

Hepatic Angiosarcoma: Clinicopathologic Study With an Investigation of ROS1 Gene Rearrangements

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Abstract. *Background/Aim: Primary hepatic angiosarcoma (PHA) is a rare disease entity with variable morphologic features. Recent findings regarding ROS1 gene rearrangements in PHA may lead to new targeted therapies. Patients and Methods: Thirteen cases (4 resected specimens and 9 biopsy samples) underwent histologic review and morphologic patterns were classified according to a previous study as 1) sinusoidal, 2) peliotic, 3) vasoformative, and 4) solid (epithelioid/spindled). ROS1 immunohistochemistry and investigation of the presence of a ROS1 fusion gene by reverse transcription-polymerase chain reaction were performed in available cases. Results: Eight of 13 cases (62%) showed vasoformative patterns. Three cases (23%) were classified as sinusoidal and two (15%) as solid patterns. Mortality rate was 90% (9/10) except for three patients lost in follow up. Only one patient is still alive and has survived for 8 months with the disease. All cases tested did not have ROS1 expression (0/9) or a ROS1 fusion gene (0/4). Conclusion: We report 13 cases of PHA with 90% mortality. Vasoformative PHA is the most common histologic type. New findings on ROS1 fusion gene rearrangements could lead to the development of novel targeted therapeutics for PHA patients with dismal prognosis.*

Angiosarcoma is a malignant vascular tumor arising from vessel endothelial cells. The liver is the second most

common angiosarcoma site (1). However, primary hepatic angiosarcoma (PHA) is quite rare, comprising less than 2% of all primary hepatic tumors (2). A previous multi-institutional study of about 44 hepatic angiosarcoma cases revealed a poor prognosis with 5.8 months median overall survival and only two patients achieving 5-year survival (3).

Although no standard treatment has been established, surgical resection is the only treatment option that can provide long-term survival (4). According to a previous review article, partial hepatectomy in small size tumors (particularly, <10 cm) is a favorable factor (5). Chemotherapy can be an option for unresectable cases but liver transplantation is not recommended (5). Therefore, if possible, molecular therapy can be an alternative for the treatment of advanced tumors or those larger than 10 cm. Little is known about the genetic abnormalities in hepatic angiosarcoma, but a recent study by Marks *et al.* reported the first case of a ROS1 fusion gene rearrangement with *GOPC/FIG* (6). Crizotinib has now been used for pulmonary non-small cell carcinoma with a ROS1 rearrangement. In hepatic angiosarcoma with ROS1 rearrangement, ROS1 tyrosine kinase inhibitors including crizotinib can be an optional treatment. A *CEP85L-ROS1* fusion was also identified in another study (7). Therefore, the presence of a ROS1 gene fusion in this disease should be further investigated.

Variations in hepatic angiosarcoma have been found at the microscopic level in several studies. Yasir *et al.* from the Mayo clinic, classified the morphologic patterns of 21 hepatic angiosarcoma cases into two major groups that considered whether the tumor has mass formation or not (8). “Mass-forming” tumors are further subclassified as vasoformative or non-vasoformative by the recognition of vascular space on microscopic examination (8). Non-vasoformative solid tumors can have epithelioid/or spindled morphology (8). “Non-mass-forming” tumors can be divided into a “sinusoidal” or “peliotic” pattern (8). Overall four main histologic growth patterns have been suggested: 1) sinusoidal, 2) peliotic, 3) vasoformative, and 4) solid (epithelioid/spindled) (8).

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Table I. Clinicopathologic data of 13 hepatic angiosarcomas.

Case No.	Gender/Age (year)	Location/Number	Symptom	Management	Metastasis (Detected time from diagnosis)	Status	Survival period	Morphological pattern	ROS1 (IHC/RT-PCR)
1	M/57	S4 & S3/Multiple	Epigastric discomfort	Left lobectomy	Multiple (2 months)	Dead	2 months	Vasoformative	Negative/Failed
2	F/2	Both lobes/Multiple	Abdominal distension	Liver transplantation, postoperative chemotherapy and auto-PBSCT	Multiple (1 month)	Dead	3 years	Vasoformative with spindled	Negative/Negative
3	M/52	S4, 5, 8 & S6/Multiple	Abdominal pain	Trisegmentectomy (Postoperative chemotherapy refuse) (local recurrence)	None	Dead	6 months-	Solid (spindled)	Negative/Negative
4	M/60	S7/Single	None	Liver transplantation and postoperative radiation therapy	Bone (11months)	Dead	11 months	Sinusoidal	Negative/Failed
5	F/96	Both lobes/Multiple	Abdominal pain	Supportive treatment	None	Dead	2 months	Solid (spindled)	Negative/Negative
6	M/66	Right/Single	Indigestion, back pain	ND	None	Dead	2 weeks	Vasoformative	Negative/ND
7	M/76	S5/Multiple	None	Palliative chemotherapy and paracentesis	None	Dead	7 months	Vasoformative	Negative/Negative
8	F/67	S6/Multiple	RUQ pain	ND (chemotherapy refuse)	None	Dead	5 months	Vasoformative	Negative/ND
9	M/83	S5/Multiple	Abdominal distension	ND	Adrenal (initial)	Dead	1 month	Sinusoidal	Negative/ND
10	F/78	S8/Multiple	Abdominal distension	Supportive treatment	None	Follow up loss	2 weeks	Vasoformative	ND/ND
11	M/62	Both lobes/Multiple	Weight loss	Palliative chemotherapy	Intrahepatic metastasis (initial)	Alive with disease	8 months	Vasoformative	ND/ND
12	M/77	Right/Two	None	Palliative chemotherapy	None	Follow up loss	5 months	Vasoformative	ND/ND
13	M/73	Left/Multiple	None	Supportive treatment	None	Follow up loss	1 year 8 months	Sinusoidal	ND/ND

Survival period: from initial histologic diagnosis to last follow up (dead or alive). ND: Not done; PBSCT: peripheral blood stem cell transplantation; IHC: immunohistochemistry; RT-PCR: reverse transcription polymerase chain reaction.

In our study, we aimed to describe morphologic findings in 13 cases of PHA presented at a single institute over 19 years, accompanied by clinicopathological analysis. Additionally, our study investigated *ROS1* gene rearrangements by immunohistochemical and molecular analyses.

Patients and Methods

Patient selection. From June 2000 to March 2019, the pathology database of Samsung Medical Center, Seoul, Korea, was searched for all biopsy and resected specimens identified as ‘angiosarcoma’ of the liver. In total, 13 cases were selected and hematoxylin and eosin stained slides were reviewed. After examining the residual formalin-fixed paraffin-embedded (FFPE) blocks, nine of them had sufficient tissue for further ROS1 immunohistochemical analysis and six for molecular analysis. Electronic medical records were reviewed for clinical information, including demographic features, radiologic findings, treatment history, and clinical outcomes. The

Institutional Review Board of the Samsung Medical Center approved this study and waived the informed consent.

Histologic classification. Histologic review of 13 cases was performed, and cases were classified according to four main classifications: 1) sinusoidal, 2) peliotic, 3) vasoformative, and 4) solid (epithelioid/spindled), as suggested by Yasir *et al.* (8).

ROS1 immunohistochemistry. ROS1 immunohistochemical studies were performed on 4-µm thick representative sections of FFPE tissues. Appropriate samples were obtained from nine cases of hepatic angiosarcoma. A Bond 3 Automated Immunostainer (Leica Microsystems, IL, USA) was used to retrieve antigen with ER2 solution for 20 min, and the sections were incubated with anti-ROS1 antibody (clone: D4D6, 1:100; Cell Signaling Technology, Danvers, MA, USA).

Reverse transcription-polymerase chain reaction (RT-PCR) for ROS1 fusion gene detection. Total RNA was prepared using a RNeasy FFPE kit (Qiagen, MD, USA) according to the manufacturer’s protocols. A

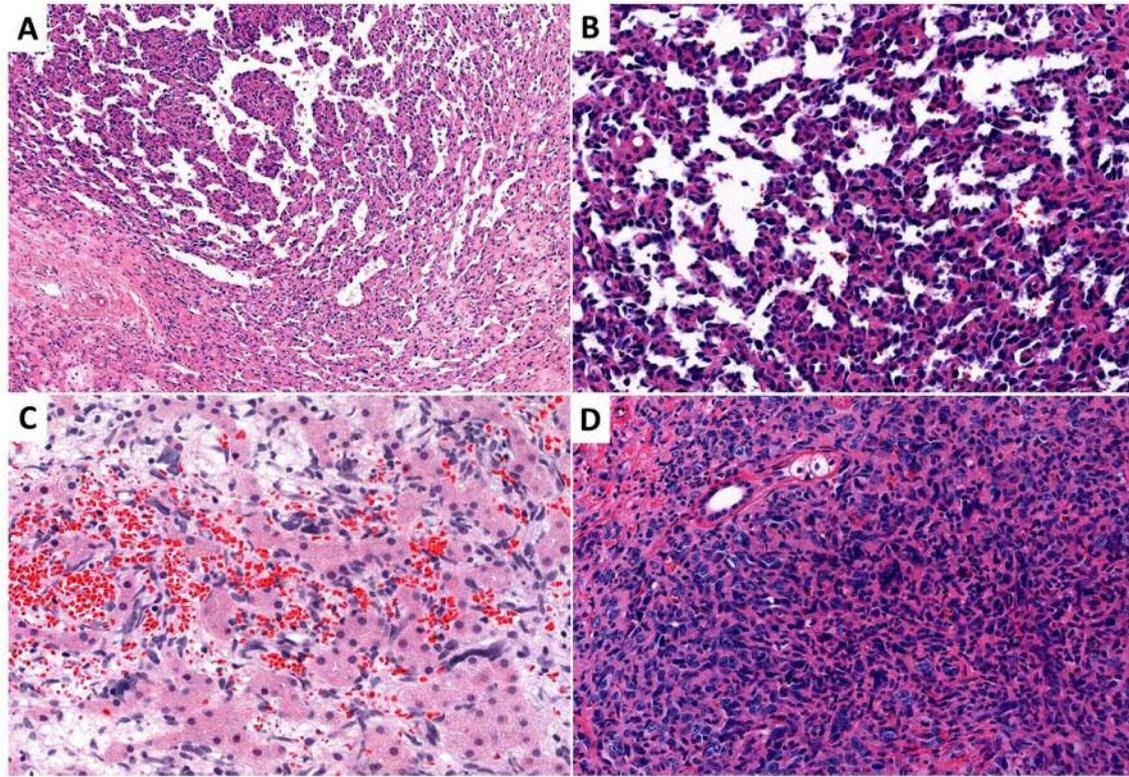


Figure 1. Histologic classification of primary hepatic angiosarcoma. (A, B) Vasoformative pattern, (C) sinusoidal pattern, and (D) solid pattern.

total of 2 µg of RNA was reverse transcribed using random hexamer and SuperScript III Reverse Transcriptase (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA). RT-PCR amplification reactions were performed using the following conditions: an initial denaturation for 10 min at 95°C and then 40 cycles for 30 sec at 95°C, 30 sec at 59°C, and 60 sec at 72°C, followed by 72°C for 10 min. The primers for the *CEP85L-ROS1* fusion gene were 5'-GCCAACAAGATGAGATTGACAGA-3' (forward) and 5'-TAAGCACTGTCACCCTTCC-3' (reverse). The primers for the *FIG-ROS1(S)* fusion gene were 5'-CCCTTCGTAGACATATAGCTGTT-3' (forward) and 5'-CCACTGCTGTTCCCTTCATACA-3' (reverse). The primers for the *FIG-ROS1(L)* fusion gene were 5'-TGGACATCGTTACCGTTTGTA-3' (forward) and 5'-GCATTAGCCAGGCCTACTC-3' (reverse).

Results

Clinicopathological characteristics. The clinicopathological features of 13 angiosarcomas are summarized in Table I. Nine of the 13 patients were male and the median age at initial histologic diagnosis was 67 years old. Eleven patients had multiple masses at the initial radiologic examination. Nine patients had symptoms at the first visit, including abdominal pain and distension. Four of the 13 underwent surgical treatment, which included two liver transplantations, one lobectomy, and one trisegmentectomy. The patient who had the trisegmentectomy experienced local recurrence after the

operation but refused further chemotherapy. Chemotherapy was received by four patients including one operated patient. Treatment was not possible in two patients due to the advanced stage of disease; one of these patients died 2 weeks and one at 1 month subsequent to their initial diagnoses. One patient refused treatment and died after 5 months. Initial work up findings revealed that two patients had metastatic lesions, one patient had adrenal metastasis and the other had multiple intrahepatic metastasis. After the diagnosis, multiple metastases were identified in two patients (after 2 months and 1 month). One patient who underwent transplantation and postoperative radiation therapy developed bone metastasis after 11 months. Nine died of disease progression, one patient is still alive with the disease (Case No. 11) after 8 months, and three (Case No. 10, 12, and 13) were lost to follow up.

Histological classification. Eight of the 13 cases (61.5%) showed a vasoformative pattern (Table I). They had vessel formation without parenchymal liver tissue (Figure 1A and B). Between the vascular forming area, fibrotic stroma was identified in most cases. One of the cases had a focal solid area with spindled morphology. There were three cases of sinusoidal pattern and two solid tumors with a spindled pattern. In our case series, the peliotic type was not found.

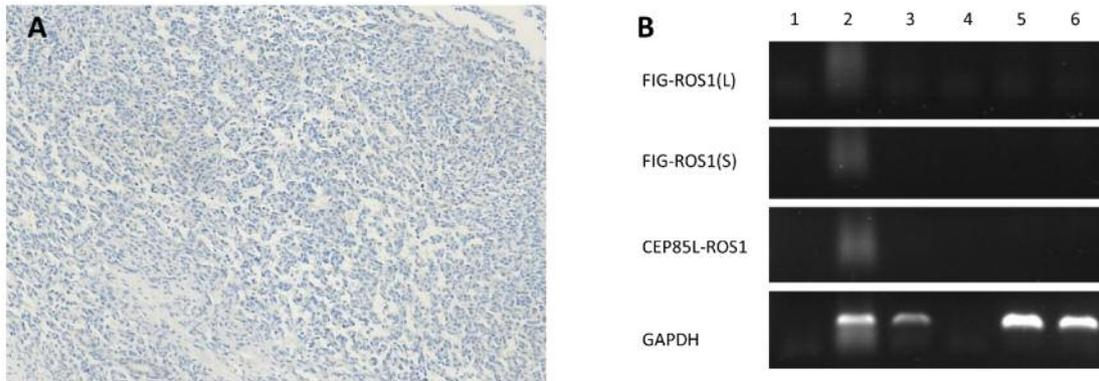


Figure 2. *ROS1* immunohistochemistry (A) and reverse transcription-polymerase chain reaction for the *ROS1* fusion gene (B). *ROS1* expression or the *ROS1*-fusion gene was not detected in any of the samples tested.

The sinusoidal type showed dilated sinusoidal spaces with congestion of red blood cells (Figure 1E). Atypical cells with hyperchromasia were lined along the sinusoids and hepatic parenchyma was identified between the spaces. Two solid tumors had spindled morphology with mild whorling or storiform architecture (Figure 1F).

ROS1 immunohistochemistry (IHC) and RT-PCR for *ROS1* fusion gene detection. Nine cases had sufficient remnant tissue for further study. *ROS1* IHC was used to assess them, and all cases were negative (Figure 2A). After immunohistochemistry, RT-PCR was used to investigate the presence of *ROS1* fusion transcripts in six cases. Two failed due to inappropriate RNA quality that prevented cDNA synthesis, and the remaining four cases did not indicate the presence of a *FIG-ROS1* or *CEP85L-ROS1* fusion (Figure 2B).

Discussion

In this study, we reviewed histopathologic features of 13 PHAs over 19 years at our institute, and classified them into four categories, 1) sinusoidal, 2) pelioidic, 3) vasoformative, and 4) solid (epithelioid/spindled), that were suggested by Yasir *et al.* (8). As a result, the most common histologic type was vasoformative (8 of 13 cases). Three of the PHAs were classified as sinusoidal, and the remaining two cases as solid. There was no typical pelioidic type PHA in this study. PHA has morphologic diversity, thus pathologists should consider the variation of histologic features when facing vascular or mesenchymal tumors with suspicion of PHA. Clinical data were similar to previous studies of PHA. Males were predominant. Only one patient remains alive with the disease and nine of the 13 patients have died. The mortality rate was 90% (9 of 10), with the exception of three patients who were lost to follow up. The treatment of PHA has not been defined. In previous studies, complete surgical resection was shown

to be the key to improving prognosis (9, 10). In the cases we studied, two patients went through surgical resection, but they died within 6 months with multiple metastases and local recurrence after surgery. Transplantation is now abandoned due to the high recurrence rate and poor survival (11) and PHA is now a contraindication to liver transplantation by the European Liver Transplant Registry (12). Two patients (Cases No. 2 and 4) in this study underwent transplantation in 2001 and 2008, before the recommendation. They died of the disease after postoperative chemotherapy with auto-peripheral blood stem cell transplantation and postoperative radiation therapy. Palliative chemotherapy was performed in three patients. One of the patients remains alive and has survived with the disease for 8 months so far, although intrahepatic multiple metastatic lesions were detected at the initial diagnosis. The median survival period was 5 months. The overall prognosis of PHA is dismal, compared to hepatic hemangioendothelioma with a mortality rate of 25% (13).

Recently, the *ROS1* fusion genes with *GOPC/FIG* (6) and *CEP85L* (7) have been reported in angiosarcomas. We can anticipate the application of *ROS1*-targeted therapy in angiosarcomas with poor prognosis. Therefore, we tested *ROS1* IHC in nine cases and RT-PCR for *FIG-ROS1* or *ROS1-CEP85L* fusion transcripts in four cases, but could not find either *ROS1* expression or the *ROS1*-fusion gene. The major limitation of this study is that we performed RT-PCR without a positive control sample. *FIG-ROS1* or *CEP85L-ROS1* is a very rare variant, that have been reported only in a few previous reports, and we could not find any positive control harboring such variants. Actually, the results of IHC and RT-PCR for *ROS1* rearrangements have been reported to be highly concordant, and IHC is regarded as a reliable screening method for fusion gene detection (14-16). In our lab, we perform IHC, RT-PCR, and next generation sequencing in daily practice, and the results of these modalities are highly concordant. In our study, all tested

samples were IHC-negative, so they could also be negative for a *ROS1* rearrangement. Further studies regarding the *ROS1* fusion gene are necessary in more PHAs.

Conclusion

We report 13 cases of PHAs with 90% mortality. Vasoformative PHA is the most common histologic type. Pathologists should consider variable morphological patterns including sinusoidal, solid (epithelioid/spindled), and peliotic type, in addition to vasoformative type. Although all cases tested did not express *ROS1* or a *ROS1* fusion gene, new findings on *ROS1* fusion gene rearrangements could be key for the development of targeted therapy for PHA with dismal prognosis.

Conflicts of Interest

None of the Authors has any conflicts of interest to declare regarding this study.

Authors' Contributions

Conception and design: HJ, SYH; Acquisition of data: HJ, SS, CP, SYH; Analysis and interpretation of data: HJ, SYH; Drafting the article: HJ, SYH; Revising and final approval of the article to be published: HJ, HK, YJ, SS, CKP, SYH.

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