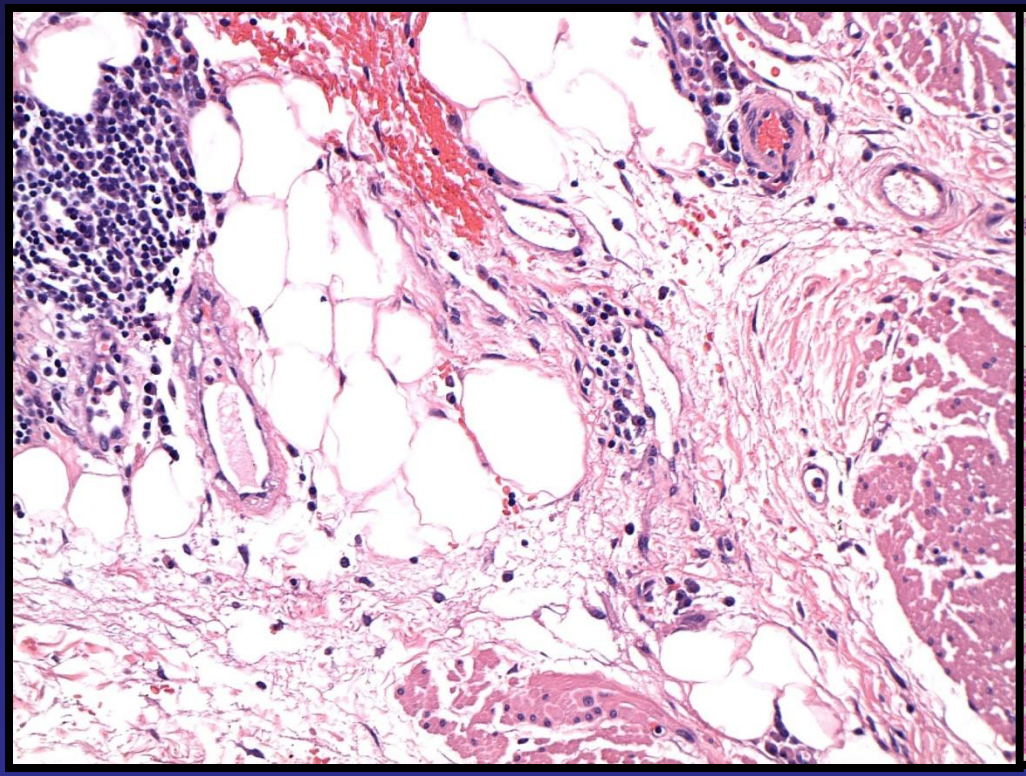


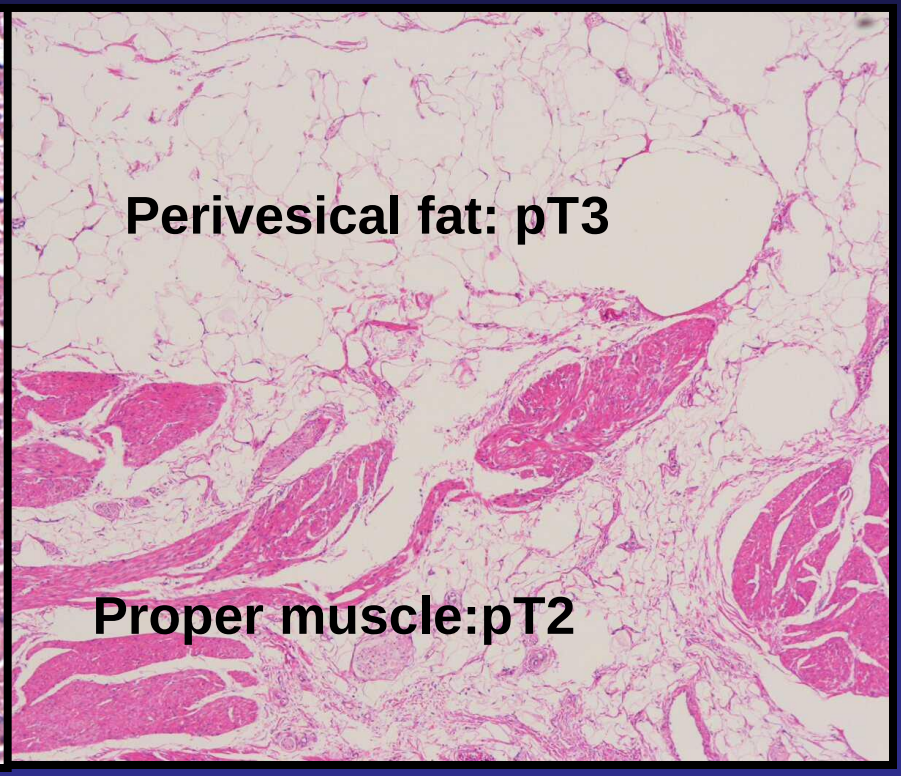




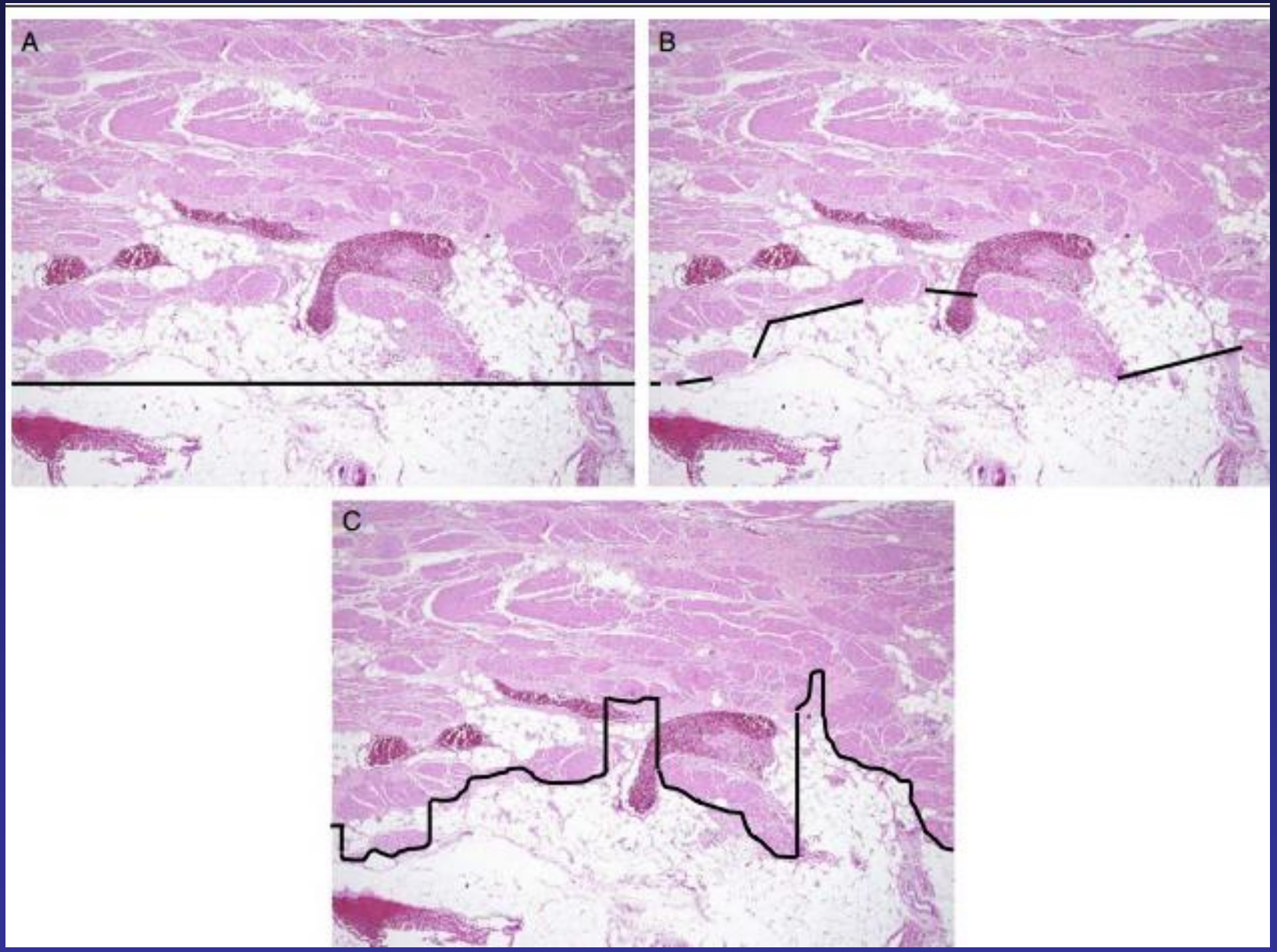
Fat invasion


T1 vs. T3



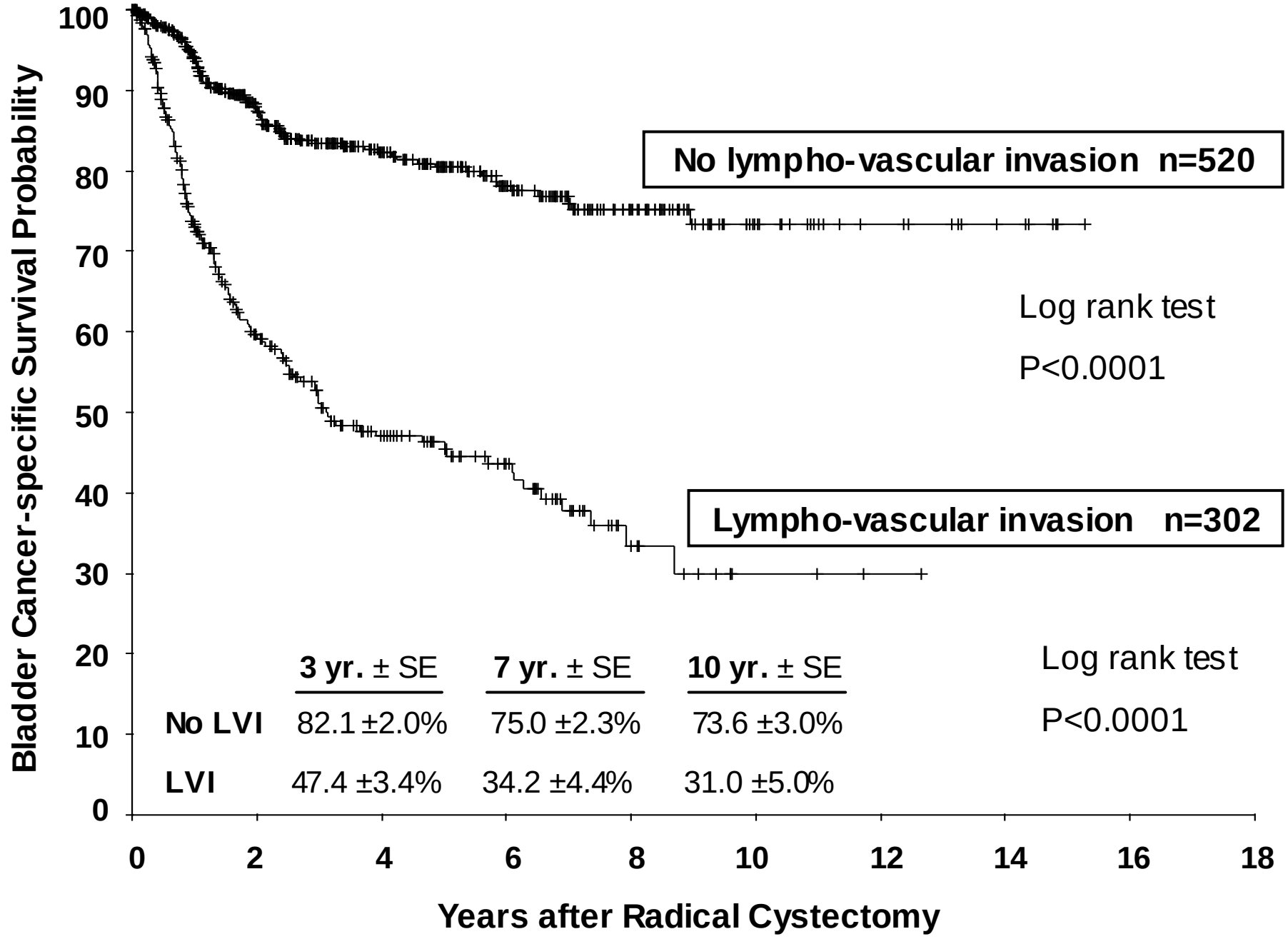


Am J Surg Pathol. 2000 Sep;24(9):1286-90. (Amin and Ro)

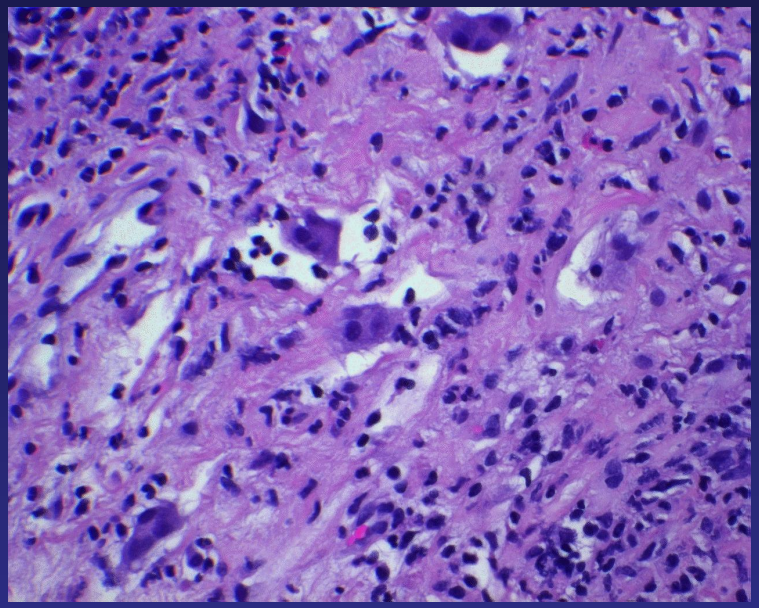


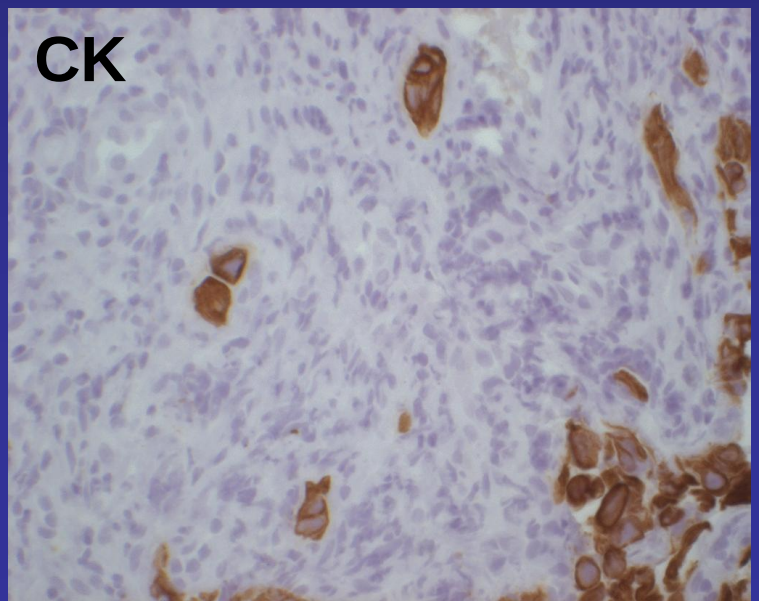
Am J Surg Pathol. 2013 Oct 18. [Epub ahead of print]

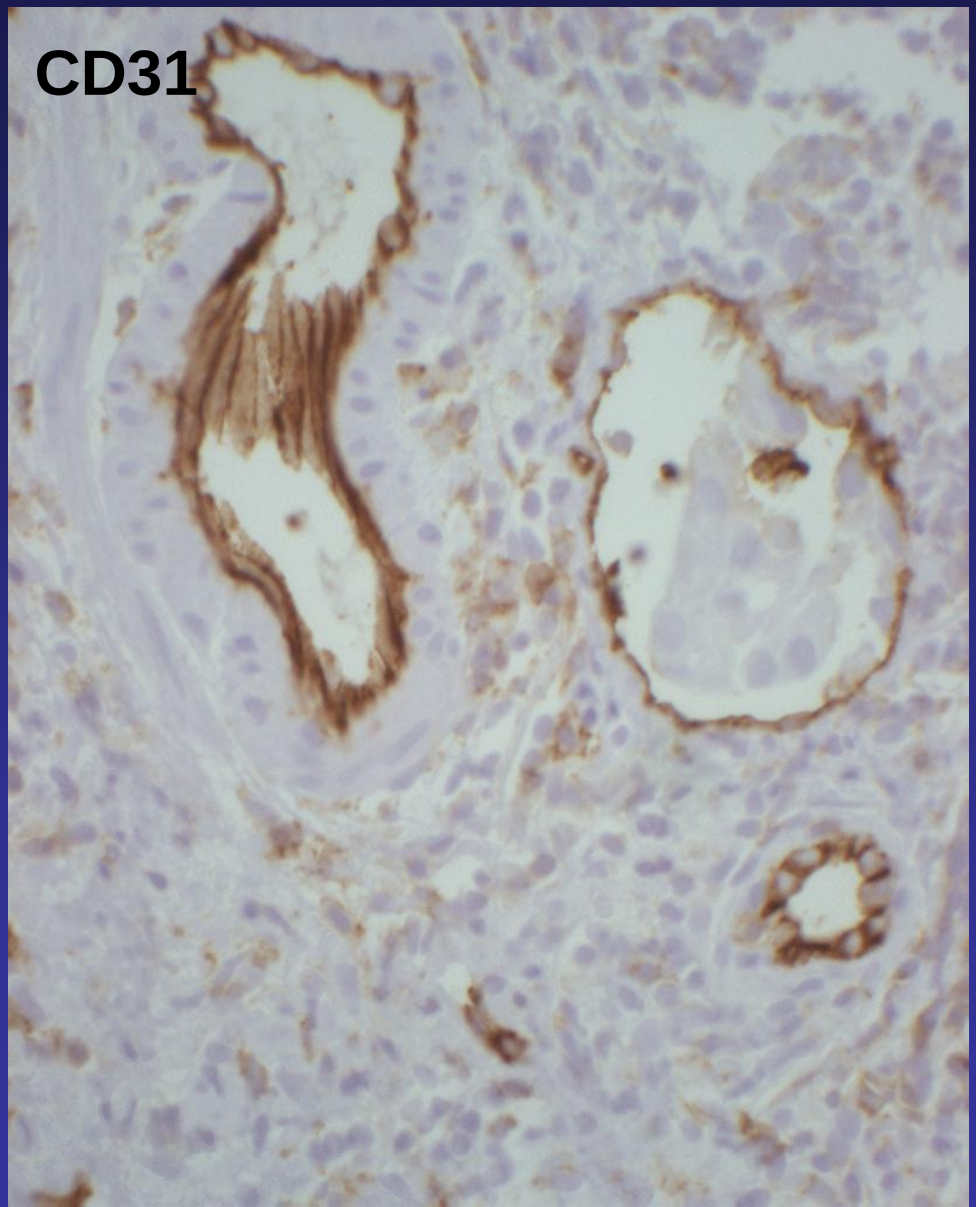
Lymphovascular Invasion

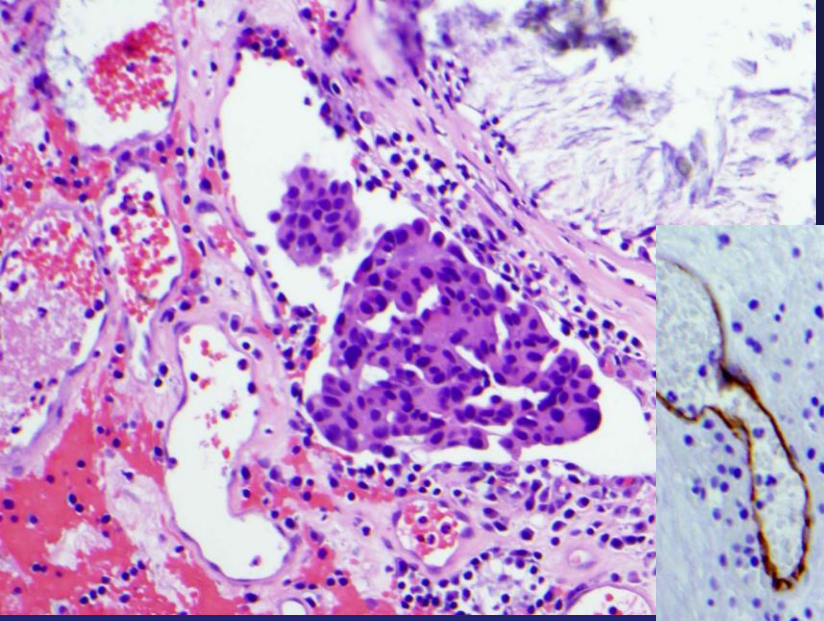

Bladder Cancer-specific Survival Probability
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
Years after Radical Cystectomy
No lympho-vascular invasion n=520
Lympho-vascular invasion n=302
Log rank test
P<0.0001
3 yr. ± SE
7 yr. ± SE
10 yr. ± SE
No LVI
82.1 ±2.0%
75.0 ±2.3%
73.6 ±3.0%
LVI
47.4 ±3.4%
34.2 ±4.4%
31.0 ±5.0%
Log rank test
P<0.0001

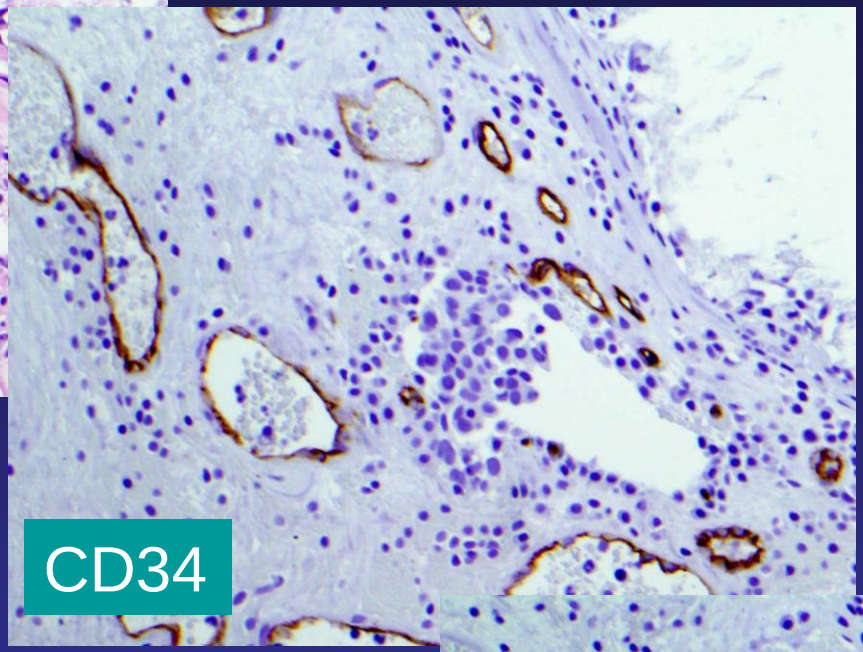
LYMPHOVASCULAR INVASION

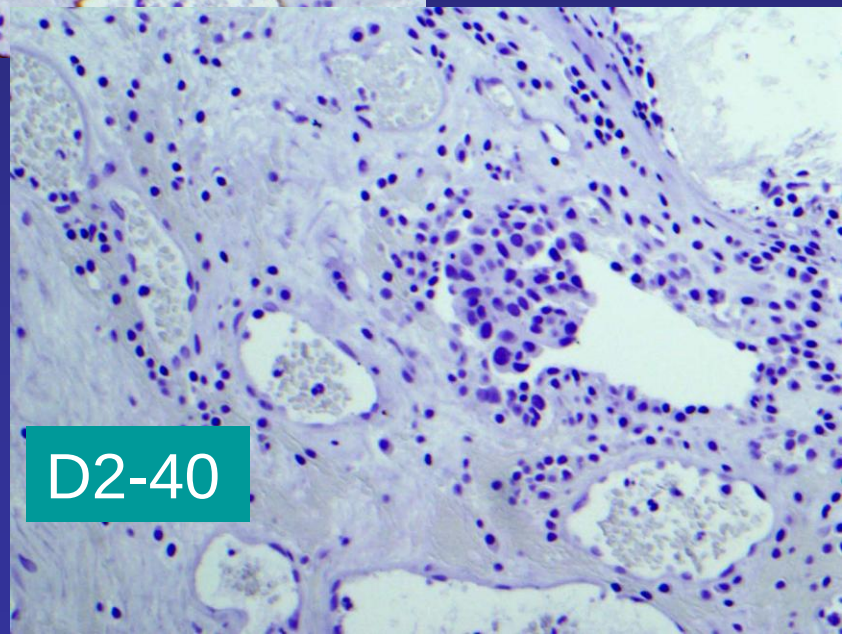












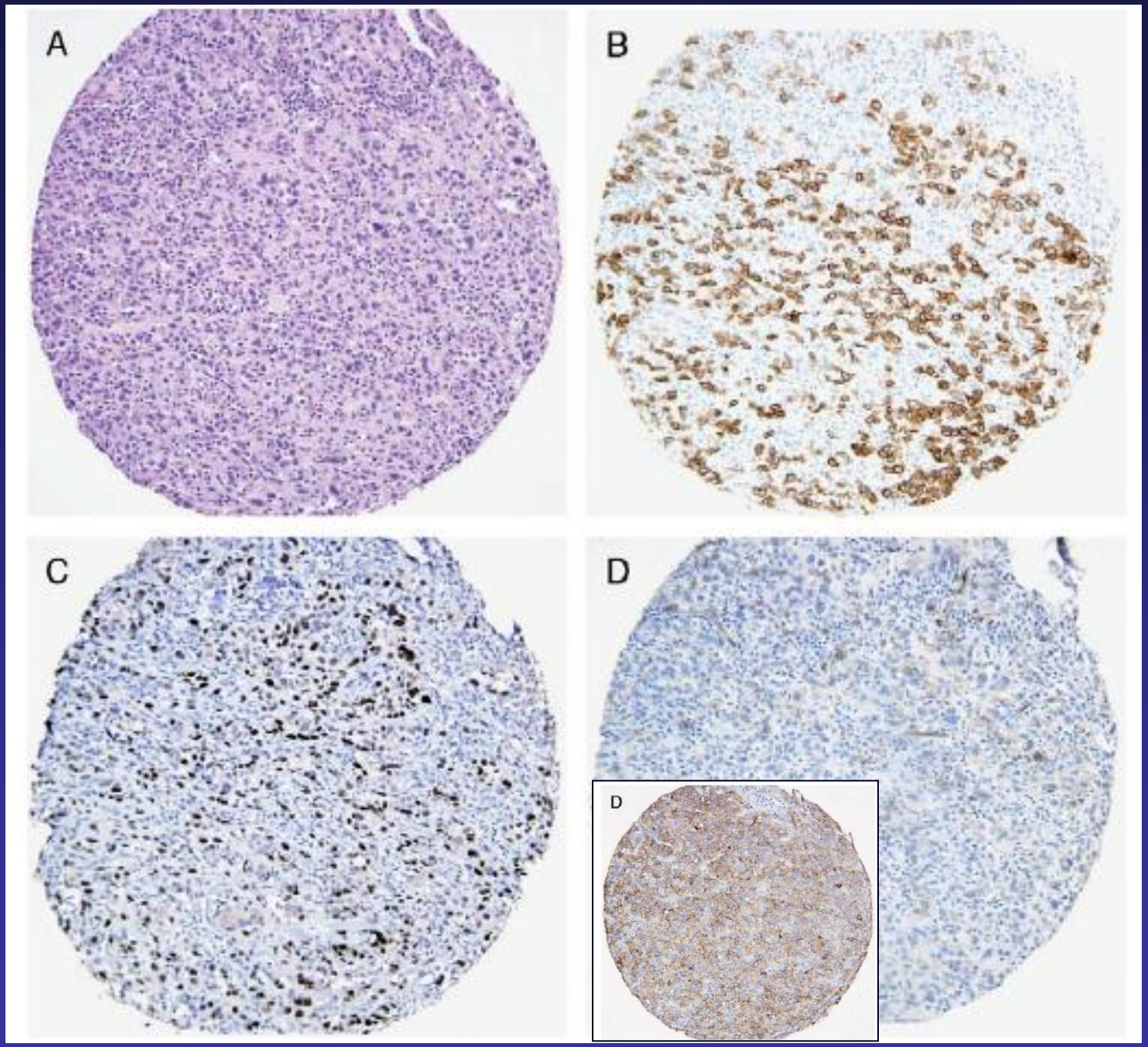
LVI that is not proved by IHC using vascular marker

PITFALLS VASCULAR-LYMPHATIC INVASION

- Retraction of stroma is common
- Large vessel invasion: carry-over due to fragmentation of neoplasm
- One study showed that LVI identified initially was confirmed in only 14% of the cases by using immunohistochemistry

High grade Undiff Ca

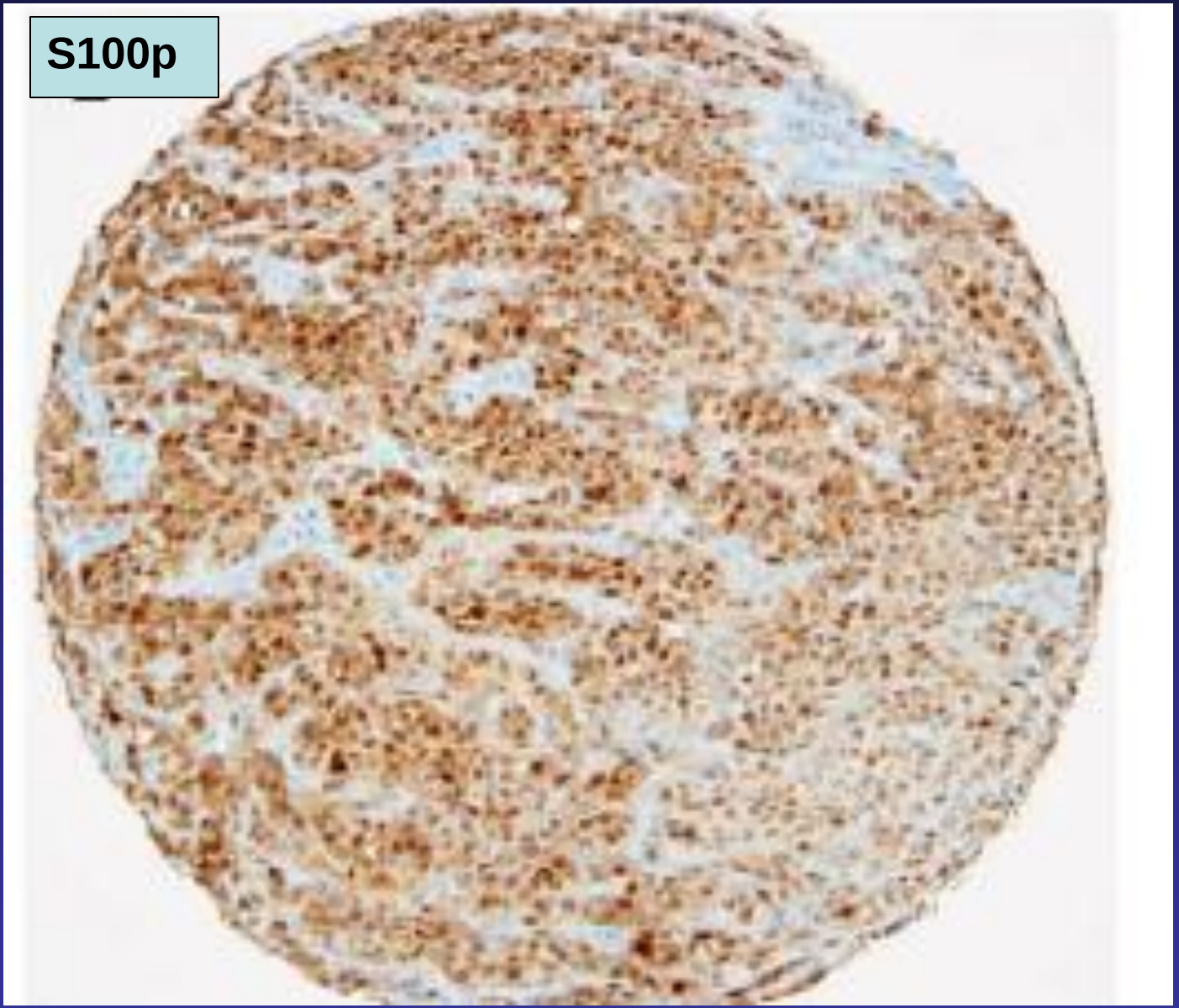
	Urothelial	Prostate
PSA/PAP/PSMA	-	+
HMCK	+	-/+ (6-10%)
Uroplakin	+ (65-100%)	-
Thrombom	+ (49-69%)	-
P63	+ (70-75%)	-/+ (-18%)
CD57	-/+ (- 17%)	+
S100p	+ (78-86%)	-
GATA	+ (67%)	-



HMCK

P63

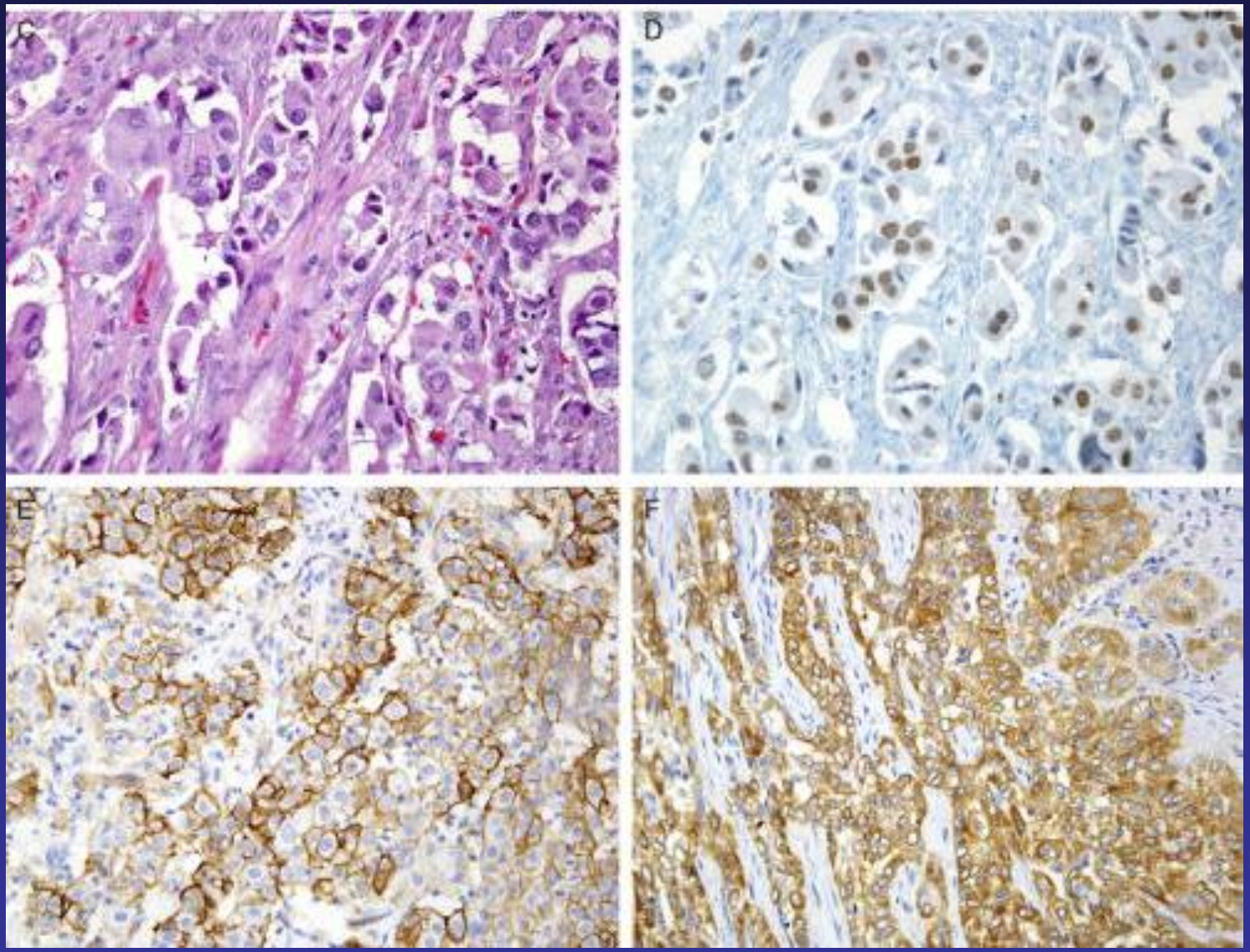
Throm



Am J Surg Pathol 2007;31:1246–1255

UC

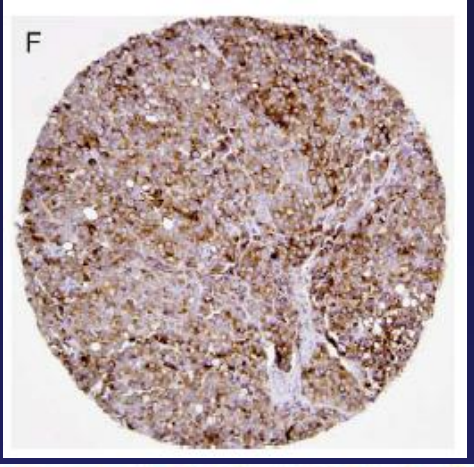
GATA



Throm

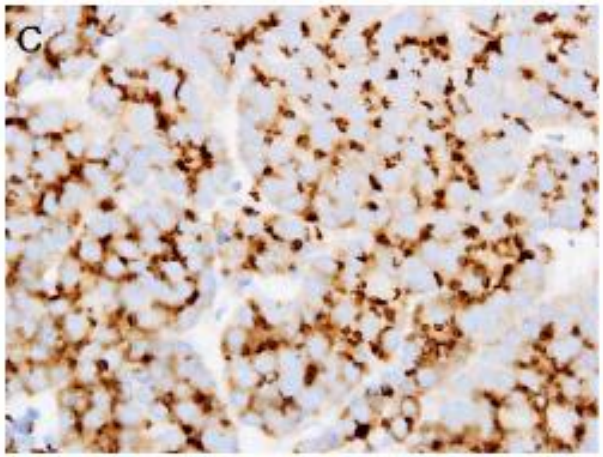
Uroplakin

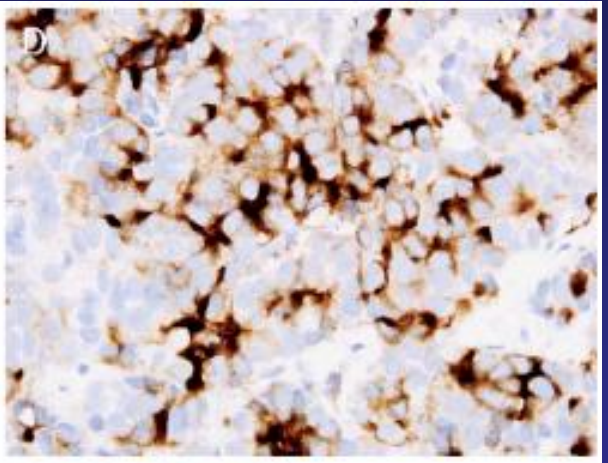
AJSP 2012;36:1472–1476



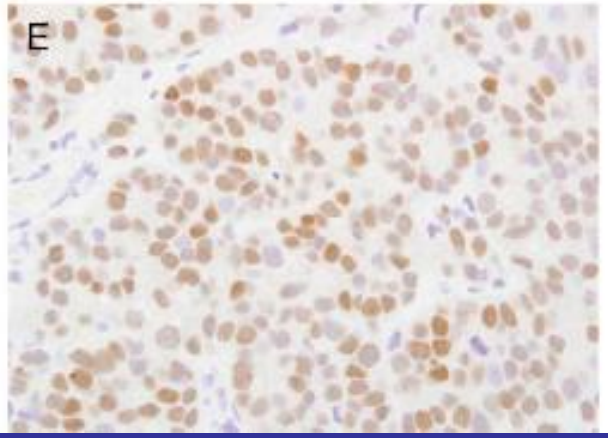
PSA

P501S





PSMA



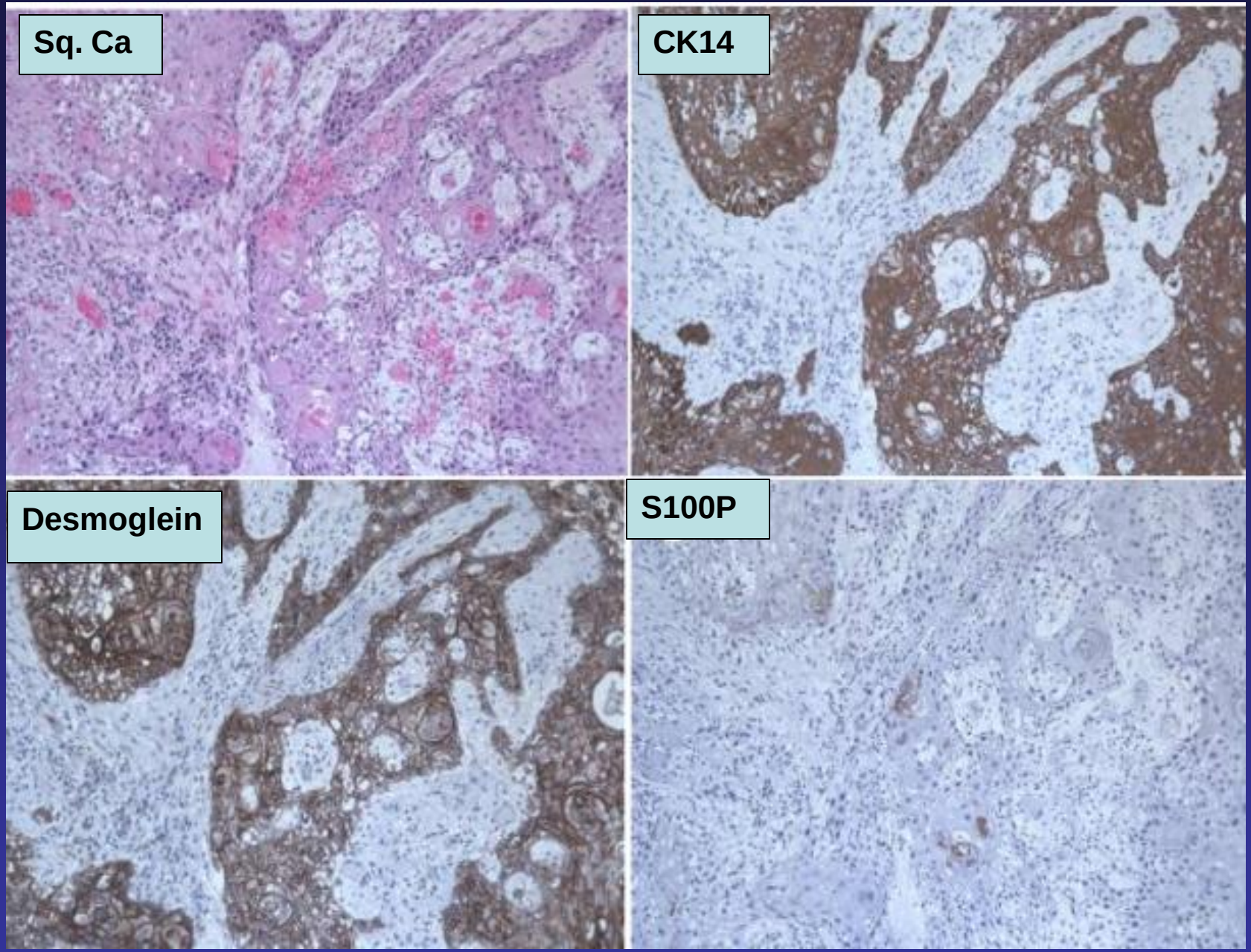
NKA3.1

Am J Surg Pathol 2007;31:1246–1255

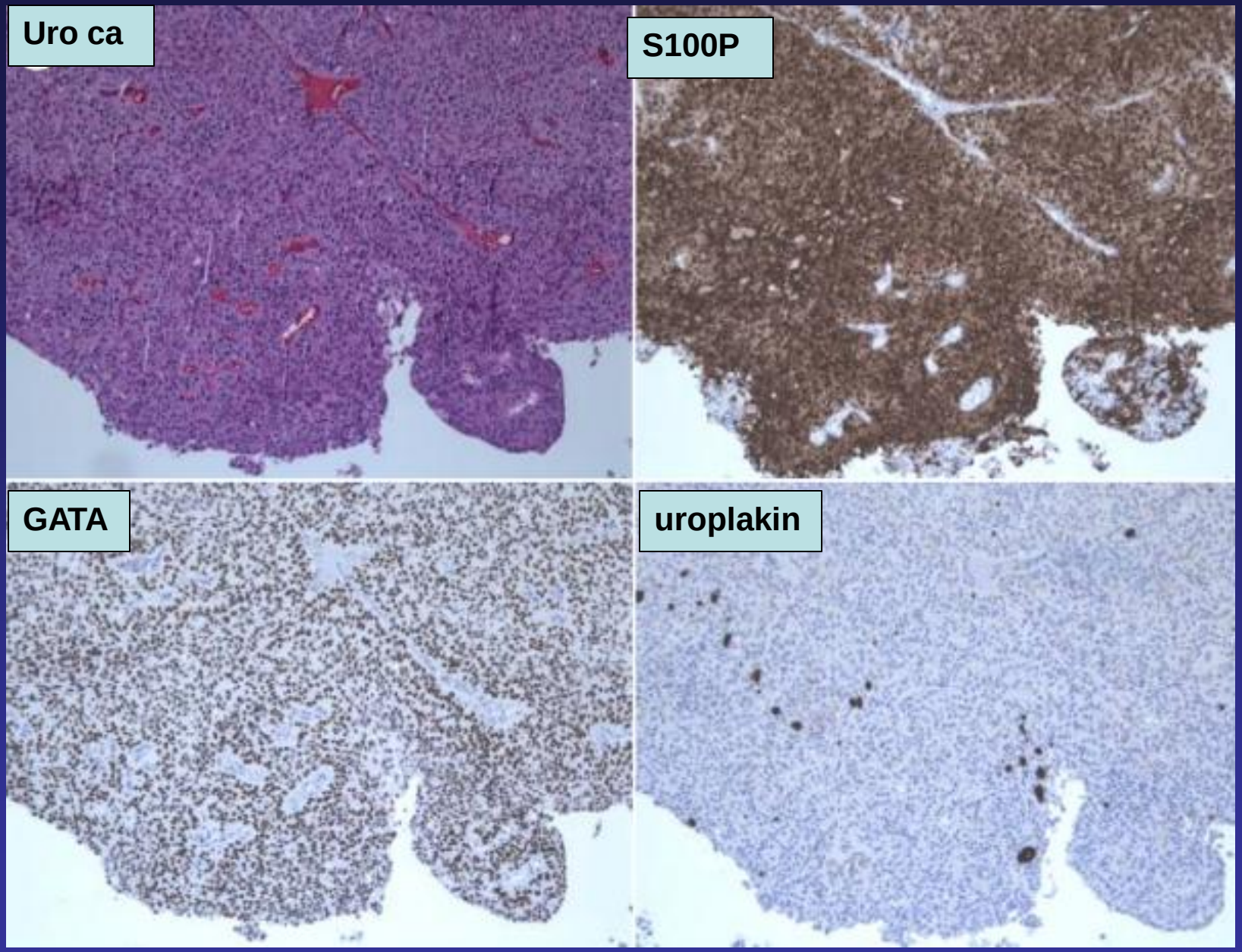
High grade Urothelial Ca

	Urothelial	Squamous
Desmoglein-3	-	+ (75%)
Uroplakin	+ (67%)	-
CK14	-/+ (27%)	+ (100%)
S100p	+ (93%)	+/- (9%)
GATA	+ (93%)	-

Hum Pathol. 2013 Feb;44(2):164-72.



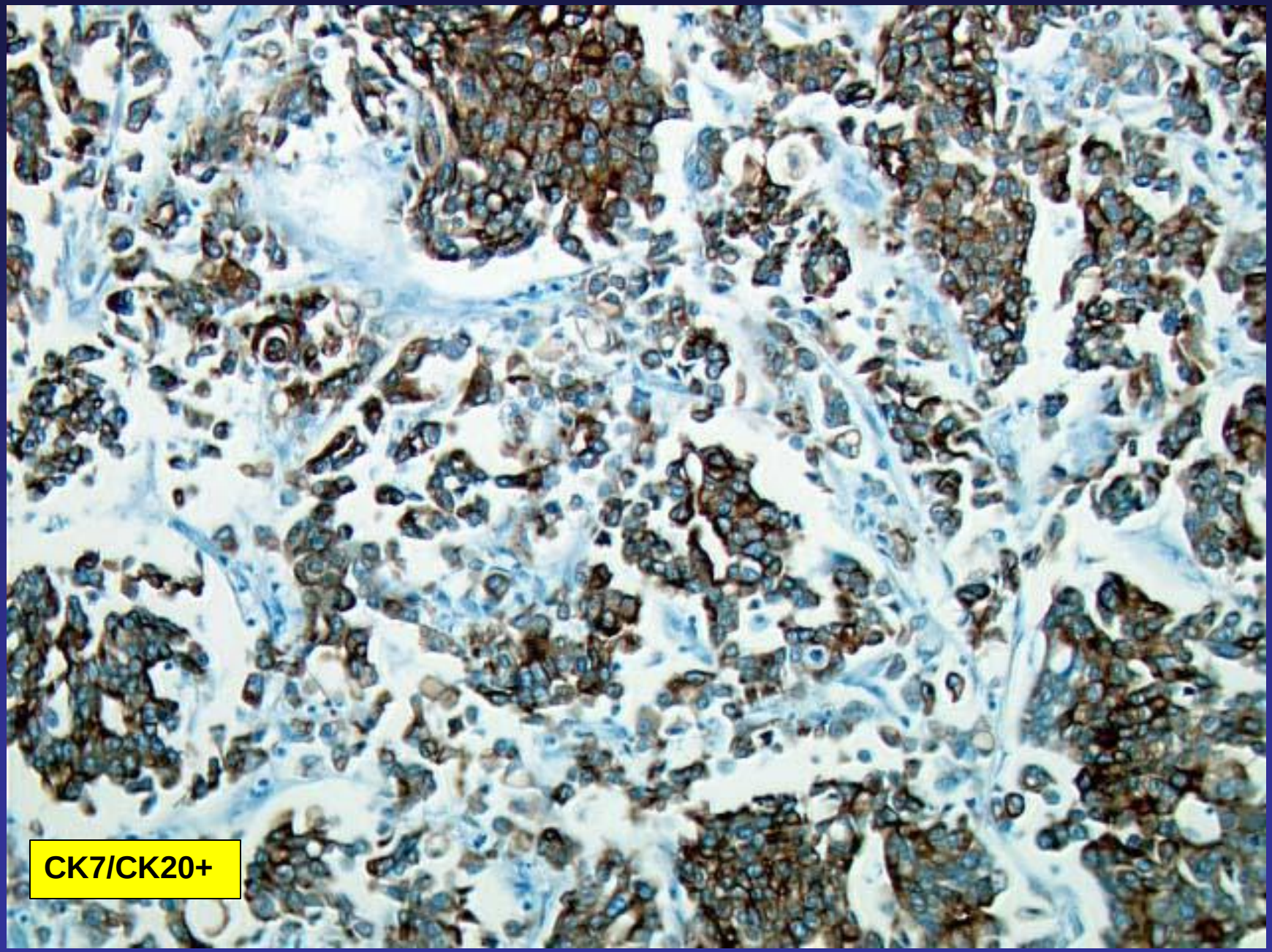
Hum Pathol. 2013 Feb;44(2):164-72.

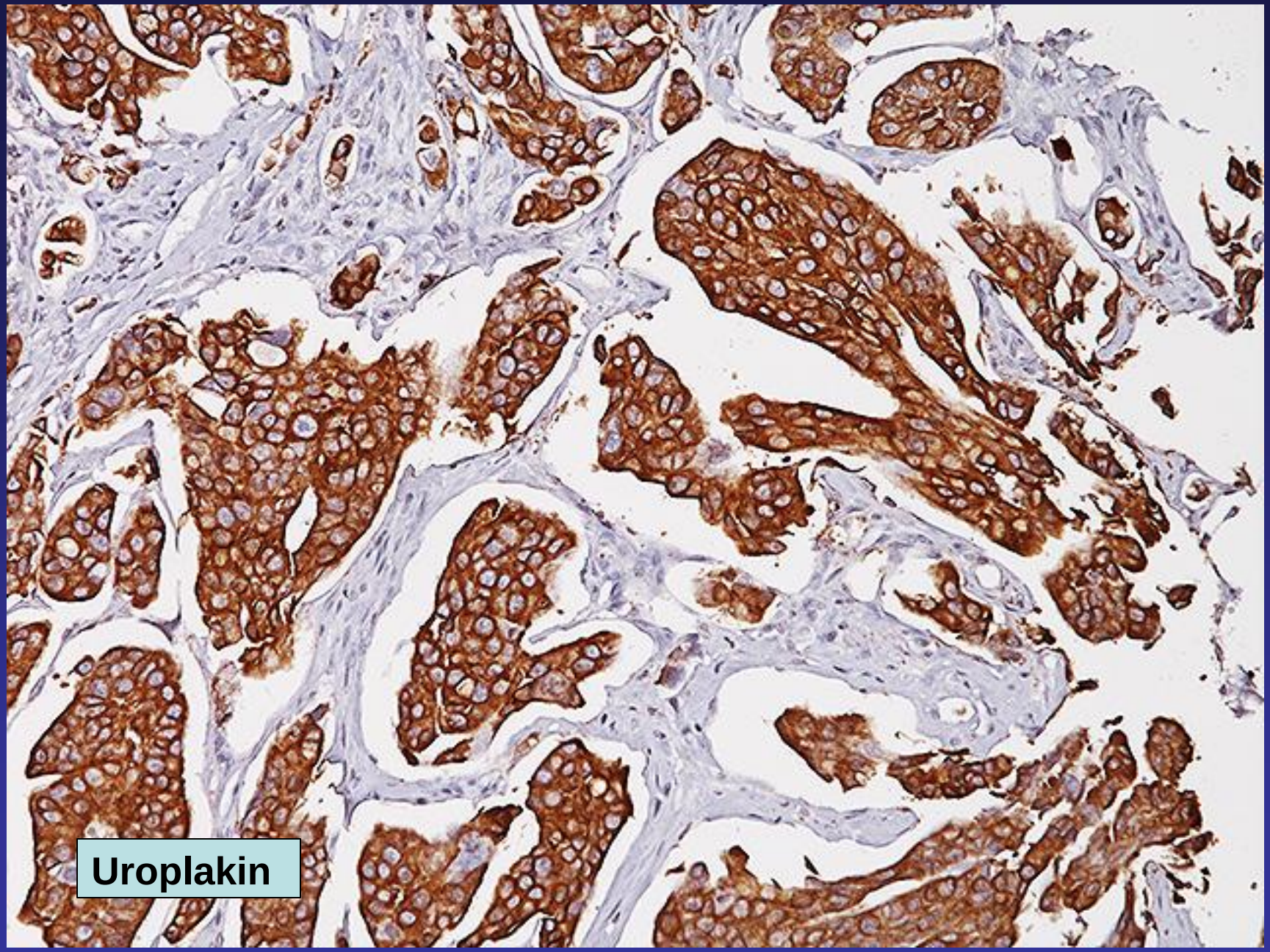


Hum Pathol. 2013 Feb;44(2):164-72.

Metastatic Carcinoma

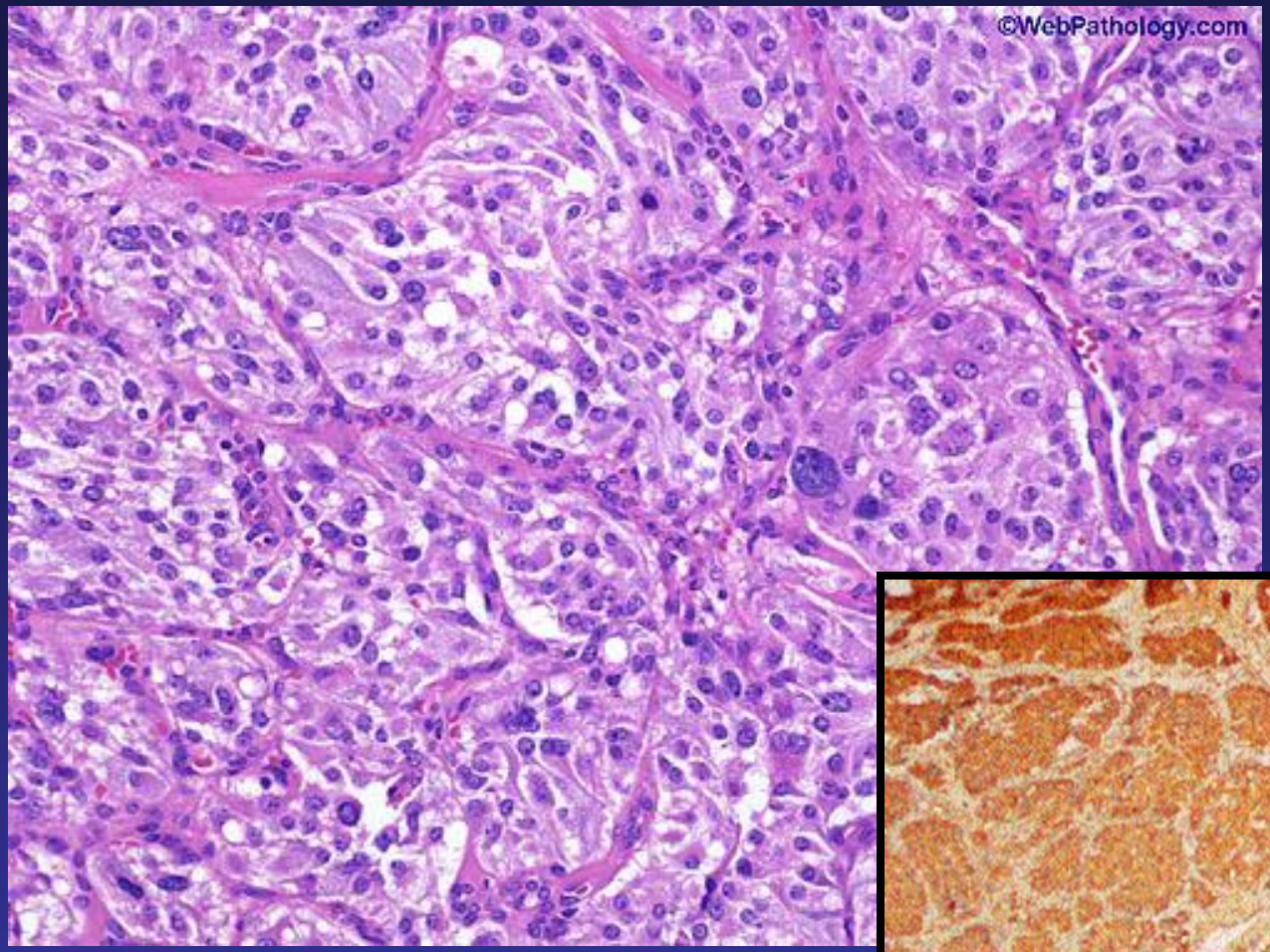
	Uroth	Other
CK7+/CK20+	65%	V
CK7+/CK20-	37%	V
CK7-/CK20+	3%	V
CK7-/CK20-	10%	V
Uroplakin	57-60%	0%
Thrombomodulin	49-69%	V
34betaE12	65-100%	V
P63, S100p, Gata3	60-90%	0% to V

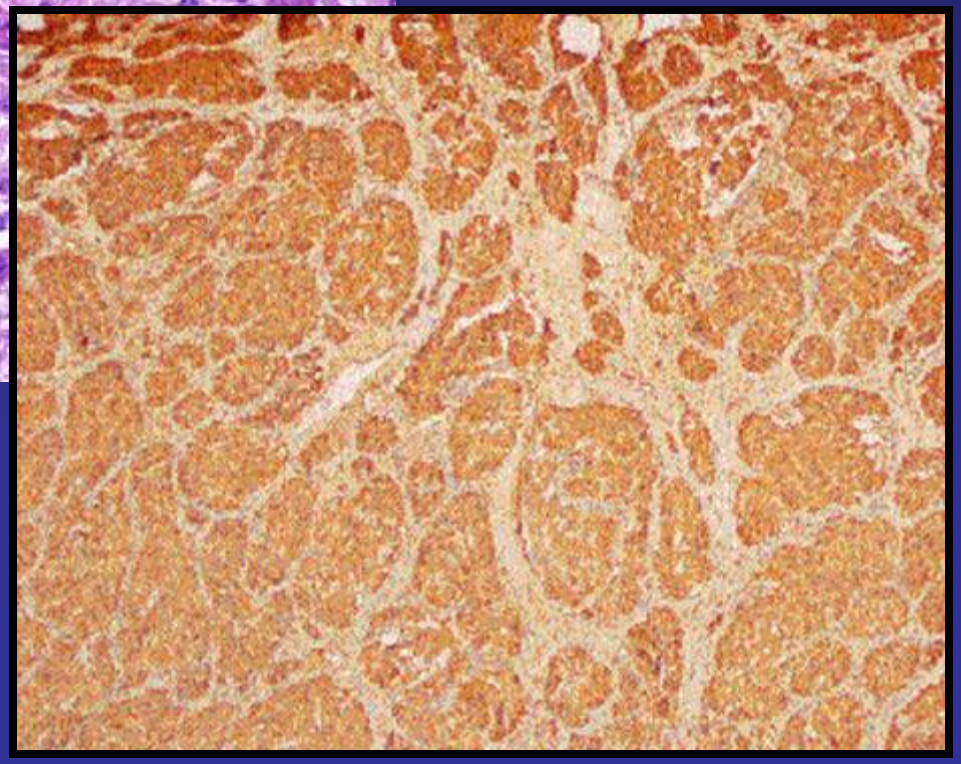

CK7/CK20+


Uroplakin

Urothelial ca vs. Paraganglioma

	Uroth	Para
AE1/AE3	+	-
Synapto	-	+
Chromo	-	+
S100	-	+


©WebPathology.com



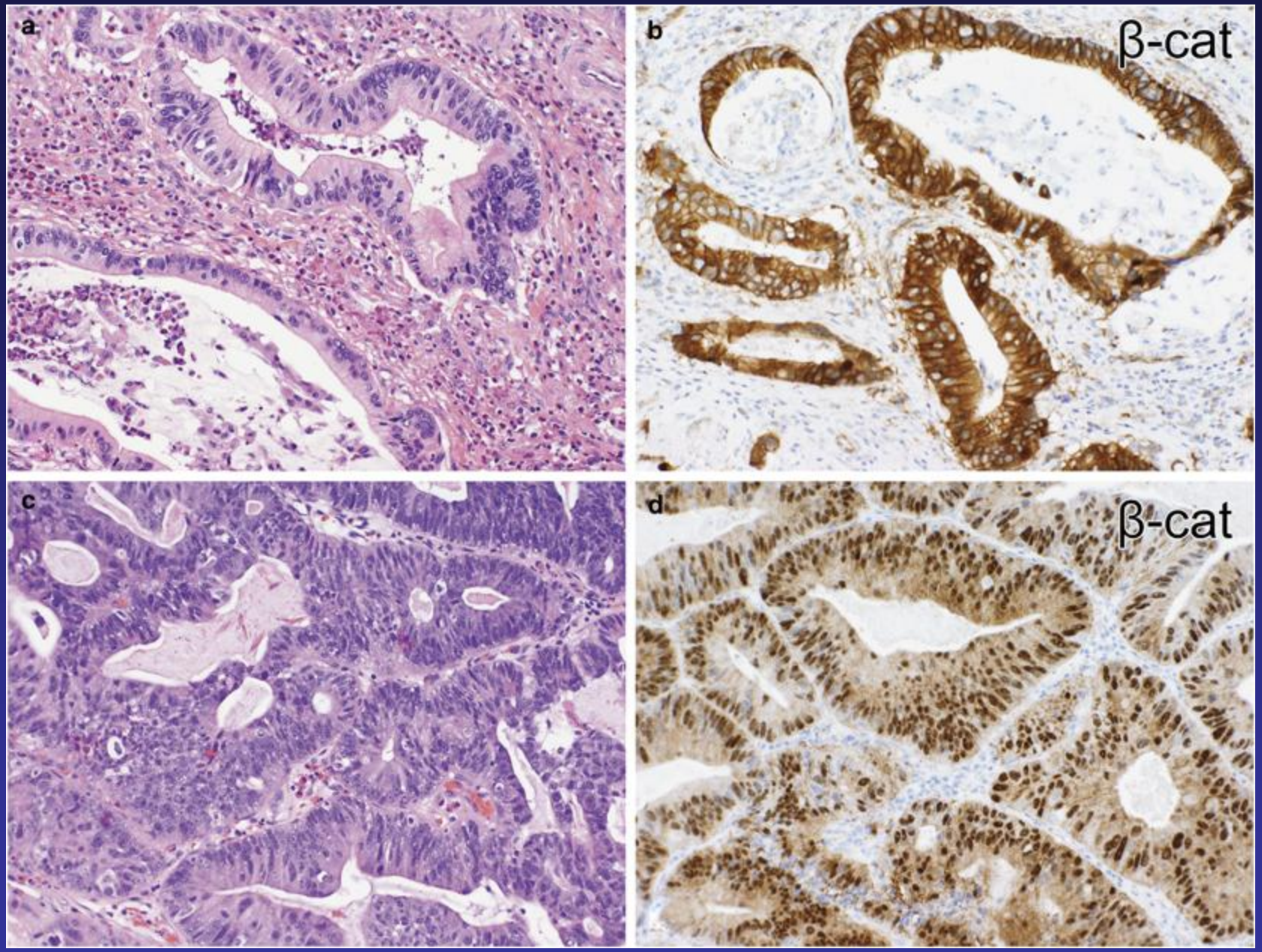
Enteric-type AdenoCa

	Bladder	Colon
CK7+/CK20+	24%	8%
CK7+/CK20-	41%	0%
CK7-/CK20+	29%	82%
CK7-/CK20-	6%	10%
CDX2	47-100%	99-100%
Beta-Catenin	Cy/Mem	Nu/Cy
villin	65-100%	82-98%
AMACR	65%	70%
Thrombomodulin	59%	0%

Mod Pathol 2013;26:725-732

Bl. A

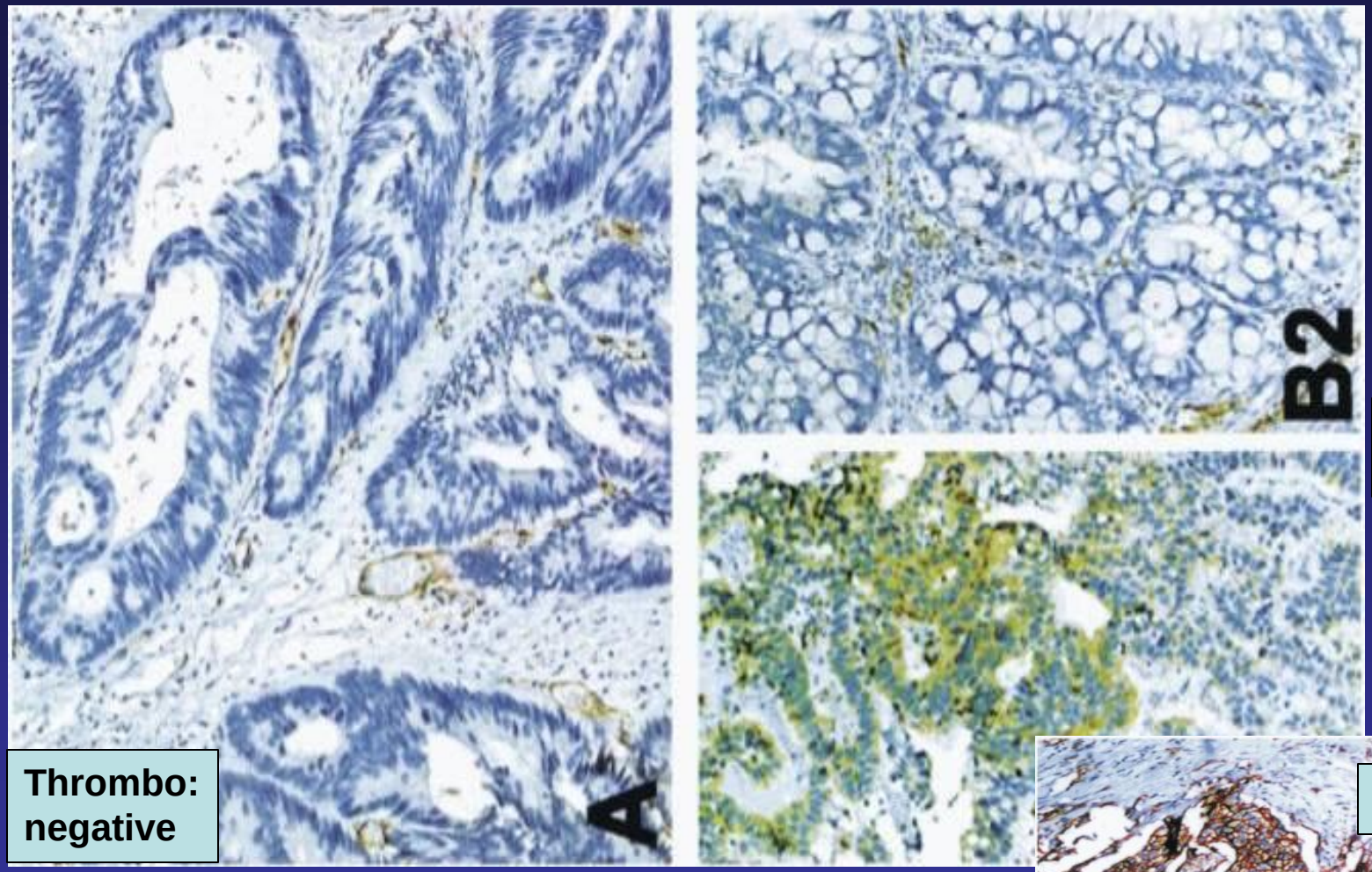
Co.A.



Mod Pathol 2013;26:725-732

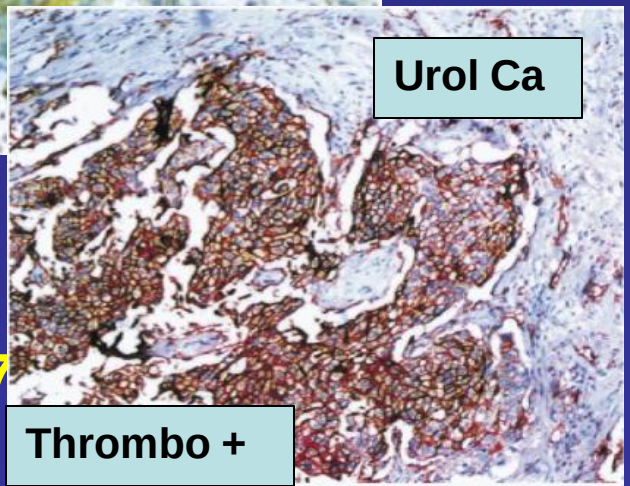
Colorectal adenoca

Bladder adenoca



Thromo:
+ or -

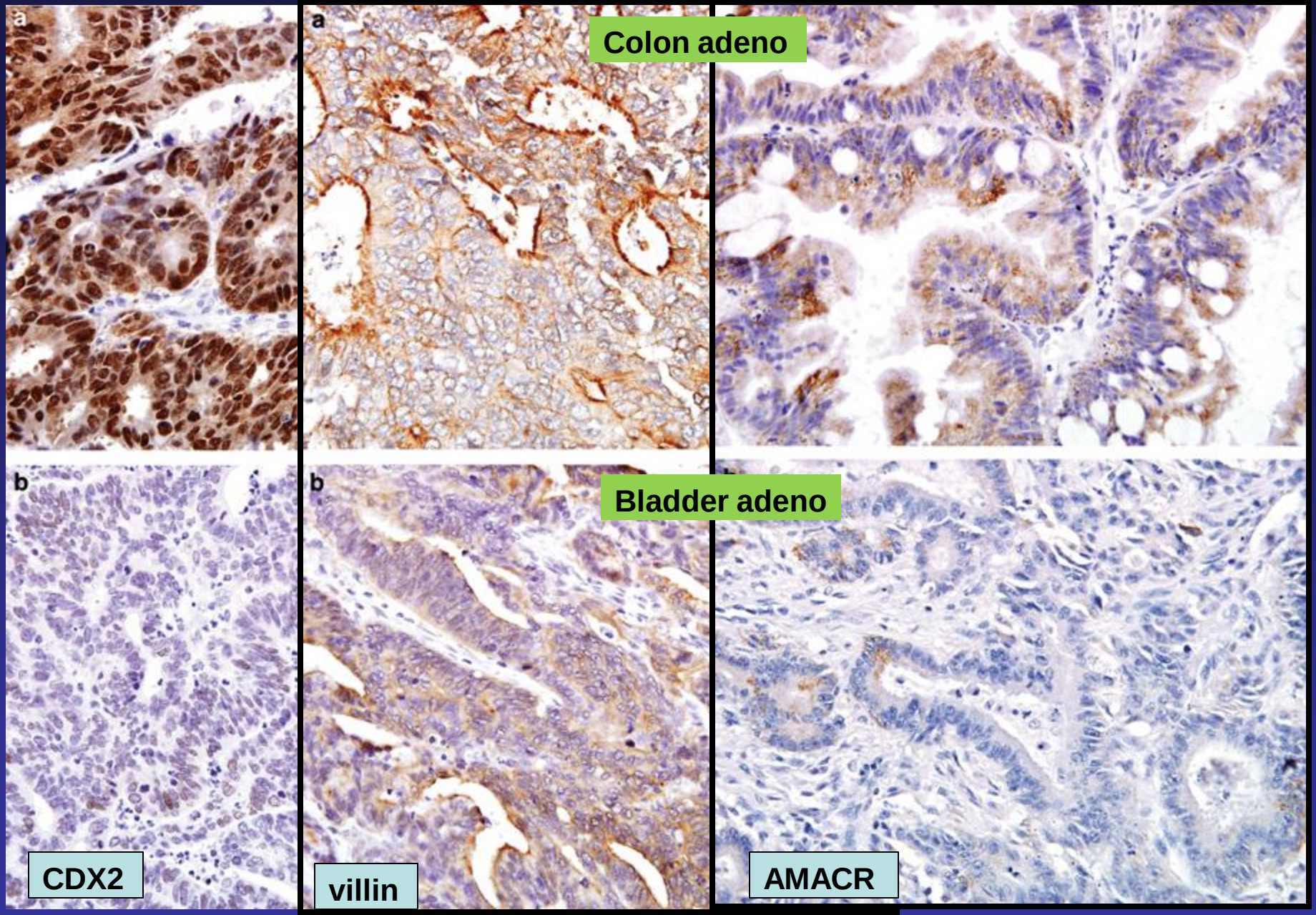
Thrombo:
negative



Urol Ca

Thrombo +

AJSP 2001;25:1380-87



Modern Pathol 18:1217, 2005

Marker	Immunoreactivity	No. (%) positive			
		PBA (n = 17)	SCA (n = 17)	PCA (n = 23)	UC (n = 14)
CDX2	Negative (<5%)	9 (53)	0	0	14 (100)
	5–25%	0	0	0	0
	26–50%	3 (18)	0	1 (4)	0
	51–75%	1 (6)	1 (6)	1 (4)	0
	>75%	4 (24)	16 (94)	21 (91)	0
Villin	Negative (<5%)	6 (35)	1 (6)	0	14 (100)
	5–25%	1 (6)	2 (12)	0	0
	26–50%	2 (12)	1 (6)	0	0
	51–75%	5 (29)	4 (24)	1 (4)	0
	>75%	3 (18)	9 (53)	22 (96)	0
AMACR	Negative (<5%)	6 (35)	4 (24)	8 (35)	12 (86)
	5–25%	2 (12)	3 (18)	1 (4)	1 (7)
	26–50%	3 (18)	1 (6)	1 (4)	0
	51–75%	4 (24)	4 (24)	3 (13)	0
	>75%	2 (12)	5 (29)	10 (44)	1 (7)

The % denotes the percentage of tumor cells positively stained.

PBA, primary bladder adeno; SCA, secondary colon ca; PCA, primary colon; UC, urothelial ca

Modern Pathology 2005;18:1217–1222

3. Think

- Prognosis
- ✓Stage
- ✓Grade
- Non-anatomic prognostic factors

Table 4 – Weighting used to calculate recurrence and progression scores*

Factor	Recurrence	Progression
No. of tumours		
Single	0	0
2–7	3	3
≥8	6	3
Tumour diameter		
<3 cm	0	0
≥3 cm	3	3
Prior recurrence rate		
Primary	0	0
≤1 recurrence per year	2	2
>1 recurrence per year	4	2
Category		
Ta	0	0
T1	1	4
Concomitant CIS		
No	0	0
Yes	1	6
Grade (1973 WHO)		
G1	0	0
G2	1	0
G3	2	5
Total score	0–17	0–23

CIS = carcinoma in situ; WHO = World Health Organisation.

* Electronic calculator for Table 4 is available at http://www.eortc.be/tools/bladdercalculator/.

Table 5 – Probability of recurrence and progression according to total score*

Recurrence score	Probability of recurrence at 1 yr		Probability of recurrence at 5 yr		Recurrence risk group
	%	(95% CI)	%	(95% CI)	
0	15	(10–19)	31	(24–37)	Low risk
1–4	24	(21–26)	46	(42–49)	Intermediate risk
5–9	38	(35–41)	62	(58–65)	
10–17	61	(55–67)	78	(73–84)	High risk
Progression score	Probability of progression at 1 yr		Probability of progression at 5 yr		Progression risk group
	%	(95% CI)	%	(95% CI)	
0	0.2	(0–0.7)	0.8	(0–1.7)	Low risk
2–6	1	(0.4–1.6)	6	(5–8)	Intermediate risk
7–13	5	(4–7)	17	(14–20)	High risk
14–23	17	(10–24)	45	(35–55)	

CI = confidence interval.

* Electronic calculator for Table 5 is available at http://www.eortc.be/tools/bladdercalculator/.

Urinary Bladder, TNM Stage

- **TX** tumor cannot be assessed
- **T0** no evidence of primary tumor
- **Ta** papillary non-invasive carcinoma
- **Tis** carcinoma in situ
- **T1** tumor invades subepithelial con. tissue
- **T2** tumor invades muscle proper
  T2a inner half; T2b outer half
- **T3** perivesical fat (T3a, micro; T3b, gross)
- **T4** involving prostatic stroma,
  seminal vesicles, uterus, vagina (4a);
  pelvic/abdominal wall (4b)

Lymph Nodes (N) and Metastasis (M)

N: Lymph nodes

- Nx lymph nodes cannot be assessed
- N0 no LN metastasis
- N1 single LN metastasis in true pelvic LN (hypogastric, obturator, external iliac, presacral LN)
- N2 multiple LN metastasis in true pelvic LN
- N3 common iliac LN (was considered as distant met)

M: distant metastasis

- M0 no distant metastasis
- M1 distant metastasis

Anatomic Stage/Prognostic Groups

• Stage 0a	Ta	N0	M0
• Stage 0is	Tis	N0	M0
• Stage I	T1	N0	M0
• Stage II	T2a, 2b	N0	M0
• Stage III	T3a, 3b	N0	M0
	T4a	N0	M0
• Stage IV	T4b	N0	M0
	Any T	N1-3	M0
	Any T	Any N	M1

Prognostic factors

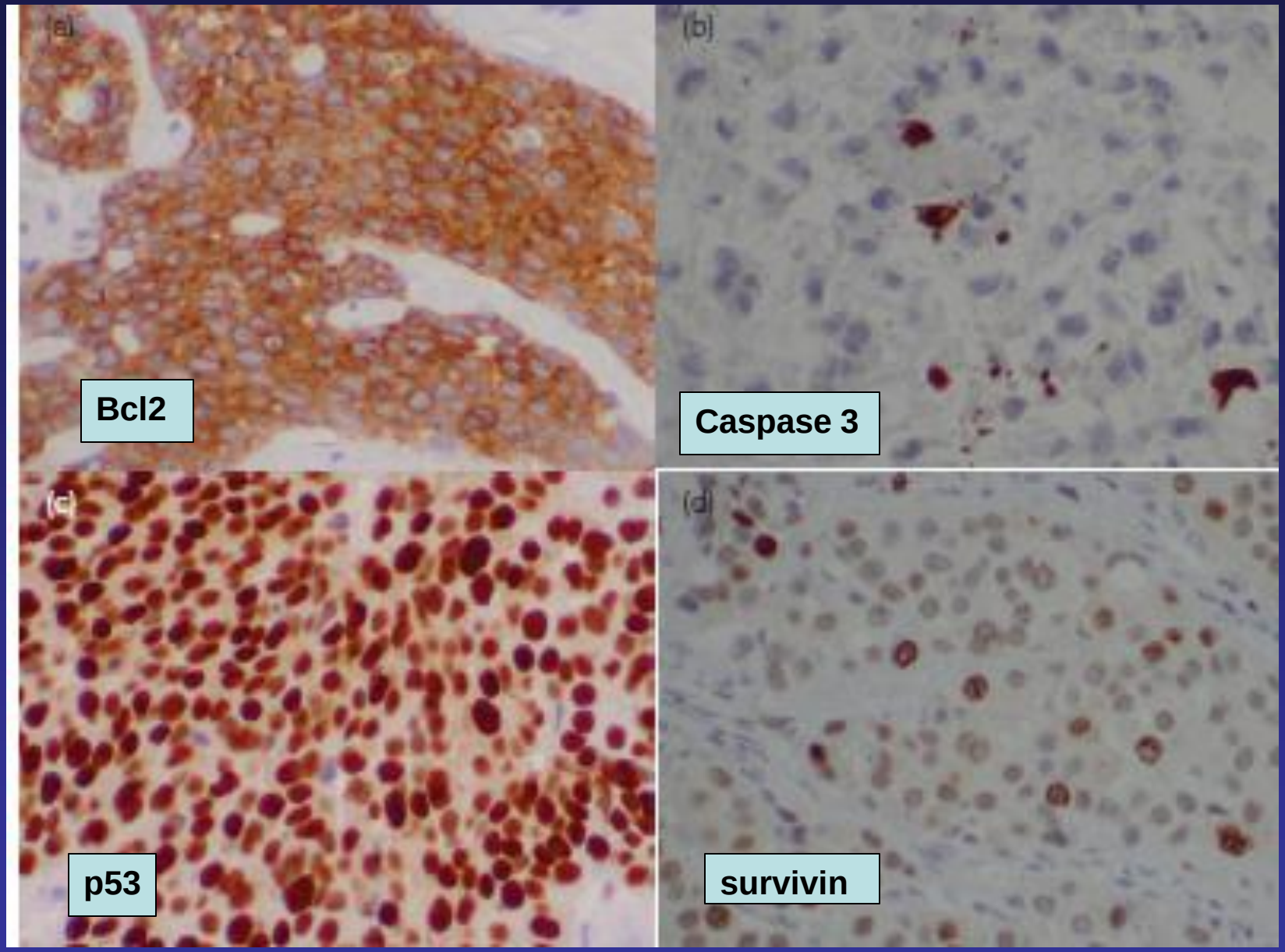
- TNM stage
- 2004 WHO/ISUP grade
- Presence or absence of extranodal extension
- Size of largest tumor deposit in LN
- New molecular data/genomics

AJCC staging, 2010 (7th ed)

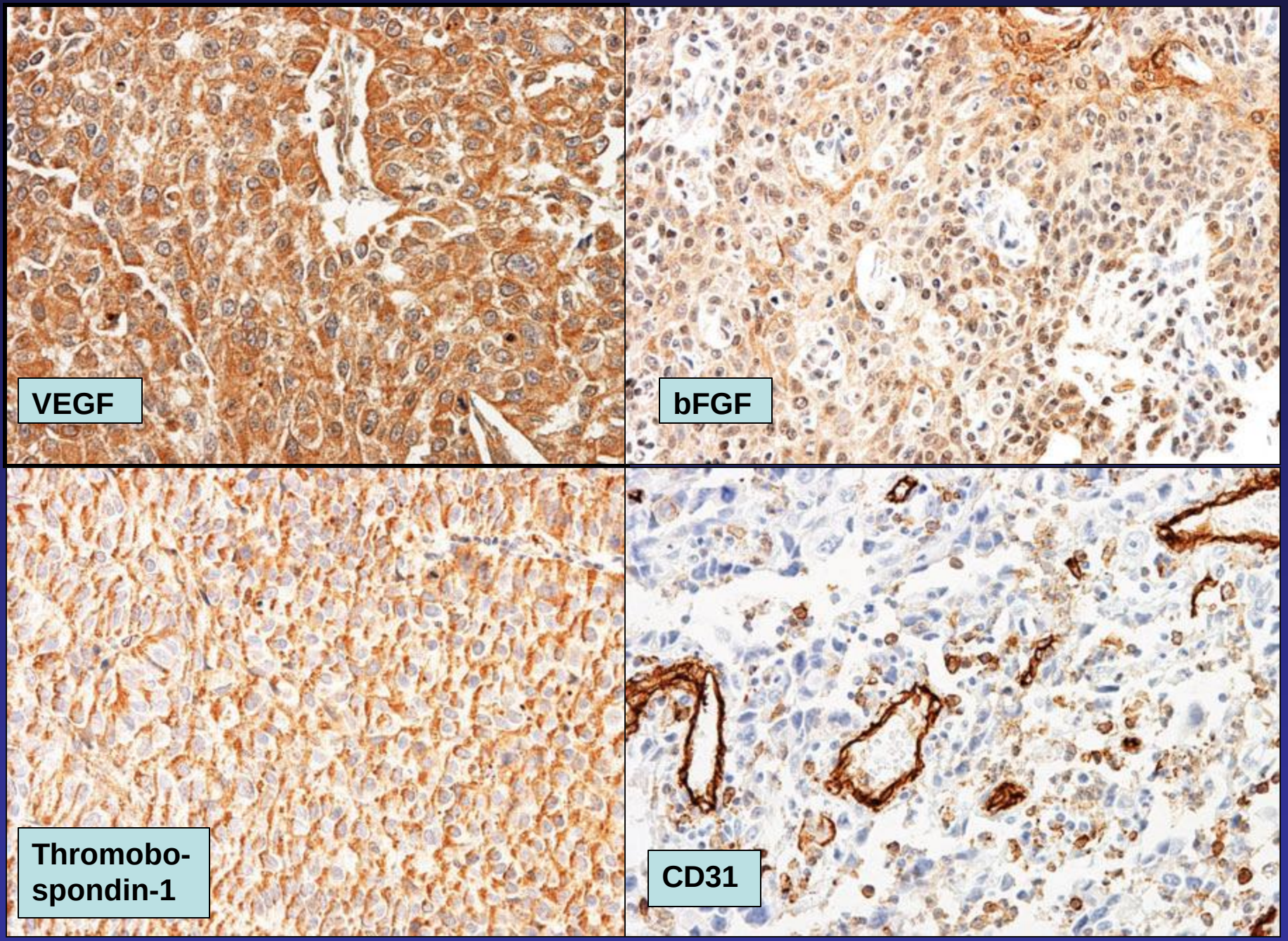
Cell cycle				
Cote *et al.*[18]	pRb, p53	RC	185	Altered p53 and pRb associated with recurrence and ACM
Stein *et al.*[45]	p21, p53	RC	242	Altered p21 independently associated with recurrence and ACM
Grossman *et al.*[19]	p53, pRb	TUR, pT1	45	Altered p53 and pRb associated with progression
Chatterjee *et al.*[22]	p53, p21, pRb	RC	164	Altered p53, p21, and pRb associated with recurrence and ACM; significant after stratification according to stage
Shariat *et al.*[23]	p53, p21, pRb, p16	RC	80	Altered p53, p21, pRb, and p16 associated with recurrence and CSM; p53 and p21 independent predictors in multivariate models
Shariat *et al.*[12]	p53, pRb, p21, p27, cyclin E1	RC, pTa-3N0M0	191	Number of altered cell cycle biomarkers associated with recurrence and CSM
Apoptosis				
Shariat *et al.*[70]	Survivin	RC	222	Survivin expression independently associated with recurrence, CSM, ACM
Karam *et al.*[59]	Bcl-2, caspase-3, p53, survivin	RC	226	Number of altered apoptosis biomarkers independently associated with recurrence and CSM
Angiogenesis				
Bochner *et al.*[73]	MVD (CD34)	RC	164	MVD independently associated with recurrence and ACM
Grossfeld *et al.*[80]	TSP-1 MVD (CD34) p53	RC	163	TSP-1 independently associated with recurrence and ACM TSP-1 associated with p53 status and MVD
Miyata *et al.*[82]	LVD (D2–40) MVD (CD34) VEGF-A, VEGF-C, VEGF-D	TUR	126	LVD associated with metastasis-free survival, but not independently High LVD + high MVD independently associated with metastasis-free survival VEGF-C associated with LVD and MVD
Shariat *et al.*[76]	VEGF bFGF TSP-1 MVD (CD31)	RC	204	bFGF and TSP-1 independently associated with recurrence and CSM
Signaling and hormones				
Mellon *et al.*[92]	EGFR	TUR	212	EGFR status independently associated with progression and mortality
Kassouf *et al.*[106]	EGFR/ErbB-1 ErbB-2, ErbB-3, ErbB-4	TUR, RC	248	Altered EGFR and ErbB-4 associated with grade, stage, recurrence, and ACM
Jimenez *et al.*[107]	HER-2	RC	80	HER-2/neu overexpression not associated with ACM Some HER-2/neu-negative primaries may have overexpression in corresponding metastasis
Kruger *et al.*[112]	HER-2	RC	138	HER-2 overexpression independently associated with CSM
Bolenz *et al.*[115]	HER-2	RC	198	HER-2 expression independently associated with recurrence and CSM
Boorjian *et al.*[116]	AR	TUR, RC	49	AR expression inversely correlated with tumor stage
Mir *et al.*[118]	AR	TUR, RC	472	AR expression not associated with stage, grade, or clinical outcome

Immunohistochemical Biomarkers

- Cell cycle markers: p53, Rb, p21, p27, cyclins (D and E)
- Apoptosis: Fas (CD95), caspase 3, Bcl-2, survivin
- Angiogenesis: Microvessel density, thrombospondin-1, VEGF, bFGF
- Uroplakins
- Signaling proteins: ErbB family, PI3K, FGFR
- Hormone receptors: EGFR2, AR, ER, PR



International Journal of Urology (2011) 18, 616–629



International Journal of Urology (2011) 18, 616–629

4. Enjoy

Take home message (BeST!)

- **Basic:** Normal histology & anatomy
- **Study (effort)**
  - ✓ Flat lesions with carcinoma in situ
  - ✓ Diagnostic approach and grading of papillary tumors
  - ✓ Invasion and variants of TCC
- **Think:** Prognostic factors
- **Enjoy**