A Point-based Histologic Scoring System for Hepatocellular Carcinoma Can Stratify Risk of Posttransplant Tumor Recurrence

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常用国际标准

- 1996 年 Mazzaferro等提出米兰标准：

  单个肿瘤结节直径 ≤ 5 cm；

  多结节者 ≤ 3 个，且最大直径 ≤ 3 cm；

  无大血管浸润，无淋巴结或肝外转移。

- 2001年，University of California at San Francisco（UCSF）提出**UCSF标准**：

  单个肿瘤直径 ≤ 6.5 cm；

  多个肿瘤 ≤ 3 个且最大者 ≤ 4.5 cm（同时肿瘤总大小≤ 8 cm）；

  无大血管浸润，无淋巴结或肝外转移。

- 2009年，米兰团队自身也发现这一问题，在不影响总体生存率的情况下，提出了“新米兰标准”：

  最大肿瘤直径与肿瘤个数之和不大于 7；

  无主要血管侵犯，无肝外转移。

常用国内标准

- **上海复旦标准**：

  单发肿瘤直径 ≤ 9 cm；

  多发肿瘤 ≤ 3 个且最大直径 ≤ 5 cm、全肿瘤直径总和 ≤ 9 cm；

  无大血管侵犯 、淋巴结转移及肝外转移。

- **成都标准**：

  肿瘤总直径 ≤ 9 cm，无肝外转移，无血管侵犯。

- **杭州标准**：

  所有肿瘤结节直径之和 ＞ 5 cm 且 ≤ 8 cm；

  所有肿瘤结节直径之和 ＞ 8 cm，但 AFP ≤ 400 ng /mL，且组织

  学分级为高、中度分化；

  无大血管侵犯和肝外转移。

INTRODUCTION

- 对于国际常用的米兰标准与 UCSF 标准，仅考虑了肿瘤的个数与大小，而且，术前影像测量的大小与肿瘤实际大小会存在不一致性。
- 目前，多项研究认为，肿瘤的个数及数量并不是肝癌肝移植术后复发的唯一危险因素，而肿瘤的分化程度被认为是影响术后的相关危险因素，低分化肝癌往往较高分化肝癌术后复发的可能性要大。
- 本研究的目的是将组织学特征与移植后肝癌复发相关联，并建立临床相关的评分系统。

MATERIALS AND METHODS

- 1061 liver transplants (1997 – 2014)

  University of California, San Francisco Medical Center

- 351 contained HCC
- 190 had adequate follow-up and tissue
- 184 had available preoperative imaging reports
- The median follow-up time was 6.9 years, ranging from 52 days to 19.7 years

MATERIALS AND METHODS

- The following histologic parameters were evaluated in 109 explants:
  - architectural pattern
  - nuclear-to-cytoplasmic ratio
  - nuclear pleomorphism
  - cytoplasmic amphophilia
  - presence of fatty change
  - macronucleoli
  - mitotic activity
  - cytoplasmic granularity
  - CK19
- After the initial evaluation, in which Recurrence Risk Assessment Score (RRAS) criteria were established, an additional 81 explants were evaluated; the performance of the RRAS in predicting tumor recurrence was compared with WHO grade in both the independent cohort and the cumulative total of 190 explants.

WHO的组织学分级

- 高分化：

病变由轻微异型和核/浆比增高的细胞组成，排列成细小梁状，常见假腺样结构。脂肪变常见。

- 中分化：

由三层或更多层细胞排列成小梁状，肿瘤细胞胞浆丰富嗜酸性，核圆形、核仁明显，常见假腺样结构，且假腺体中常含有胆汁或蛋白性液体。

- 低分化：

肿瘤呈实性生长，无明显血窦样腔隙，仅能在大的癌巢中见到裂隙样血管，肿瘤细胞核/浆比增高并常有中等或明显的多形性。

- 未分化：

肿瘤细胞含少量胞浆，梭形或圆形，实性生长。

MATERIALS AND METHODS

- 本文机构分五级：

  高分化

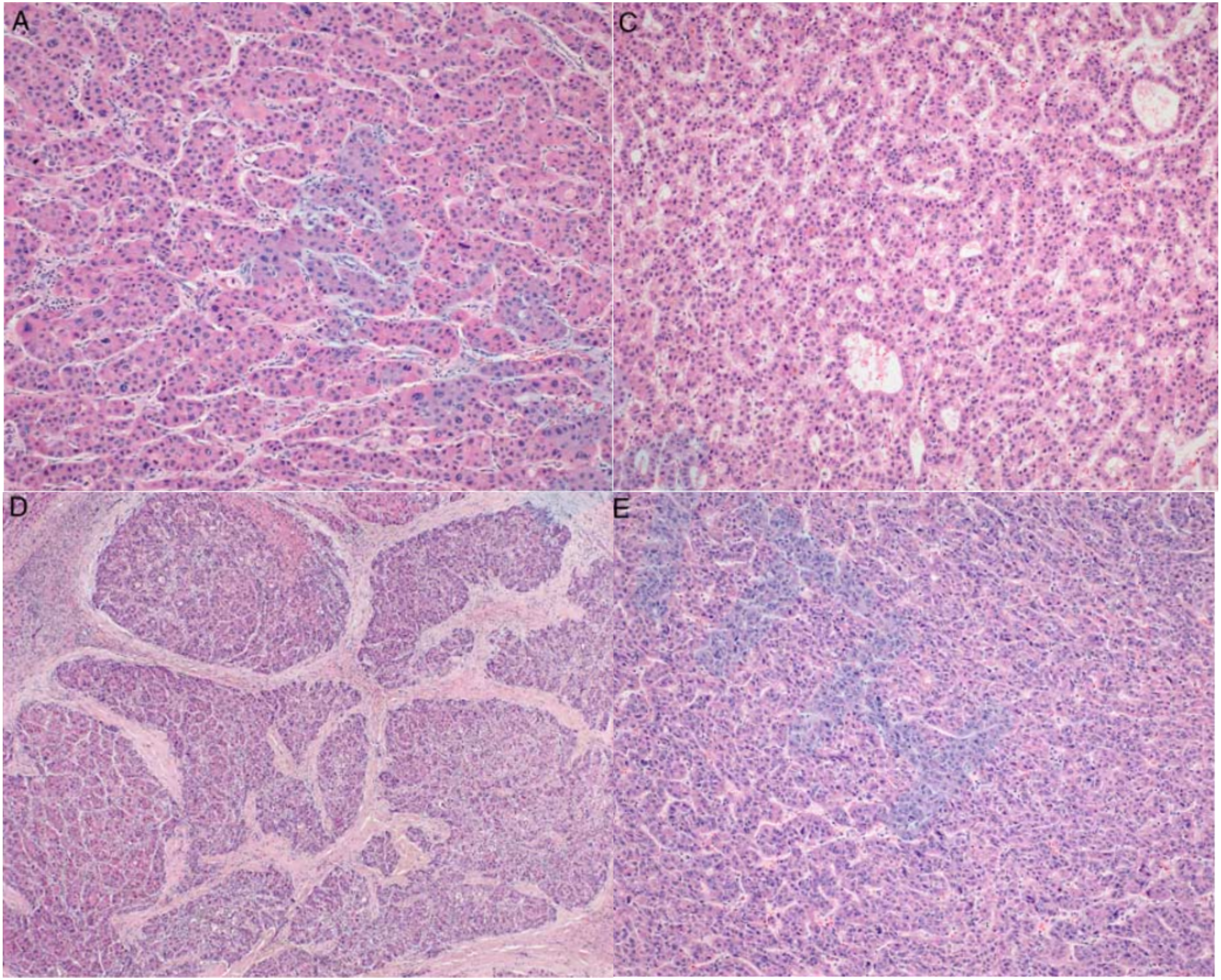
  高-中分化 （ WHO 中分化 ）

  中分化

  中-低分化（ WHO 低分化 ）

  低分化（ WHO 低分化、未分化 ）

- 病例由两位病理学家独立评估
- 对于分级差异较严重的 20 个病例，由第三个亚专科肝胆病理学家再次评估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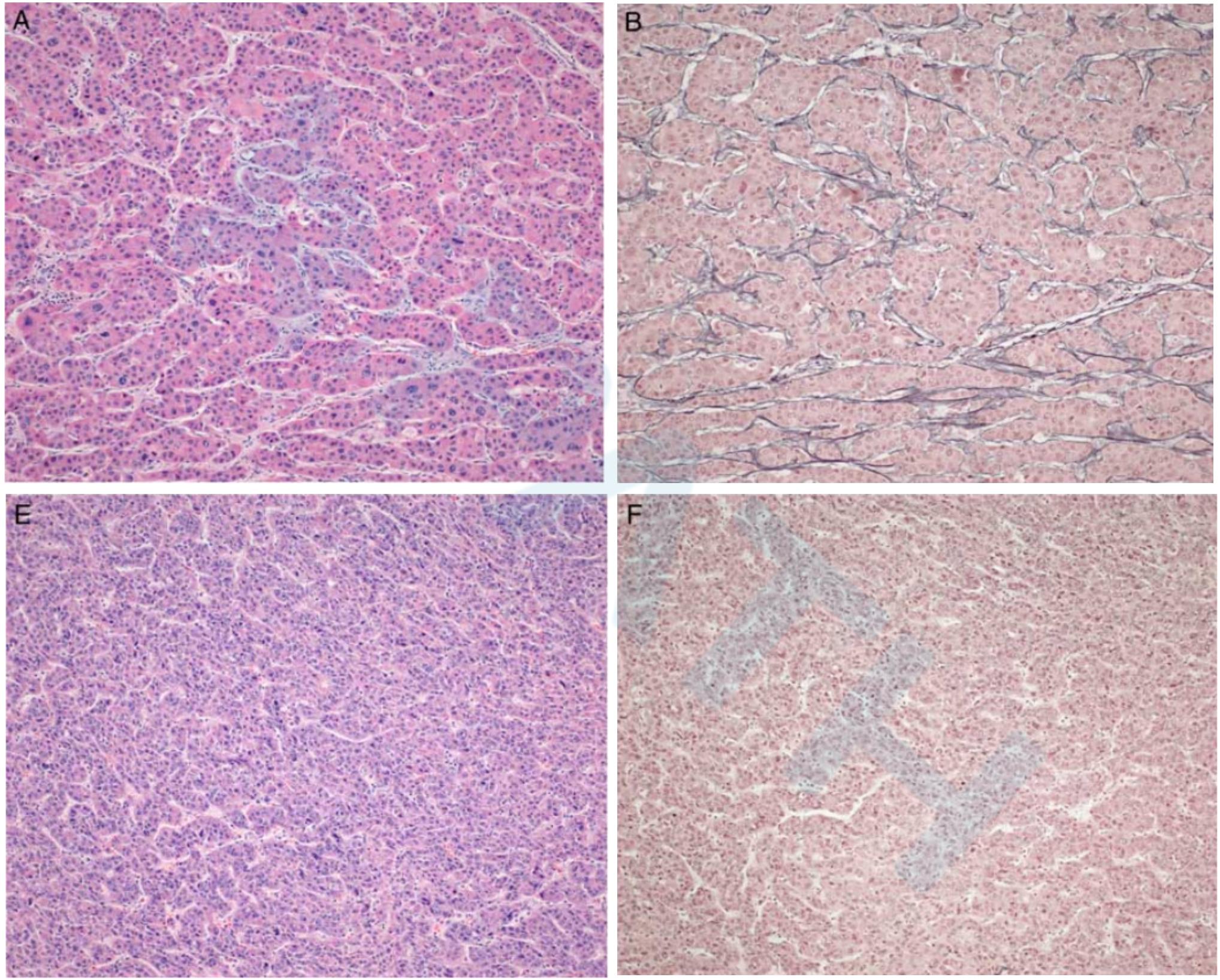
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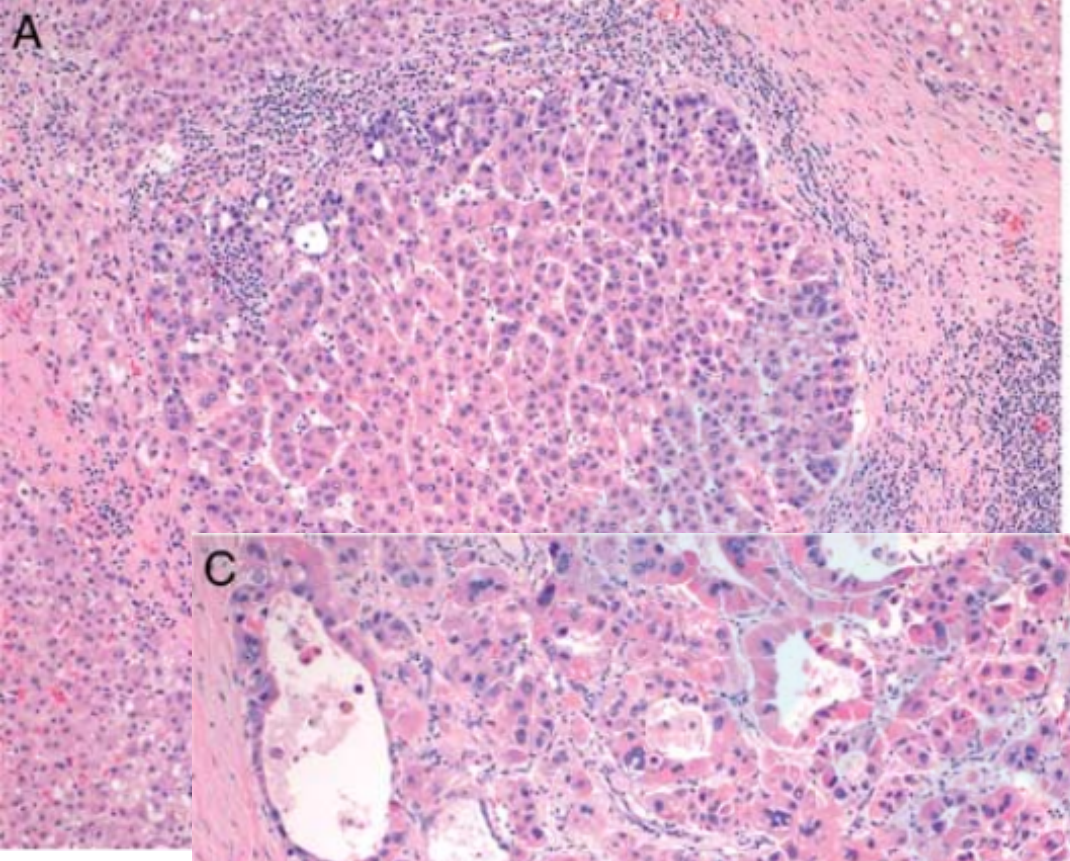
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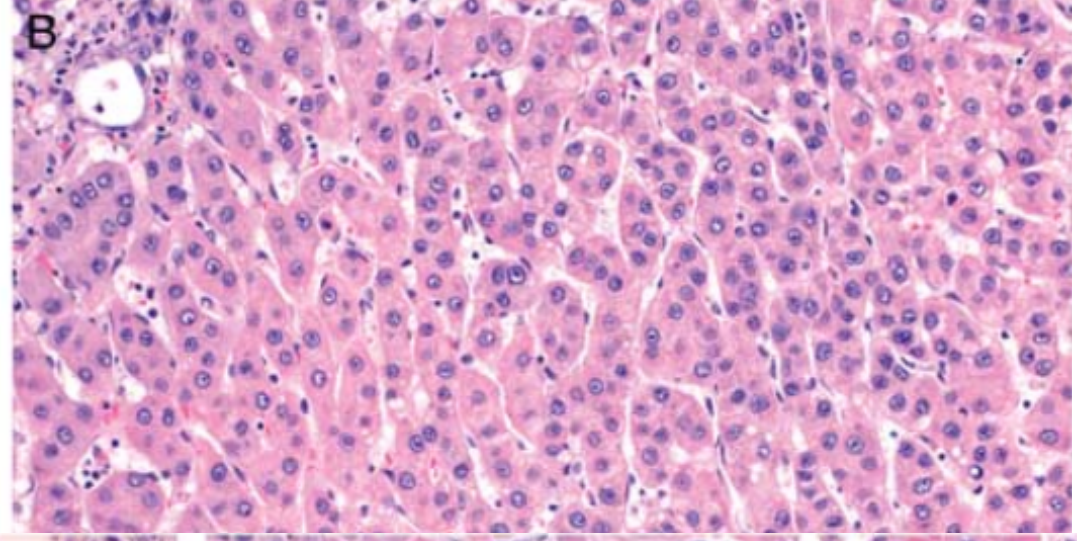
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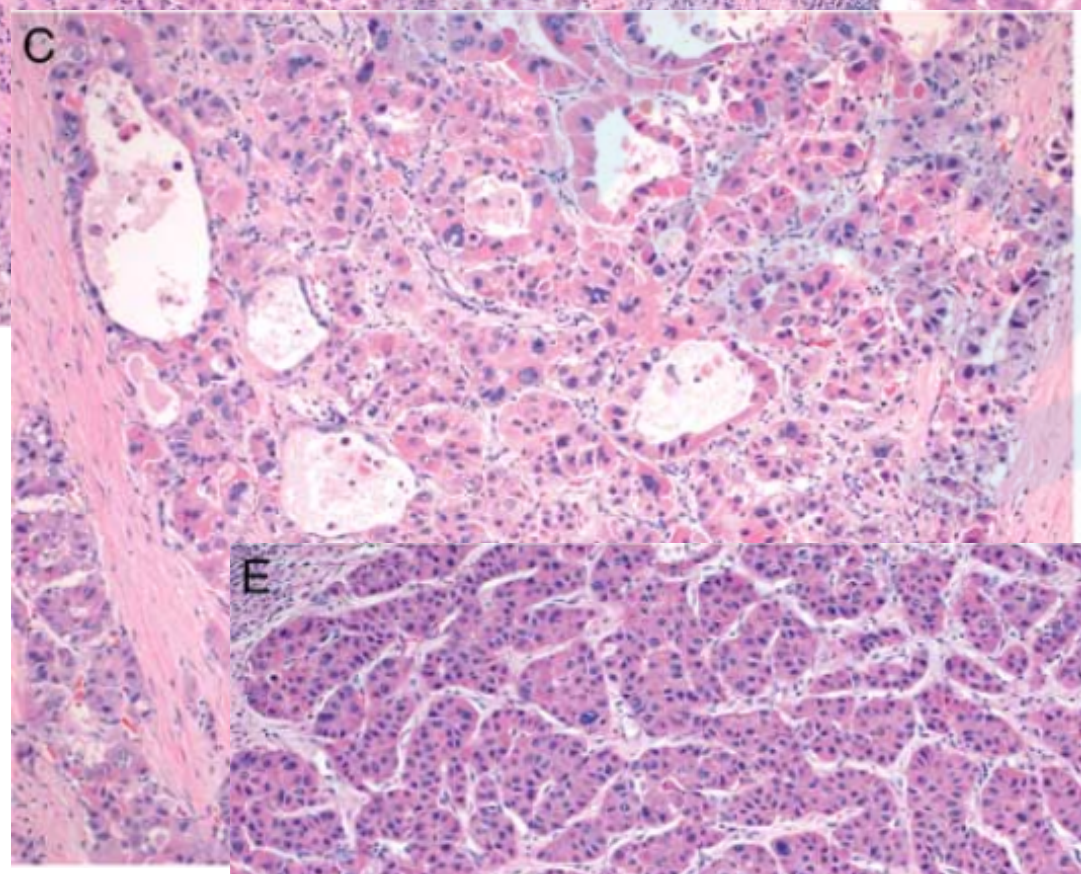
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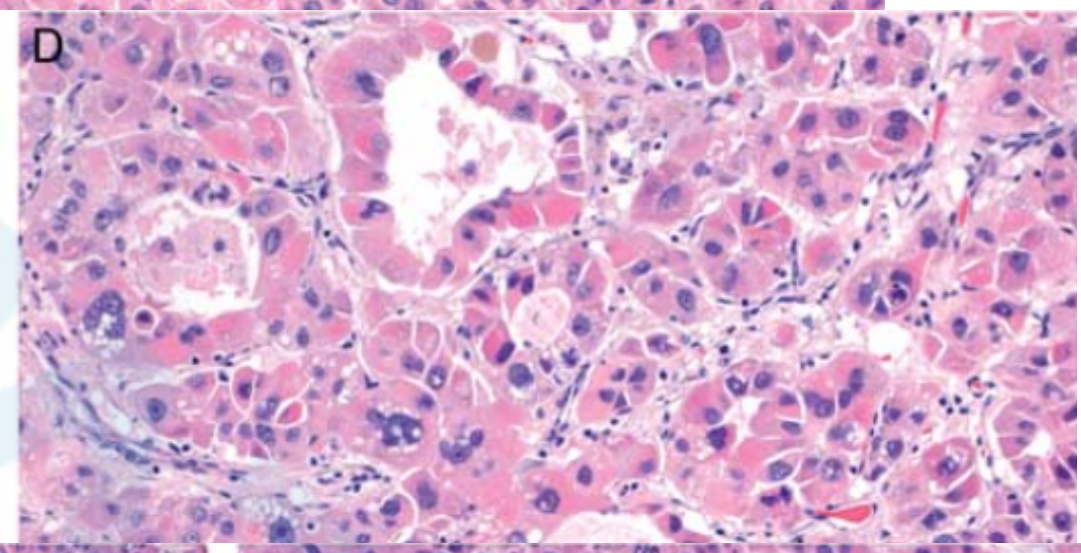
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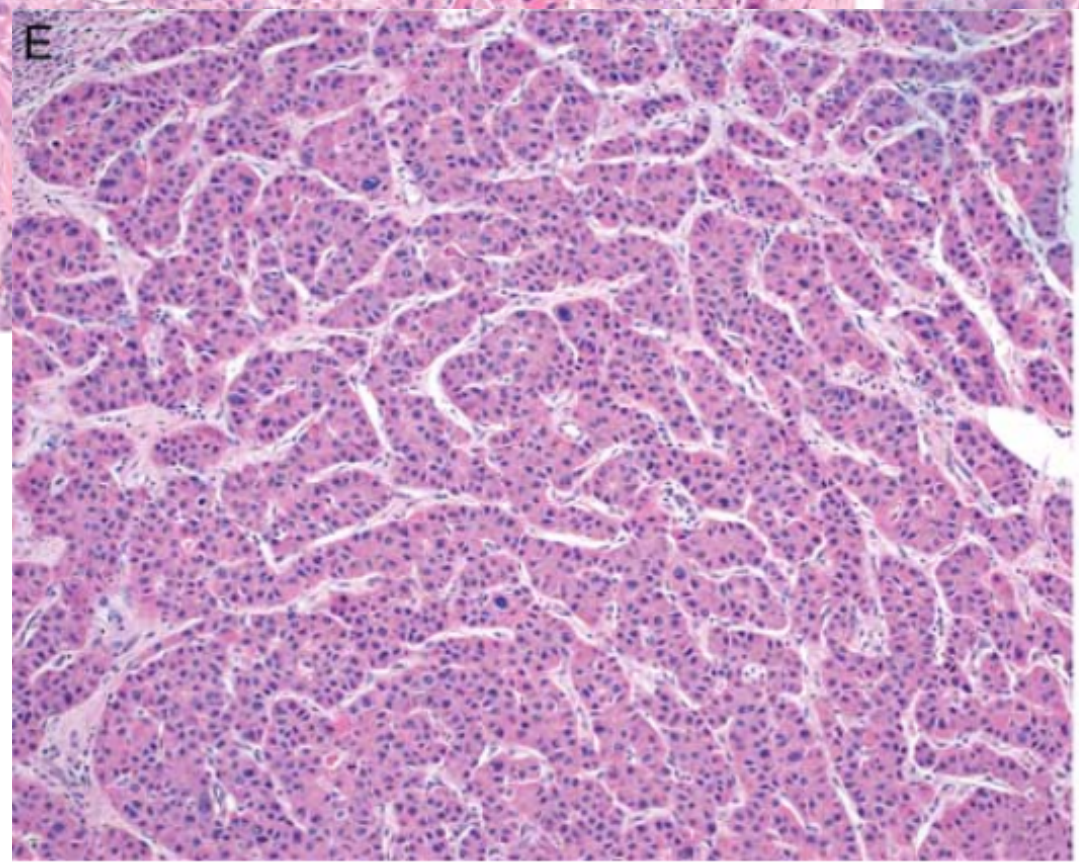
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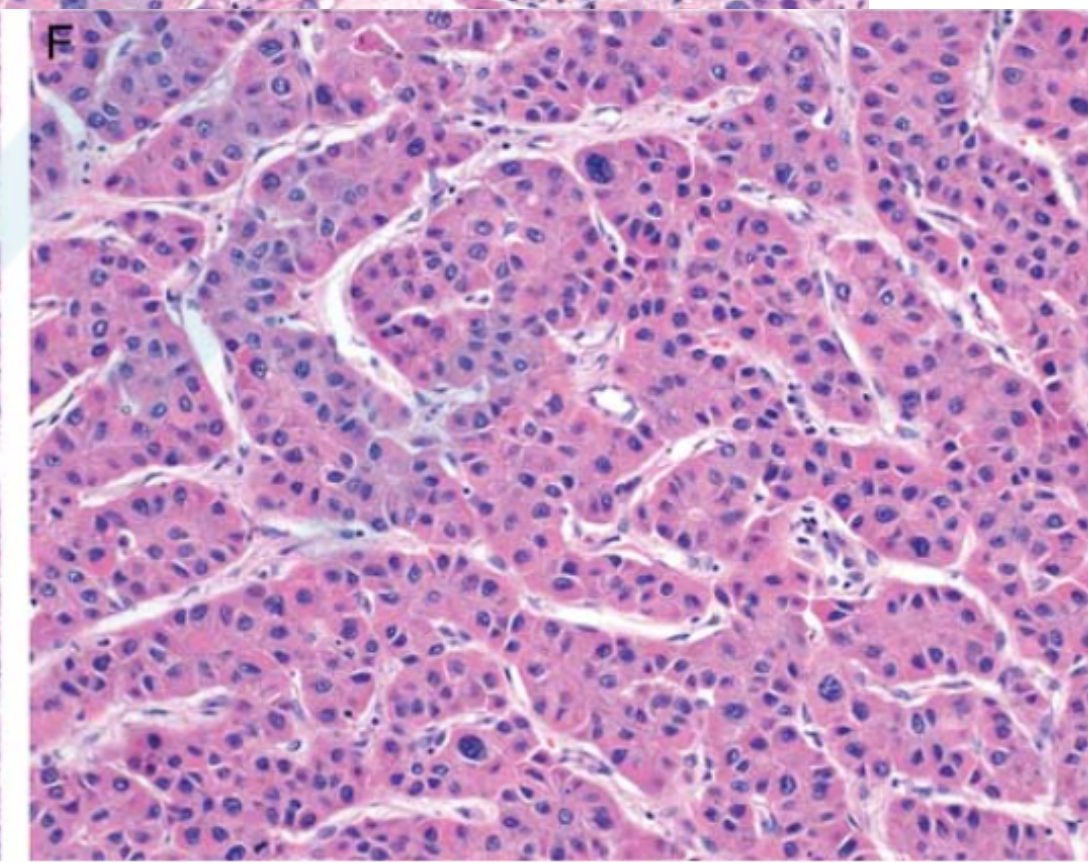
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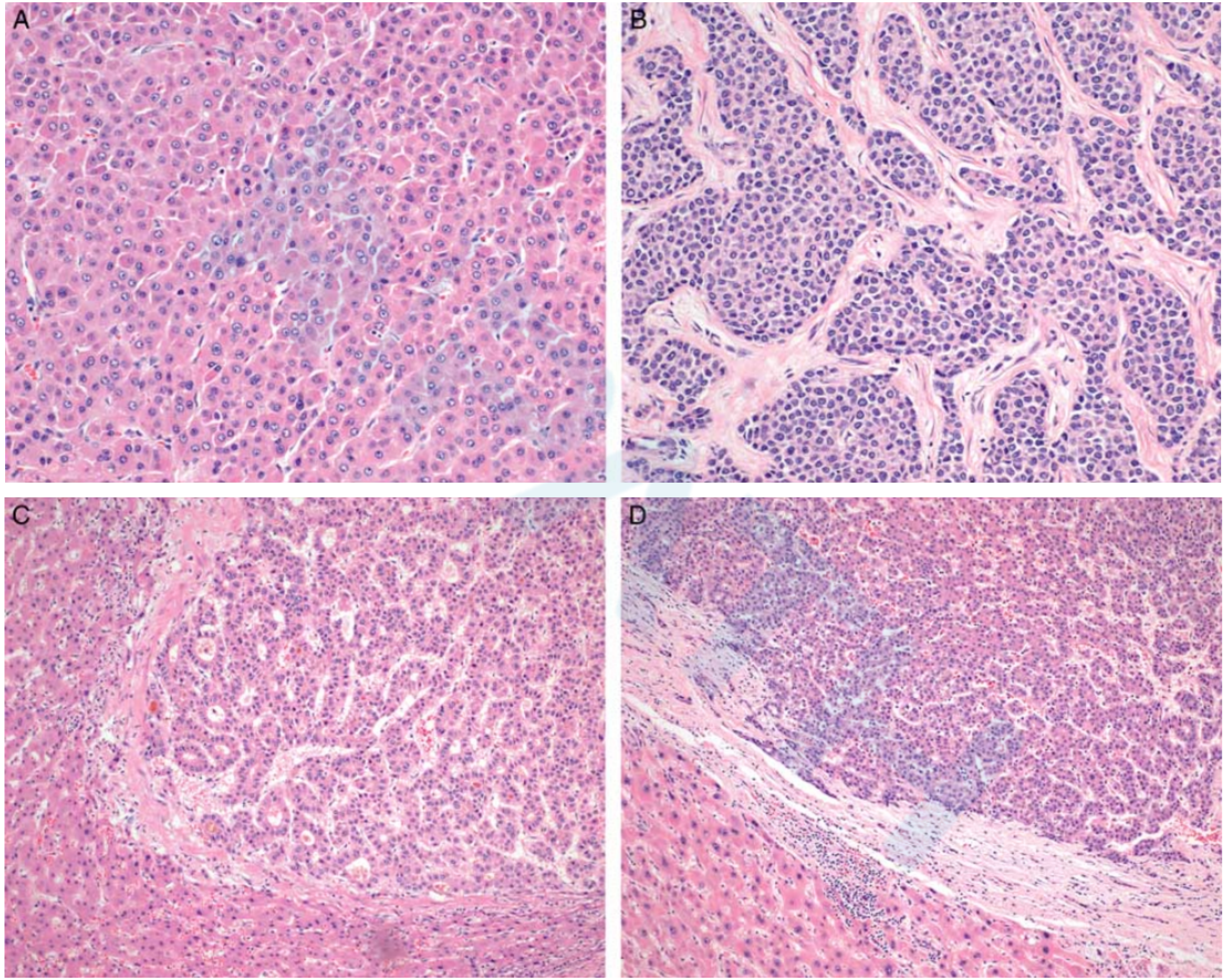
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Supplementary Table 1: Correlation of Histologic Features with HCC Recurrence Following Liver Transplantation

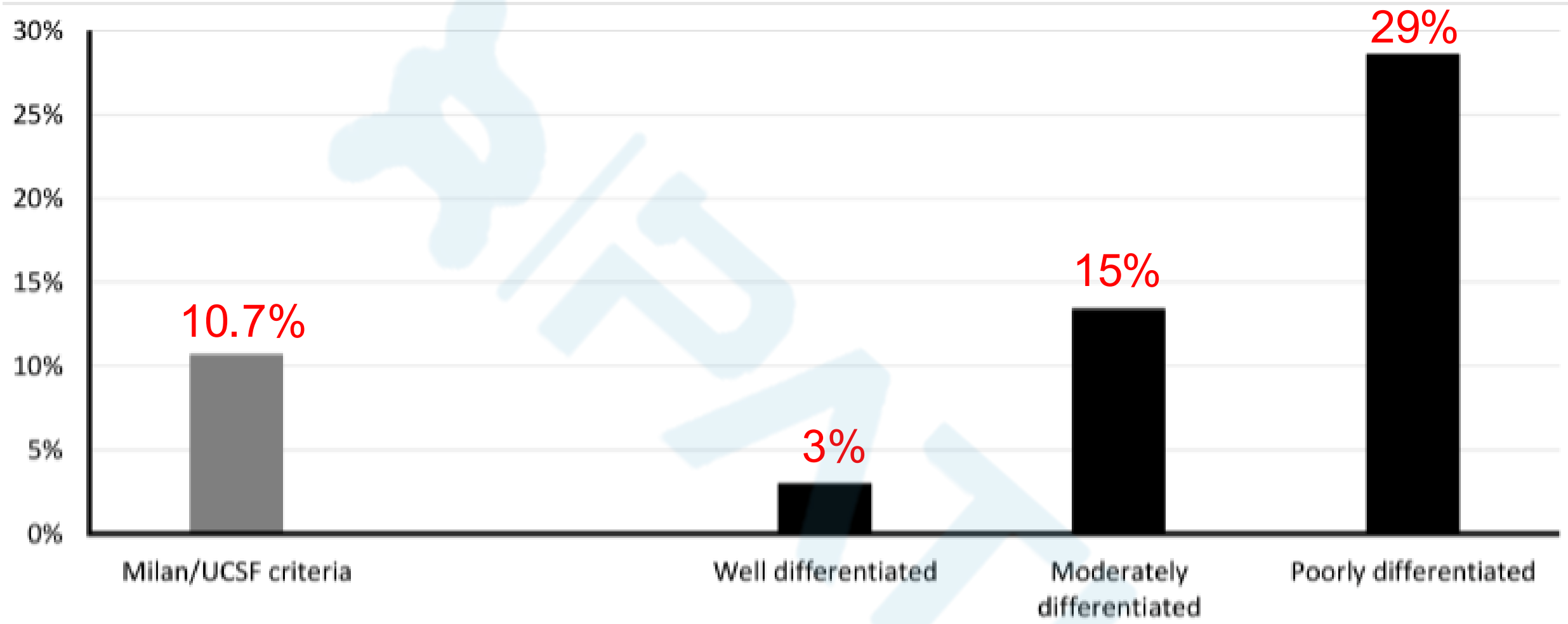
		Recurrences	Total Cases	Percentage	Significance
Architecture					0.0139
	Trabecular	2	53	3.8%	
	Acinar	3	20	15.0%	
	Scirrhous	8	30	26.7%	
	Solid	2	6	33.3%	
Nuclear-to-cytoplasmic ratio					0.0462
	Less than 0.5	8	81	9.9%	
	0.5 and greater	7	28	25.0%	
Nuclear pleomorphism					0.0010
	Absent	5	76	6.6%	
	Present	10	33	30.3%	
Cytoplasm					0.0009
	Eosinophilic	1	51	2.0%	
	Amphophilic	14	59	23.7%	
Mitotic activity					0.0135
	< 10 per 10 HPF	8	85	9.4%	
	⩾ 10 per 10 HPF	7	24	29.2%	

Supplementary Table 1: Correlation of Histologic Features with HCC Recurrence Following Liver Transplantation

		Recurrences	Total Cases	Percentage	Significance
CK19 labeling					0.1350
	Negative	14	107	13.1%	
	Positive	1	2	50.0%	
Fatty change					0.9868
	Present	3	22	13.6%	
	Absent	12	87	13.8%	
Macronucleoli					0.3320
	Present	6	32	18.8%	
	Absent	9	77	11.7%	
Cytoplasmic granularity					0.0803
	Fine	11	57	19.3%	
	Granular	4	52	7.7%	

TABLE 1. Radiologic and Pathologic Characteristics of Liver Explants With HCC

	Recurrences	Total Cases		Percentage	Significance
Total number of liver explants from 1997 to 2015		1061			
With HCC		351			
With available paraffin-embedded tissue		190			
With tissue and preoperative radiology reports		184			
Transplant criteria on imaging					0.1850
Meets Milan criteria	18	161	87.5%	11.2	
Meets UCSF criteria	1	17	9.2%	5.9	
Exceeds Milan/UCSF criteria	2	6	3.3%	33.3	
Transplant criteria on gross examination					0.0179
Meets Milan criteria	10	128		7.8	
Meets UCSF criteria	5	35		14.3	
Exceeds Milan/UCSF criteria	6	21		28.6	
Disagreement between imaging and gross examination					0.4552
Upstaged from Milan to UCSF criteria by gross examination	4	26		15.4	
Upstaged beyond Milan/UCSF criteria by gross examination	5	20		25.0	
Downstaged by gross examination	1	12		8.3	
Tumor differentiation					0.0167
Well differentiated	2	74		2.7	
Well-to-moderately differentiated	4	21		19.0	
Moderately differentiated	11	81		13.6	
Moderately-to-poorly differentiated	1	5		20.0	
Poorly differentiated	3	9		33.3	



Supplementary Figure 1: Comparison of HCC recurrence rates by histologic grade and radiographic criteria. *Classification of tumor grade using WHO methodology better stratifies post-transplant HCC recurrence than Milan or UCSF imaging criteria (n=178, p=0.0082).*

在符合米兰或 UCSF 标准的病例中，移植后 HCC 复发率为 10.7%。

而通过 WHO 分级后，不同分化程度与肿瘤复发有显着相关性（P = 0.0082）。

TABLE 1. Radiologic and Pathologic Characteristics of Liver Explants With HCC

	Recurrences	Total Cases	Percentage	Significance
Vascular invasion				<0.0001
Absent	11	166	6.6	
Present	10	24	41.7	
Number of masses on imaging				0.0204
0	4	62	6.5	
1	13	74	17.6	
2	1	37	2.7	
3+	3	11	27.3	
Size of largest mass on gross examination (cm)				0.0008
0-2	1	55	1.8	
2-4	9	92	9.8	
≥4	11	43	25.6	
Aggregate size of masses on gross examination (cm)				0.0408
0-2	0	39	0.0	
2-4	6	62	9.7	
4-6	6	40	15.0	
≥6	9	49	18.4	

血管侵犯、肿瘤数目、肿瘤最大径和肿瘤总直径与移植后复发也有显著相关性

TABLE 2. Multiple Logistic Regression Model for Histologic Features

Variables	Coefficient (β)	SE	Wald (χ^2)	Significance	Odds Ratio	95% CI
Nuclear pleomorphism*	1.449	0.602	5.791	0.016	4.260	1.309-13.871
Cytoplasmic amphophilia	1.395	0.832	2.809	0.094	0.248	0.049-1.267
N:C ≥ 50%	1.012	0.599	2.851	0.091	2.751	0.850-8.904
Architecture: solid	1.304	0.939	1.926	0.165	3.683	0.584-23.225
Architecture: scirrhous	1.021	0.713	2.054	0.152	2.777	0.687-11.222
Architecture: acinar	0.736	0.878	0.703	0.402	2.088	0.374-11.671
Macronucleoli	0.570	0.622	0.841	0.359	1.768	0.523-5.979
Mitotic index	0.337	0.596	0.320	0.571	1.401	0.436-4.507
CK19 labeling	−1.229	1.350	0.828	0.363	0.293	0.021-4.127
Intratumoral steatosis	−0.486	0.706	0.473	0.492	0.615	0.154-2.457
Intercept	−3.477	0.750				

*Nuclear pleomorphism is a statistically significant independent predictor of tumor recurrence after transplant.
95% CI indicates 95% confidence interval for estimated odds ratio; intercept, mathematical constant; N:C, nuclear-to-cytoplasmic ratio; Wald, Wald test statistic.

Multiple logistic regression demonstrated strong correlation between tumor recurrence and nuclear pleomorphism, cytoplasmic amphophilia, solid and scirrhous architecture, and high nuclear-to-cytoplasmic ratio; however, **mitotic activity** showed only weak correlation and was excluded from our scoring system.

TABLE 3. Recurrence Risk Assessment Score

Feature	Score
Architecture	
Trabecular or acinar	0
Scirrhous or solid	1
Nuclear-to-cytoplasmic ratio (%)	
< 50	0
≥ 50	1
Nuclear pleomorphism	
Absent	0
Present	1
Cytoplasm	
Eosinophilic	0
Amphophilic	1
RRAS category	
Low risk	0 points
Intermediate risk	1-3 points
High risk	4 points

评分是根据整个肿瘤中得分最高的区域进行评估的，并且每一个区域都是独立的。如果一个区域包含核多形性而没有其他不利特征，则为1分，另一个单独的区域包含除核多形性之外的所有不利特征，则为3分，即使所有4个不利特征都出现在该肿瘤中，但最终报告的类别仍是3分，中风险。

	WHO Grade			RRAS Category		
Case	Grader 1	Grader 2	Grader 3	Grader 1	Grader 2	Grader 3
1	M	P	M	L	L	I
2	M	P	M	I	I	I
3	P	P	P	H	H	H
4	M	P	P	I	H	H
5	M	P	W	L	L	L
6	M	P	M	I	I	L
7	M	P	P	I	I	H
8	M	P	P	H	H	I
9	M	P	P	I	H	I
10	M	P	M	I	I	I
11	M	P	P	I	I	I
12	M	M	M	I	L	I
13	W	P	W	L	L	L
14	M	P	P	I	I	I
15	M	W	M	I	L	L
16	M	P	W	I	I	L
17	M	P	P	H	H	I
18	M	P	M	I	I	I
19	W	P	M	L	L	I
20	M	P	M	I	H	I
	K = 0.136			K = 0.543		

TABLE 4. Posttransplant HCC Recurrence Stratified by RRAS and WHO Grade

	Recurrences	Total Cases	Percentage
RRAS raw score			
0	0	60	0
1	3	52	5.8
2	5	43	11.6
3	6	22	27.3
4	7	13	53.9
RRAS category			
Low risk	0	60	
Intermediate risk	14	117	
High risk	7	13	
WHO grade			
Well differentiated	2	74	2.7
Moderately differentiated	15	102	14.7
Poorly differentiated	4	14	28.6

2例低分化
20例中分化
38例高分化
34例高分化
2例高分化

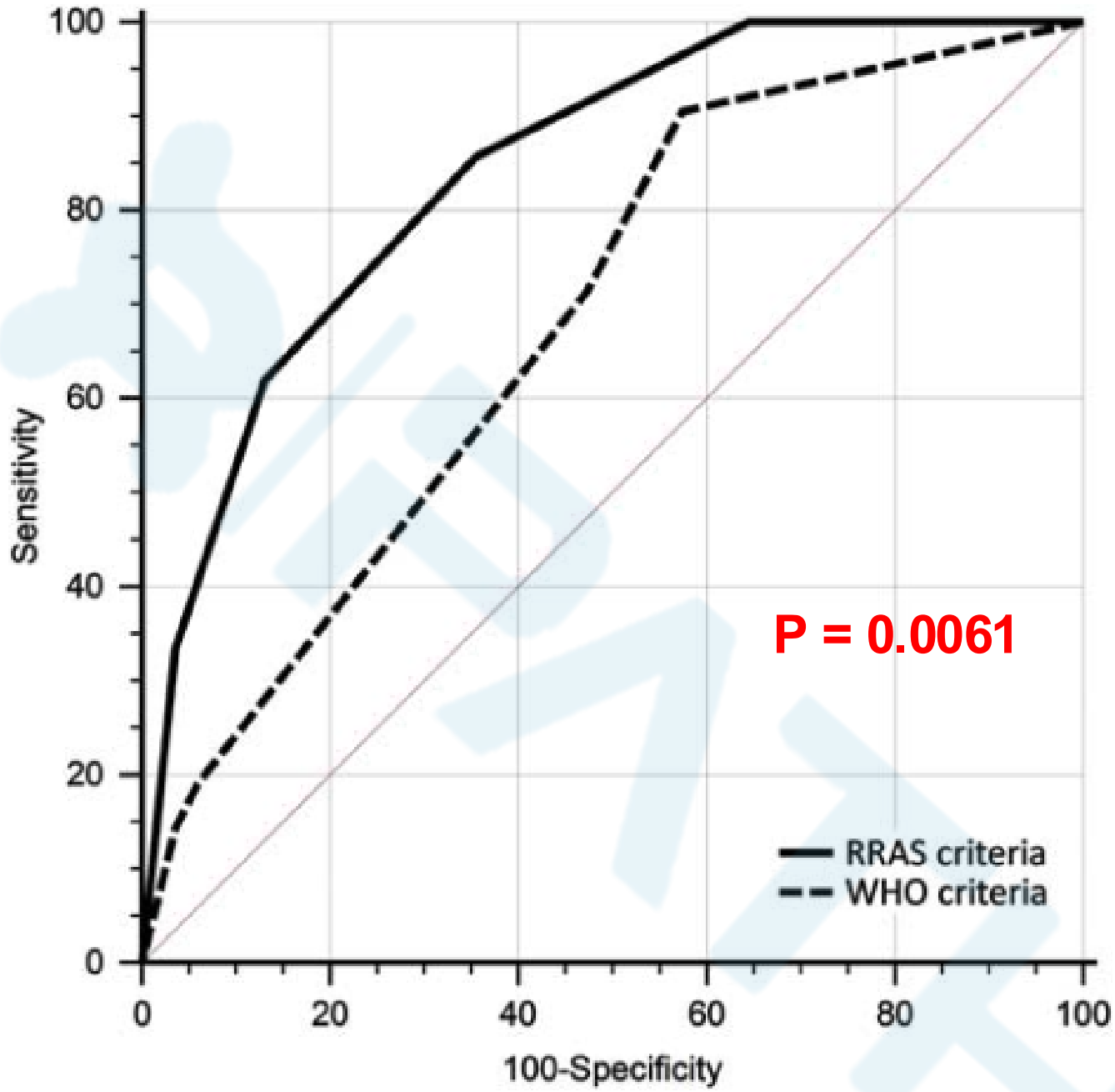
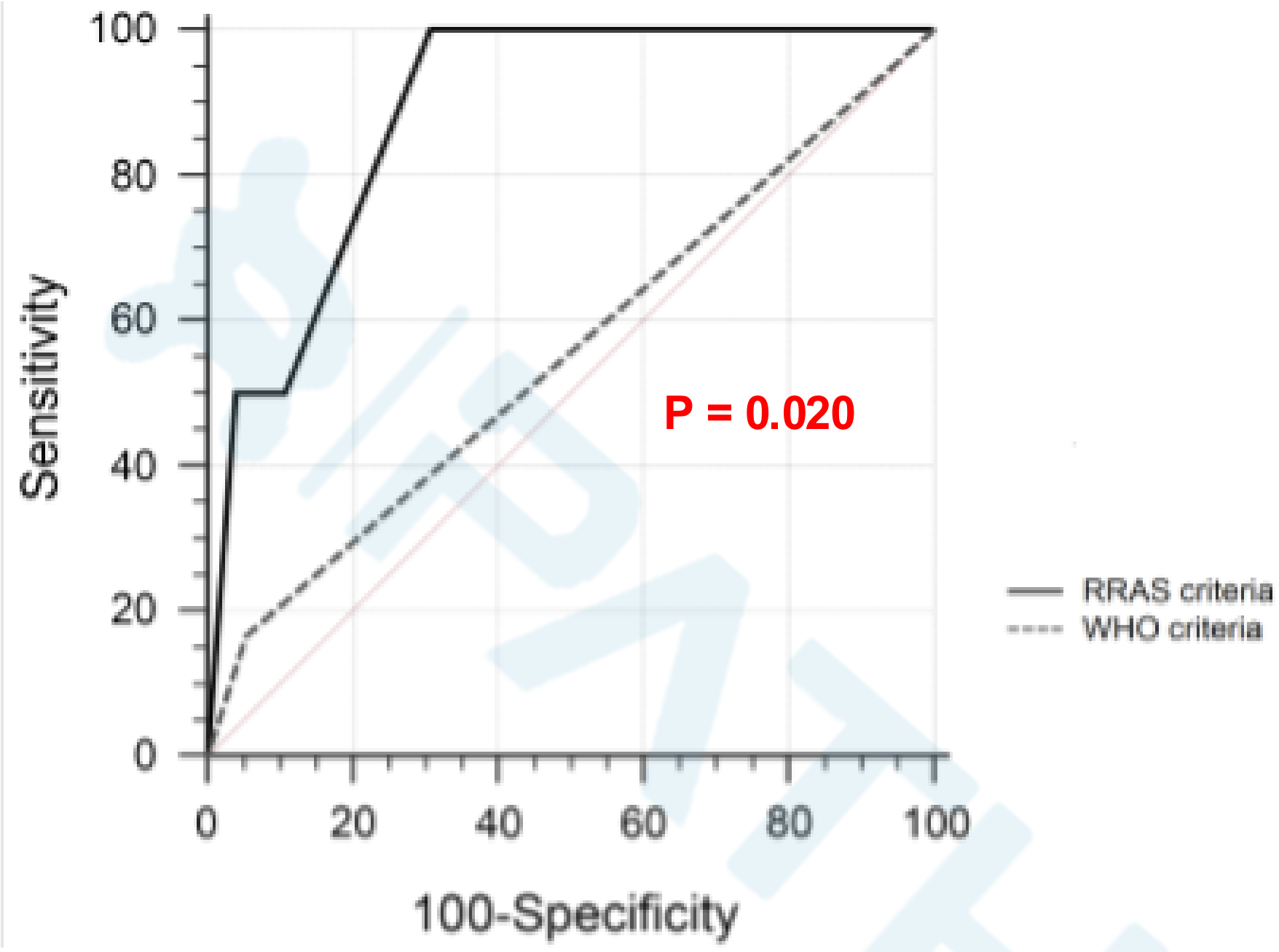
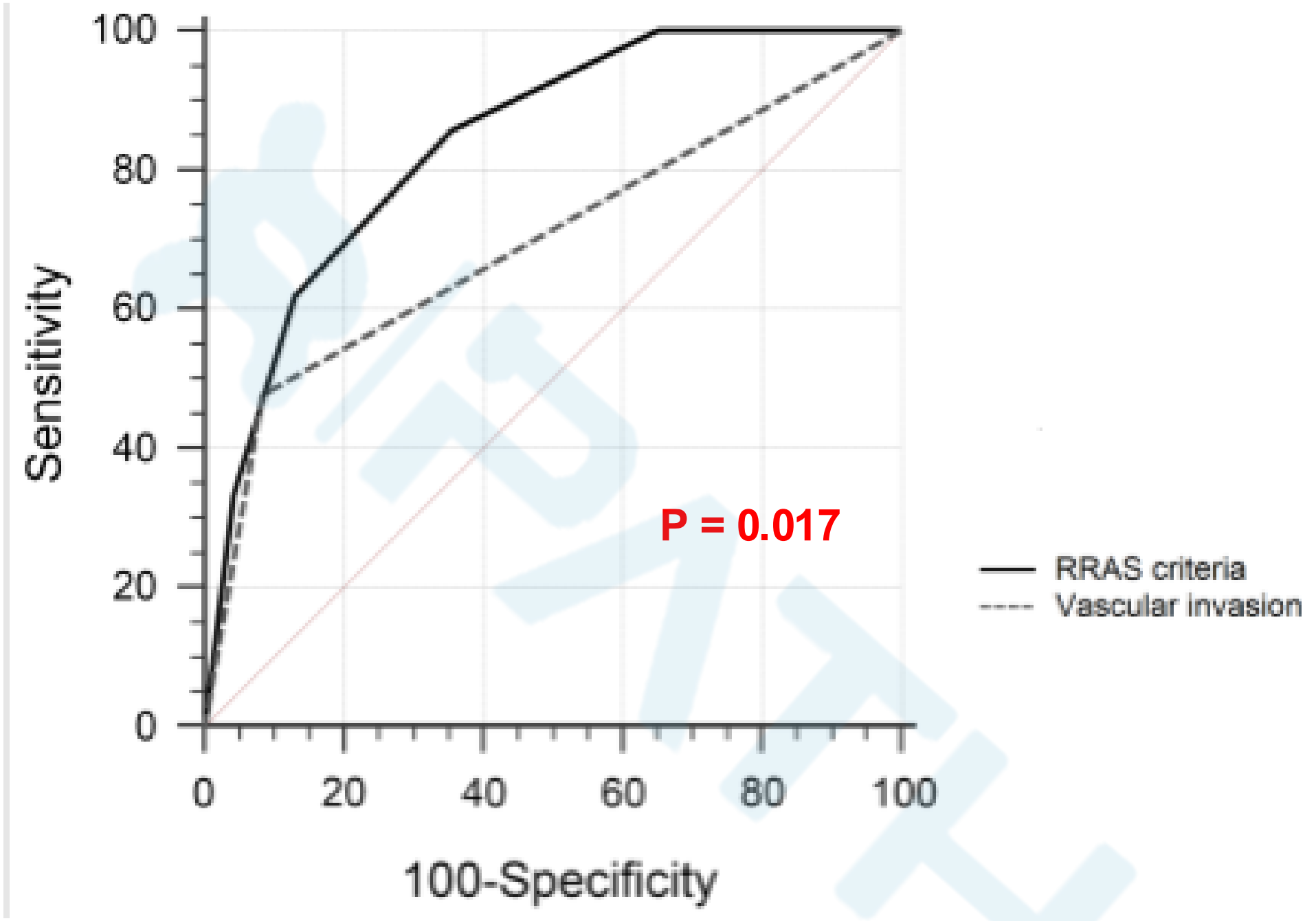


FIGURE 4. ROC curves were analyzed to assess the performance of the WHO tumor grade and RRAS in predicting posttransplant HCC recurrence (area under curve = 0.617 and 0.841, respectively). RRAS allowed for a more accurate prediction of tumor recurrence following transplantation (P = 0.0061, n = 190).



Supplementary Figure 3: Validation of RRAS performance in independent cohort. *In 81 explants that were not used to establish the scoring criteria, ROC curve analysis was used to validate the diagnostic performance of the RRAS in predicting post-transplant HCC recurrence when compared to WHO tumor grade (WHO criteria (dashed line, AUC=0.554), RRAS criteria (solid line, AUC=0.887), n=81). In this independent cohort, RRAS allowed for a more accurate prediction of tumor recurrence following transplantation (p=0.020).*



Supplementary Figure 4: Comparison of recurrence prediction by vascular invasion and RRAS. *Post-transplant recurrence prediction using vascular invasion status and RRAS criteria was assessed by ROC curve analysis (vascular invasion (dashed line, AUC=0.697), RRAS criteria (solid line, AUC=0.839), n=190). RRAS more accurately predicts tumor recurrence following transplantation than vascular invasion status alone (p=0.017).*

DISCUSSION

- 确定肝移植资格的最常用方法是基于影像学测量的肿瘤范围，这种方法在过去二十年中基本保持不变。但是，完全依赖影像学特征来确定移植资格可能会对患者的复发风险产生不可靠的预测。
- 最近的多项研究已将肿瘤组织学确定为移植后强有力的预测指标，并且一些机构已将组织学形态纳入移植标准。例如，杭州标准。
- WHO 是最广泛使用的 HCC 分类指南，由于主观因素，一致性不强，相比之下，RRAS 标准的一致性更好。
- RRAS 标准对肿瘤复发的预测优于米兰标准、UCSF 标准。

DISCUSSION

- 本文有 2 例高分化的肿瘤，归类为高风险，这是因为 2 例均有小灶高风险的结构特征，RRAS 评分是根据具有最差的组织学特征的肿瘤区域决定的。

- 本文有 2 例低分化的肿瘤，归类为低风险。这主要是因为这两例评为低分化的标准主要是 RRAS 标准中与移植后复发无关的结构特征，如两例均含有大核仁，染色质密度不同，腺泡的形成，核轮廓稍不规则，但没有明显的核大小差异。

DISCUSSION

- CK19 是肝祖细胞的标志物，其在分化成肝细胞时下调，但维持在成熟的胆管细胞中。具有 CK19 表达的 HCC 可能源自肝祖细胞而非成熟肝细胞，并且多项研究已将其表达鉴定为预测肿瘤复发。然而，在我们的分析中，CK19 仅在 1.8% 的肿瘤中表达，并且未显示与肿瘤复发的显着相关性。因此，本研究没有将 CK19 作为 RRAS 标准的一部分。

- 移植后肿瘤复发已成为移植患者中最常见的死亡原因，更准确的量化患者的复发风险可以帮助指导移植后的 HCC 监测策略。此外，RRAS 分类可能影响移植后免疫抑制的选择。

CONCLUSION

- RRAS 评分系统比 WHO 分级具有更好的预测性能。

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Cytoplasm	
Eosinophilic	0
Amphophilic	1
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Low risk	0 points
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THANK YOU