Rate of Seeding With Biopsies and Ablations of Hepatocellular Carcinoma: A Retrospective Cohort Study

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Biopsies of liver masses that prove to be hepatocellular carcinomas (HCCs) are associated with a risk of seeding the abdominal or chest wall with tumor cells. The reported frequency of seeding varies greatly in the literature. We performed a retrospective cohort study in a large integrated health care system to examine rates of seeding in patients with HCC who had targeted liver biopsies, ablations, or both performed by community radiologists. We reviewed pathology and radiology records to determine the occurrence of wall seeding, defined as a chest or abdominal wall lesion along a definite or probable needle tract. A total of 1,015 patients had targeted liver biopsies (795), ablations (72), or both (148). Multiple procedures were done in 284 patients (28%). Six cases of seeding were identified. The rate of wall seeding was 2/795 patients (0.13%; 95% confidence interval [CI], 0.00%-0.60%) if only biopsies were done versus 4/220 (1.82%; 95% CI, 0.05%-3.58%) if ablations were performed ($P = 0.01$). The rate was 0/72 (0.00%; 95% CI, 0.00%-0.04%) with ablations alone and 4/148 (2.70%; 95% CI, 0.74%-6.78%) if both procedures were done ($P = 0.31$). Of those with 1 year follow-up (n = 441), the rate of seeding was 2/269 (0.74%; 95% CI, 0.00%-1.77%) if biopsies alone were done and 4/172 (2.33%; 95% CI, 0.07%-4.58%) if ablations were done. In none of the cases was the seeding a proximate cause of death. *Conclusion:* Biopsies of liver masses are associated with a low rate of wall seeding when performed in a community setting and when they are the sole procedures. Ablations may have a higher rate of seeding, particularly if done with biopsies, but are still rare. (*Hepatology Communications* 2017;1:841-851)

Introduction

Hepatocellular carcinoma (HCC) is common in patients with cirrhosis, reaching a yearly incidence of 1%-4% in those with hepatitis C virus,[1] 1%-2% in patients with hepatitis B virus, and over 1% with other causes of cirrhosis.[2] The frequency of HCC with cirrhosis is sufficient to make screening for HCC cost effective,[3] and screening is recommended by various liver society guidelines.[4,5]

The goal of screening for HCC is to diagnose these tumors when they are small and potentially curable.

When HCC tumors are 1-2 cm in diameter, the 5-year survival after surgery or ablation is between 70% and 90%.[4,6] When tumors are larger than 2 cm, the risk of vascular invasion and satellite lesions rises exponentially, and survival is lower.[7]

When HCC tumors are over 2 cm, they can often be diagnosed without a biopsy because their predominantly arterial supply gives them a distinctive radiologic appearance. This arterial blood supply typically does not occur until they are close to 2 cm in size,[8] and the smaller the tumor, the less likely it is to have this typical radiologic appearance. For example, two studies of tumors between 1 and 3 cm in size found

that about 20% of the documented HCC did not meet American Association for the Study of Liver Disease (AASLD) imaging criteria. [9,10] Thus, the most curable tumors that are the prime target for surveillance are also the ones most in need of a biopsy for diagnosis. [9-13] Further, even larger tumors are not always classic in appearance and may require a biopsy for diagnosis. Finally, the increasing incidence of intrahepatic cholangiocarcinoma makes an accurate diagnosis critical in deciding which treatments to offer.

Percutaneous biopsies of HCC carry multiple risks, including the risk of tumor seeding. The published risk varies greatly, with the most quoted study being a 2008 meta-analysis comprising 1,340 biopsies that showed an overall incidence of 2.7%, with individual studies ranging from 1.5% to 5.8%. [14] These data have led to frequent warnings to be cautious in recommending biopsies of liver lesions. The AASLD guideline on liver biopsies discusses this caution and notes that "uncertainty regarding these issues underlies the reported wide practice variation." [15] It also concludes that the risks of seeding are "almost certainly overstated." Supporting this assertion, a subsequent large Korean study found a much lower rate of seeding (0.12%). [16]

Seeding can also occur when tumors are treated with percutaneous ablation techniques. That risk also varies greatly in the literature, with incidences in a 2007 meta-analysis varying from 0% to 12.5%, with an overall risk of 1.21%. The time to diagnosis of seeding in these studies ranged up to 58 months for biopsies and 108 months for ablations, with a median time of 13 and 16 months, respectively. [17]

Given the wide range of seeding risk described in the literature and the overall small numbers in the meta-analysis above, [14] we undertook a study to determine the rate of seeding when biopsies and ablations were performed in a large community setting in the United States.

Patients and Methods

A retrospective cohort study was conducted at a Northern California integrated health care delivery system by examining electronic data on patients who underwent biopsies or ablations for HCC between January 1996 and December 2010. Patients were included if they had undergone a targeted liver biopsy of a radiologically detected lesion that eventually proved to be HCC, or if they underwent a percutaneous image-guided ablation procedure (radio frequency ablation [RFA] or cryoablation or ethanol injection) of an HCC lesion. Procedures were included only if done by staff radiologists at one of our 16 medical centers. Patients were included under International Classification of Diseases, Ninth Revision, diagnosis codes 50.11, 50.22, 50.24, 50.26, and 50.29 and procedure codes 47000, 47001, 47382, 76003, 77002, 77012, and 77013. Chart reviews were done of all biopsies to determine whether a biopsy was targeted or random. The diagnosis of HCC was made in accord with criteria and guidelines at the time the diagnosis was made. The study was approved by the Kaiser Permanente Northern California Institutional Review Board.

Seeding was defined as a chest or abdominal wall lesion along a definite or probable needle tract. Biopsy confirmation was not essential to the diagnosis. Candidate cases were identified by a hepatologist's (J.L.S.) review of all pathology and radiology records subsequent to the index biopsy or ablation, supplemented by available electronic hospital and clinic records. Possible cases of seeding underwent detailed scrutiny by an interventional radiologist (T.E.D.) to insure that the candidate seeding was along a needle tract. Cases were not excluded if the patient had additional surgical procedures as long as they had a targeted percutaneous biopsy or ablation.

Follow-up of tumors was provided by the treating physicians, variably oncologists, gastroenterologists, hepatologists, or transplant physicians. The date of

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TABLE 1. DEMOGRAPHICS, FULL COHORT (N = 1015): AGE AT STUDY ENTRY, SEX, RACE/ETHNICITY

	Biopsy Only (n = 795) n (%)	Ablation Only (n = 72) n (%)	Biopsy + Ablation (n = 148) n (%)	Total (N = 1,015) n (%)
Sex				
Male	581 (73.1)	53 (73.6)	108 (73.0)	742 (73.1)
Female	213 (26.8)	19 (26.4)	40 (27.0)	272 (26.8)
Transsexual	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)
Race				
White	404 (50.8)	27(37.5)	82 (55.4)	513(50.5)
Hispanic	106 (13.3)	14 (19.4)	19 (12.8)	139 (13.7)
Asian/Pacific Islander	206 (25.9)	22 (30.6)	28 (18.9)	256 (25.2)
Black	74 (9.3)	6 (8.3)	18 (12.2)	98 (9.7)
Other/Unknown	5 (0.6)	3 (4.2)	1 (0.7)	9 (0.9)
Age, year (mean ± SD)	64.0 ± 12.3	60.5 ± 9.7	62.6 ± 10.2	63.5 ± 11.9

study entry was the date of first liver biopsy or ablation. The date of seeding was the first date it was noted in the record. Patients were followed until death or leaving plan membership permanently, with censoring on December 31, 2015. Thus, most patients, if alive, had a minimum of 5 years follow-up.

STATISTICAL METHODS

The primary outcome was the rate of seeding after undergoing percutaneous procedures for HCC. Fisher's exact tests were used to evaluate differences in proportions of patients undergoing biopsies only, biopsies and ablations, and ablations only, and 95% confidence intervals (CIs) were calculated for the proportions. Statistical significance was set at 0.05. We also present the outcomes as person-years per seeding case.

Kaplan-Meier curves for survival from first procedure to death were plotted for all patients in the cohort, and time of seeding was noted on the curves. SAS 9.3 (SAS Institute, Cary, NC) was used for all analyses and plots.

Results

The demographic characteristics of the patients are shown in Table 1. As is typical in series of patients with HCC, most were male and a higher proportion was Asian compared to the proportion of Asians in the general population.

Of the 1,015 patients who underwent percutaneous procedures, 795 had biopsies only, 72 had ablations only, and 148 had both (Table 1). Multiple procedures were done in 284 patients (28.0% of the cohort), with about one sixth of the biopsies and one sixth of the ablations being done multiple times (Table (2A–2C)). Of the total

ablation cohort of 220, 172 patients had RFA, 3 had ethanol injection and RFA, 12 had ethanol only, 31 had cryoablations, and two had both RFA and cyroablation.

If we limit the data to the 737 with 3 or more months of follow-up, 526 had biopsies only, 64 had ablations only, and 147 had both ablations and biopsies. Multiple procedures were done in 259 (35.1%) of these patients (Table 2C), with about one fifth of the biopsies and one fifth of the ablations being done multiple times (Table (2A and 2B)).

A total of 441 patients had 1 year or more of follow-up, 269 of whom had biopsies, 44 had ablations, and 128 had both.

Biopsy needle size was available in 807 cases. Almost all were performed with 18 gauge (54.15%), 20 gauge (29.37%), or 22 gauge (12.02%). The largest needle used per case was larger than 18 gauge in only 2.11% of cases, and only once was the largest size less than 22 gauge (Table 3).

The distribution of tumor sizes at biopsy is displayed in Table 4. As expected, tumor size was largest in those with the shortest follow-up. Those with follow-up of 3 months or less had a median tumor size of 8.0 cm, with 56% greater than 5 cm, compared to a median size of 3.7 cm in those who were followed for more than 1 year, with 33% greater than 5 cm. Forty-six percent of the former had more than two tumors, with almost 22% having macrovascular involvement compared to 13% and less than 3% in those with more than 1 year of follow-up.

The median number of cross-sectional body imaging was 1 (interquartile range 0-2) in those who were followed for less than 1 year and 8 (interquartile range 4-13) in those who were followed for more than 1 year.

Of the total cohort (N = 1,015), 54 patients went on to have liver transplants, with 22 after biopsies only, 14 after ablations only, and 18 after both.

TABLE 2A. PATIENTS WHO UNDERWENT BIOPSY: PERCUTANEOUS BIOPSIES PER PATIENT

	All Patients (N = 942) n (%)	Patients with 3+ Months Follow-Up (n = 673) n (%)	Patients with 1+ Year Follow-Up (n = 396) n (%)
Number of Biopsies			
1	786 (83.4)	539 (80.2)	302 (76.3)
2	125 (13.3)	102 (15.2)	71 (17.9)
3	29 (3.1)	29 (4.3)	21 (5.3)
4	2 (0.2)	2 (0.3)	2 (0.5)

TABLE 2B. PATIENTS WHO UNDERWENT ABLATION: ABLATIONS PER PATIENT

	All Patients (N = 220) n (%)	Patients with 3+ Months Follow-Up (n = 211) n (%)	Patients with 1+ Year Follow-Up (n = 172) n (%)
Number of Ablations			
1	181 (82.3)	173 (82.0)	137 (79.7)
2	32 (14.5)	31 (14.7)	28 (16.3)
3	5 (2.3)	5 (2.4)	5 (2.9)
4	2 (0.9)	2 (0.9)	2 (1.2)

TABLE 2C. TOTAL NUMBER OF PROCEDURES (BIOPSIES, ABLATIONS)

	All Patients (N = 1,015) n (%)	Patients with 3+ Months Follow-Up (n = 737) n (%)	Patients with 1+ Year Follow-Up (n = 441) n (%)
Number of Procedures			
1	731 (72.0)	478 (64.9)	239 (54.2)
2	209 (20.6)	184 (25.0)	138 (31.3)
3	59 (5.8)	59 (8.0)	49 (11.1)
4	12 (1.2)	12 (1.6)	11 (2.5)
5	0 (0.0)	0 (0.0)	0 (0.0)
6	3 (0.3)	3 (0.4)	3 (0.7)
7	1 (0.1)	1 (0.1)	1 (0.2)

TABLE 3. LARGEST NEEDLE SIZE USED FOR CASE

Largest Needle Size	Frequency (%)	Percentage
Unknown	208	20.49
14	2	0.20
15	2	0.20
16	5	0.49
17	8	0.79
18	437	43.05
19	15	1.48
20	237	23.35
21	3	0.30
22	97	9.56
23	1	0.10

Of the 795 patients with biopsies only, two had seeding (0.25%; 95% CI, 0.00%-0.60%) (Table 3). Of the 220 patients with ablations, four had seeding (1.82%; 95% CI, 0.05%-3.58%). All four had both biopsies and ablations (4/148, 2.70%; 95% CI, 0.74%-6.78%) with no seeding cases among those who had only an ablation (0/72, 0.00%; 95% CI, 0.00%-0.04%). Three of the four patients had RFA and 1 had cryotherapy. The rate of seeding with ablations was significantly higher than with biopsies alone ($P = 0.02$). The rate of seeding after ablations with biopsies was not significantly different from that after ablations alone ($P = 0.31$). The Kaplan-Meier survival curve (Fig. 1) displays the time from first procedure to the time of seeding, together with the length of follow-up.

Of those who had 3 months of follow-up (n = 737), the overall rate of seeding was 0.81% (95% CI, 0.17%-1.46%). For biopsy only (n = 526), the rate of seeding was 0.38% (95% CI, 0.00%-0.91%). For ablations with or without biopsies (n= 211), the rate of seeding was significantly higher (1.90%, $P = 0.01$), albeit with an overlapping CI (95% CI, 0.06%-3.74%). Of those patients with ablations who had follow-up of 3 or more months, 1/31 with cryoablation had seeding (3.23%; 95% CI, 0.00%-9.45%) compared with 3/166 with RFA (1.81%; 95% CI, 0.00%-3.83%; $P = 0.50$). Of the 2 patients who had both cyroablation and RFA, neither had seeding.

Of those who had a year or more of follow-up (n = 441), the overall rate of seeding was 1.36% (95% CI, 0.28-12.44). For biopsy only (n = 269), the rate of seeding was 0.74% (95% CI, 0.00-1.77). For ablations with or without biopsies (n = 172), the rate of seeding was significantly higher (2.33%, $P < 0.01$), albeit with an overlapping CI (95% CI, 0.07%-4.58%). Of those with cryoablation, 1/24 had seeding (4.17%; 95% CI, 0.00%-12.16%) compared with 3/139 with RFA (2.16%; 95% CI, 0.0%-4.57%; $P \leq 0.01$). Two patients had both cryoablation and RFA, and neither had seeding.

The mean time to detection of seeding was 29.8 months (range, 14 to 52 months), and the median was 29.5 months. The person-years per seeding was 350.3 for the entire cohort or 343.3 for those with at least 3 months of follow-up and 315.6 for those with at least 1 year of follow-up.

Three patients had widespread HCC close to the time when seeding was discovered, and all 3 individuals died. Four patients underwent treatment of the seeded tumor during the study period, 3 with resection of the seeded area and 1 with transarterial

TABLE 4. TUMOR SIZE AND NUMBER AT BIOPSY

	Overall	Less Than 3 Months Follow-Up	Less Than 1 Year Follow-Up	More Than 1 Year Follow-Up
Largest tumor median (interquartile)	4.9 (3.0-8.3)	8.0 (5.0-11.7)	6.8 (4.0-10.0)	3.7 (2.6-5.5)
Missing	125 (12.32%)	80 (28.78%)	114 (19.86%)	11 (2.49%)
<2 cm	53 (5.12%)	2 (0.72%)	13 (2.26%)	39 (8.84%)
2-5 cm	393 (38.72%)	41 (14.75%)	142 (24.74%)	251 (56.92%)
>5 cm	445 (43.84%)	155 (55.76%)	305 (53.14%)	140 (32.75%)
Number of tumors				
Missing	39 (3.84%)	21 (7.55%)	31 (5.40%)	8 (1.81%)
1	584 (57.54%)	106 (38.13%)	269 (46.86%)	315 (71.43%)
2	127 (12.51%)	24 (8.63%)	67 (11.67%)	60 (13.61%)
>2	265 (26.11%)	127 (45.68%)	207 (36.06%)	58 (13.15%)
PV/HV/IVC involved	111 (10.94%)	61 (21.94%)	99 (17.25%)	12 (2.72%)

Abbreviations: HV, hepatic vein; IVC, inferior vena cava; PV, portal vein.

TABLE 5. SEEDING CASE SUMMARIES*

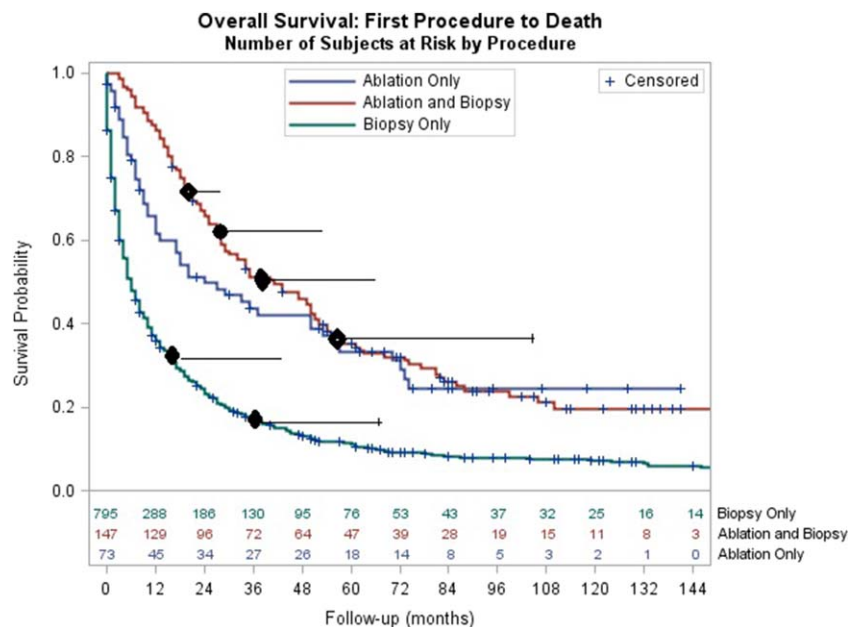| Case	Procedure	Time From First Procedure to Seeding Detection (Months)	Treatment of Seeding	Liver Transplant	Time From First Procedure to Death or Censoring (Months)	Cause of Death if Applies	Status of HCC
1	Biopsy	14	Resection	No	44	HCC	Widespread
2	Ablation and biopsy	17	None	No	27	HCC	Widespread
3	Ablation and biopsy	52	Resected	Yes	69	Alive	No recurrence
4	Ablation and biopsy	37	None	No	64	HCC	Widespread
5	Ablation and biopsy	33	TACE	Yes	70	HCC	Widespread pulmonary
6	Biopsy	48	Resection	No	64	Alive	Recurrence in liver

*First procedure

FIG. 1. Overall survival. Time from first procedure to death or censoring is shown. Seeding events are indicated as black diamonds, with length of follow-up indicated by the horizontal lines. The number of subjects at risk at each time point is indicated at the bottom.

chemoembolization (TACE). The three resections were successful, but in two of them the HCC was not controlled within the liver. The third resected patient had received a transplant before the seeding was detected and is the only one to have no other tumor recurrence. The fourth patient also underwent a liver transplant and subsequently had tumor seeding that was successfully treated with TACE. Soon thereafter, he had innumerable pulmonary metastases that eventually led to his death. Thus, two of the six seeding cases were detected after liver transplant, but in neither of them was seeding a cause of death. The individual cases of seeding are discussed below and summarized in Table 5.

CASE 1

A 1.9-cm lesion was biopsied in 2002 through an 18-gauge guiding cannula. On day 117, open surgical ablations were performed of seven lesions ranging in size up to about 2 cm, and an intra-arterial chemotherapy pump was implanted. Eight months later, multiple liver masses were noted ranging in size from 2.6 to 5.9 cm. These enlarged on subsequent scans. Computed tomography performed 14 months after the biopsy showed a new 1.4-cm lesion in the subcutaneous fat of the right chest wall. This was resected with clear margins. However the patient died of progressive HCC in month 44.

CASE 2

A 2-cm lesion in the posterior-inferior right hepatic lobe was biopsied in 2005 using fine-needle aspiration with a 22-gauge Westcott needle and 20-gauge core needle. RFA was performed 36 days later. In month 17, an enhancing lesion was first noted in the ninth intercostal chest wall interspace. By that time, the index lesion in the liver was enlarging, and despite TACE, this grew within several months to involve the portal veins and then the major splanchnic veins and the porta hepatis. The patient died of progressive HCC 27 months after biopsy.

CASE 3

Several liver masses were noted, and the largest (4.4 cm) in the anterior right lobe was biopsied in 2007 using an 18-gauge needle. Postbiopsy bleeding required transarterial embolization later that day. Almost 6 months later, the patient underwent TACE

and RFA through a right lower anterior intercostal space, and the tract was cauterized. Two years after the biopsy, a liver transplant was performed. A subcostal nodule was noted 52 months after HCC diagnosis, and biopsy confirmed it was HCC. This was resected with wide margins a month later but with angiolymphatic invasion noted. The tumor recurred and was re-resected 8 months later, again with clear margins. The patient was tumor free 17 months later.

CASE 4

A 9.4-cm mass at the right liver capsule was biopsied in 2007 with seven passes using a 17-gauge introducer and an 18-gauge needle. TACE was performed 2 months later, and cryoablation was performed in month 13. In month 37, an ill-defined subcutaneous mass was palpable. This was eventually biopsied with pathology showing HCC. By that time, the original HCC had grown within the liver along with multiple extrahepatic metastases, all of which led to the patient's death.

CASE 5

A 2.5-cm lesion in the right anterior mid-lobe was biopsied in 2009 using a 17-gauge coaxial system and 18-gauge needle, and then RFA was performed in the same sitting. A liver transplant was performed 14 months later, on day 440. An abdominal wall lesion was noted on day 1,011 (month 33), with biopsies positive for HCC 1 month later. The lesion was treated with TACE in month 37. By month 54, innumerable pulmonary metastases were evident as were adrenal metastases, and the patient died after month 70.

CASE 6

An 8-cm heterogeneously enhancing mass in the right hepatic lobe was biopsied multiple times in 2010 using a Tenmo 20-gauge core needle with an intercostal approach. The patient underwent TACE 110 days later, followed 1 month later by a right hepatectomy. Pathology showed amigo-lymphatic invasion. Forty-eight months after the biopsy, computed tomography showed a 5.3-cm mass in the mid-axillary line and apparently involving the right diaphragm. This was resected with clear margins 2 months later. However, multiple hypervascular masses were found in the left lobe of the liver soon thereafter. The patient was alive at the end of follow-up.

Discussion

Our study showed a low rate of wall seeding with HCC if biopsies were the only percutaneous procedures done. Our rate was only 0.25% overall, 0.38% in those with at least 3 months of follow-up, and 0.74% in those with more than 1 year of follow-up. This was significantly lower than the 2.7% found in the benchmark meta-analysis by Silva et al.[14] Of note in that meta-analysis was the trend for seeding to be higher in small series than in larger series, suggesting possible publication bias. Consistent with that trend, our number of patients with biopsies alone (no ablation) is 50% greater than the largest series in that meta-analysis and has a lower rate of seeding. Two Korean series published since that analysis found a rate similar to ours, one a series of 1,055 with an incidence of 0.76%[18] and the other a series of 3,391 biopsies performed with either 18- or 21-gauge needles that found an incidence of 0.12%.[16] When we added these series and our series to those of Silva et al.'s analysis, the number of cases quadrupled with a rate of seeding of 0.72% (40 out of 5,581 patients).

Our rate of seeding with ablation, 1.82%, is in the midpoint of the reported range to date, although higher than the overall rate in HCC cases in the Stigliano et al.[17] meta-analysis (54 of 4,609, 1.17%). This may be due in part to the long follow-up in our patients, with mean time to seeding of almost 30 months in our series compared with 7 months in the Stigliano et al. meta-analysis. Our rate with ablations is lower than the rate of 3.2% reported in the largest Japanese series of 1,031 patients.[19] That may be due to different definitions of what constitutes seeding, with the Japanese series using a criterion of "all newly detected tumors attached to the pleura or peritoneum." Only two thirds of their seeding cases were in the direct line of the RFA needle; using a direct-line definition would bring their rate to 2.1%, closer to ours.

Whether adding biopsy to ablation increases the risk of seeding is unclear. The only meta-analysis, done by Stigliano et al., reports that the incidence of seeding was higher in ablation with biopsy (median, 0.95%; mean, 2.50%) than without biopsy (median, 0.61%; mean, 1.73%),[17] but by reporting the median and mean of studies, it gives disproportionate weight to smaller series compared with larger ones. When the risk is recalculated on a per-patient basis, the opposite conclusion holds, with a risk of 1.08% after performing both biopsy and ablation and 1.23% if ablation was performed without biopsy. The Stigliano et al. analysis

does not contain information on numbers at risk at various intervals of follow-up, in part because it is a bouillabaisse of articles and poorly documented letters to the editor.[17]

More recent series are not consistent about the effect of antecedent or coincidental biopsy on the incidence of seeding after ablation. For example, in a study of 160 patients with subcapsular HCC, Kang et al.[20] found that the only risk factor for peritoneal seeding was biopsy before RFA. In a study of 23 patients who had RFA before liver transplant, Lopez and colleagues[21] found that 2/12 who had preceding biopsy had seeding versus none of 11 who did not have biopsy preceding the RFA. On the other hand, Shirai et al.[22] did not find that biopsy was a risk factor for seeding in their larger series of 257 ablations. Our own study provides too few cases of seeding to definitively answer this question. It is unclear why adding biopsy to ablation would increase the risk given that biopsy alone does not appear to confer a high risk for seeding.

The impact of seeding in our series is somewhat mitigated by the observation that it was not usually a significant cause of morbidity or mortality. In five of our six cases, the detection of wall seeding was followed immediately or soon thereafter by widespread disease, either metastatic or within the liver, and by themselves the seeding sites were either treatable or were not the cause of death. Of the two patients who received liver transplants, one underwent TACE before widespread metastases were discovered, and the other had surgical resections of the seeding site and has had no recurrence after another 17 months of follow-up. This finding that, in general, seeding did not affect patient survival is consistent with other series in the literature, notably the review by Maturen et al.[23] In multiple series, the seeded tumors were variously excised, embolized, ablated, or radiated. Of the 26 seeding cases included in the meta-analysis by Silva et al.,[14] 23 patients were successfully treated, 1 declined treatment, and 2 were lost to follow-up.[23-29] That seeded tumors can be successfully treated is not a given, however. Schotman reported two cases where seeding made a tumor unresectable,[30] and Lopez reported two liver transplant patients in whom seeding led to death 1.5 years and 9 years after liver transplant.[21] The latter case points to the importance of long-term follow-up in these patients in that the seeding was first apparent 4 years after transplant and the patient appeared to be tumor free after the second resection 6 years posttransplant. Three years after that,

however, a recurrence led to the patient's demise. Thus, it requires several years after treatment of a recurrence to determine whether the treatment is ultimately successful. This reflects the heterogeneity of HCC biology, with doubling time varying from 22 to 415 days in one study.[18]

The long time needed for the development of seeded tumors calls into question the reliability of series where the length of follow-up is either short or is not reported. A unique virtue of our study is the explicit delineation of how the number at risk dropped off over time due to death or leaving the health plan and the large number of patients remaining at risk years after the procedure: 462 at 1 year (45.5%,), 229 at 3 years (22.6%), and 141 at 5 years (13.9%). For those who had biopsies only, the numbers were 288 (36.2%), 130 (16.4%), and 76 (9.6%) at the 1-, 3-, and 5-year marks, respectively. It is rare for studies to include any acknowledgment of this drop off, and yet it is critical to a meaningful interpretation of the risk of seeding. In the large meta-analyses that we have been quoting,[14,17] only 2 of the 15 biopsy studies gave even the 1-, 3-, and 5-year survival,[27,31] and both of those studies looked only at a subset of patients whose disease state was limited enough to permit surgical resection. When those two series are combined, 228 patients started, 133 (58.3%) were alive at 3 years, and only 92 (40.4%) were alive at 5 years. Describing patient fallout is particularly important with a tumor such as HCC, where survival is low (5-year survival around 15%).[32,33] Our overall survival rates include both death and loss to follow-up and are similar to the reported rates for HCC survival. The rates for those who underwent biopsy were lower but similar to survival in cohorts not selected for suitability for resection.[34,35] The lack of detail on drop off and deaths in other studies of seeding leads to underestimates of the risk of seeding years after the biopsy or ablation.

Our study has several additional strengths. It is one of the larger published studies of biopsies and ablations. It is also comprehensive in that the study population consisted of members of a prepaid health maintenance organization in which virtually all biopsies and ablations are done internally by staff radiologists. There may have been exceptions where patients had additional insurance coverage or if a local facility used a nonplan hospital and radiologist, but we did not find evidence of that on chart review of our HCC patients.

In addition, the study period of 20 years meant that most patients had a lengthy follow-up after their index procedure. The minimum time period between study entry and end of study was 67 months, and almost all were followed far longer (our health plan has a core of long-term members). This allowed us to detect slower growing cases of seeding; our time to detection was a mean of 30 months, a longer interval than in most of the published literature. By contrast in one large study of 1,055 patients, the mean time between biopsy and emergence of tumor seeding was 267 days (9 months), with all diagnosed by 20 months.[18] In the Stigliano et al. meta-analysis,[17] the median time to diagnosis of seeding was 13 months (range 1-58) after biopsy, 6 months (2-48) after percutaneous ethanol injection, and 7 months (2-54) after RFA.

Our series started 20 years ago. Since that time, improvements in techniques may have decreased the risk of seeding with biopsies or ablations. For example, use of the coaxial cutting needle technique was associated in one series with no seeding cases in 101 patients, with a mean follow-up of 410 days.[23] However, in our series there were only two incidents of seeding among the 526 patients who had biopsies alone, one of which was more than 400 days after biopsy when there were still over 250 patients at risk. Thus, it would take a series magnitudes of order larger than 101 and a longer follow-up period in order to show an improvement in such an already low rate. Furthermore, four of our six seeding cases occurred after use of a coaxial-type system, although in three of them ablation procedures were also performed. Ablation of the RFA track has been said to decrease the risk of seeding[36]; however, that report was a letter to the editor, and ablation of the tract was only one of a number of measures taken to reduce seeding. Further, the decrease in seeding therein was based on a comparison to an earlier historical rate, so the real-life efficacy of the proposed technique is unclear. One of our cases occurred after the tract was cauterized, so clearly that is not a total answer to the problem of seeding, and there have been other reports of seeding after tract cauterization.[37]

One limitation of our study is that we included only cases with abdominal or chest wall seeding. We were not able to determine if there were cases of seeding of the thoracic cavity or cases of intra-abdominal or intra-hepatic seeding. It would be difficult in many cases to adjudicate whether a lung nodule was due to seeding or to the natural course of the disease, given that lung is the most common site of metastasis.[38] We did identify one case that strongly appeared to be pleural seeding from ablation procedures. Similarly,

intraperitoneal spread is the third or fourth most common site of metastasis, and spread within the liver is the rule. To apportion causality would be a daunting task, absent detailed films of the biopsy or ablation procedure. Judging whether intrahepatic seeding was a natural occurrence or due to a needle track would be the most difficult. We therefore restricted ourselves to abdominal and chest wall metastases. In this we were consistent with prior studies. Silva et al., in their frequently quoted 2008 meta-analysis,[14] excluded intrahepatic lesions due to the inability to distinguish second lesions from seeding cases and also noted that no cases were found of widespread intraperitoneal dissemination due to fine-needle biopsy. One study included in the Stigliano et al. meta-analysis[17] did specifically look at the incidence of new intrahepatic nodules along the needle biopsy tract by reviewing angiographic images to confirm that the location of the lesion would be consistent with tract metastasis, but their follow-up of 50 days was so short that it would not allow most tumors to emerge.[39] It also would be difficult to distinguish between a bony metastasis extending into soft tissue and a tract metastasis. For example, one of our cases had a chest wall metastasis near the neck, well away from any biopsy site, raising a residual question of whether the lower chest wall lesion that we attribute to seeding was part of a more general pattern of bony metastases. Two of our hepatoma cases involved a chest wall lesion in the lower thorax on the opposite side from where the biopsy was done, clearly not caused by seeding.

Our study did not address the rationale for the biopsies of the liver lesions in the fraction of our HCC cases that did come to biopsy. The diagnostic criteria for HCC have become more refined over time; radiologic criteria for diagnosis without biopsy were introduced in 2000, and AASLD first incorporated venous washout in their guidelines in 2005, near the end of our study.[40] The liver imaging reporting and data system criteria were first established in 2011, after entry into our study ended, and both those and the Organ Procurement and Transplant Network/ United Network for Organ Sharing criteria prioritize specificity over sensitivity.[41] For example, a recent analysis of HCC tumors between 10 and 30 mm in size found that almost 20% did not meet AASLD or European Association for the Study of the Liver criteria for diagnosis.[9] A recent study of 134 patients with HCC proven at surgical resection or liver transplant found that even when the liver imaging reporting and data system categories 4 and 5 (probable and definite HCC) were combined, imaging only had a combined 91% sensitivity for HCC.[42] Thus, although radiologic criteria have decreased the use of biopsy for diagnosis, a place for biopsy remains in the diagnosis of HCC.[9-13] An increased role for biopsy may emerge with the development of biomarkers to allow for personalization of chemotherapy, and data on the incidence of tumor seeding is an important part of the equation in deciding on a biopsy.[43-45]

Finally, the time to recurrence is related to the date chosen for the seeding diagnosis. We chose the date when seeding was first diagnosed as the most clinically relevant time point, even though in retrospect the tumor may have been seen at a much earlier point. Indeed, retrospective analysis allows for otherwise non-specific findings to acquire new significance. We chose a longer time to recurrence in order to give conservative guidance for the time period within which clinicians should be alert for seeding.

In conclusion, in our series in a community setting in the United States, wall seeding was a rare event, about one in 400, when biopsies were done as the only procedure. Ablations of HCC were associated with a higher incidence of seeding, although at 1.80% it was still rare. These data can be used to update risk–benefit analyses of liver biopsies and to guide decisions on whether to choose ablation, surgery, or TACE to treat a liver lesion. If ablation is chosen, our results can help inform the choice of whether to do so laparoscopically or percutaneously.

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