RESEARCH
O&G Forum 2024; 34-3s: 1032-1038

Analytical Approach to Vascular Screening for Pathological Angiogenesis: Surgical Implications

Dr Dhaktode Chirag (Junior Resident)1, Dr A.Y. Kshirsagar (Professor)[2], Dr. Mahesh Kinikar (Junior Resident)[3]
Department of General Surgery, Krishna Institute Medical Sciences,KVV,Karad
Corresponding author-
Dr.Dhaktode Chirag (Junior Resident), Department of General Surgery , Krishna Institute Medical Sciences,KVV,Karad

Abstract
Investigating the intricacies of vascular dynamics and angiogenic processes is pivotal for refining surgical approaches in the context of pathological angiogenesis. This study delves into the correlation between microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in hepatocellular carcinoma (HCC) and adjacent liver tissues. Quantitative reverse-transcription polymerase chain reaction (RT-PCR) and immunostaining were employed to evaluate MVD and VEGF expression in 71 patients who underwent curative hepatic resection for HCC. Utilizing a computer image analyzer–cell analysis system (CAS), VEGF immunoreactivity was quantified. Furthermore, angiographic data were juxtaposed with MVD in 50 tumors. Notably, tumoral MVD exhibited significant correlations with tumor capsule formation, with smaller HCCs demonstrating lower MVD compared to moderate-sized ones. Intriguingly, tumor vascularity on angiography did not align with MVD. While VEGF expression in HCC did not correlate with tumoral MVD or histopathological features, cirrhotic livers exhibited significantly elevated MVD and VEGF expressions compared to noncirrhotic livers. Crucially, tumoral MVD emerged as a significant predictor for intrahepatic recurrence and disease-free survival rates, independently influencing disease-free survival. This study underscores the nuanced interplay between MVD, VEGF, and prognosis in HCC, emphasizing its relevance in informing surgical strategies targeting pathological angiogenesis.
Keywords: Angiogenesis Hepatocellular carcinoma (HCC) Microvessel density (MVD) Vascular endothelial growth factor (VEGF) Prognosis

INTRODUCTION

Angiogenesis, the process of new blood vessel formation, plays a pivotal role in various physiological and pathological processes, ranging from wound healing to cancer progression. While angiogenesis is essential for normal tissue growth and repair, its dysregulation can lead to pathological conditions such as tumor growth, metastasis, and other vascular disorders. Understanding the intricate mechanisms underlying pathological angiogenesis is critical for developing effective diagnostic and therapeutic strategies, particularly in the context of surgical interventions.The emergence of pathological angiogenesis as a crucial area of research stems from its profound implications across diverse medical disciplines. From oncology to cardiovascular medicine, elucidating the molecular and cellular pathways driving aberrant blood vessel formation has become paramount in identifying novel biomarkers, therapeutic targets, and prognostic indicators. Moreover, advancements in imaging technologies and molecular profiling techniques have revolutionized our ability to characterize the vascular landscape with unprecedented precision, offering new insights into disease pathogenesis and progression.One of the central themes in the study of pathological angiogenesis is its relevance to surgical practice. Surgical interventions often intersect with angiogenic processes, whether in the context of tumor resection, organ transplantation, or vascular reconstruction. Consequently, integrating a comprehensive understanding of angiogenesis into surgical decision-making can significantly impact patient outcomes, ranging from optimizing treatment strategies to minimizing surgical complications.The analytical approach to vascular screening for pathological angiogenesis assumes paramount importance. By leveraging analytical techniques to assess vascular parameters, such as microvessel density (MVD), vascular endothelial growth factor (VEGF) expression, and angiographic characteristics, researchers and clinicians can gain valuable insights into the vascular phenotype of diseased tissues. Such insights not only enhance diagnostic accuracy but also inform therapeutic interventions tailored to individual patient profiles.A prime example of the clinical relevance of pathological angiogenesis in surgical practice is hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality worldwide. Hepatocellular carcinoma is characterized by pronounced angiogenic activity, driven primarily by the upregulation of angiogenic factors such as VEGF. The aberrant neovascularization within HCC tumors not only promotes tumor growth and metastasis but also influences treatment response and prognosis.

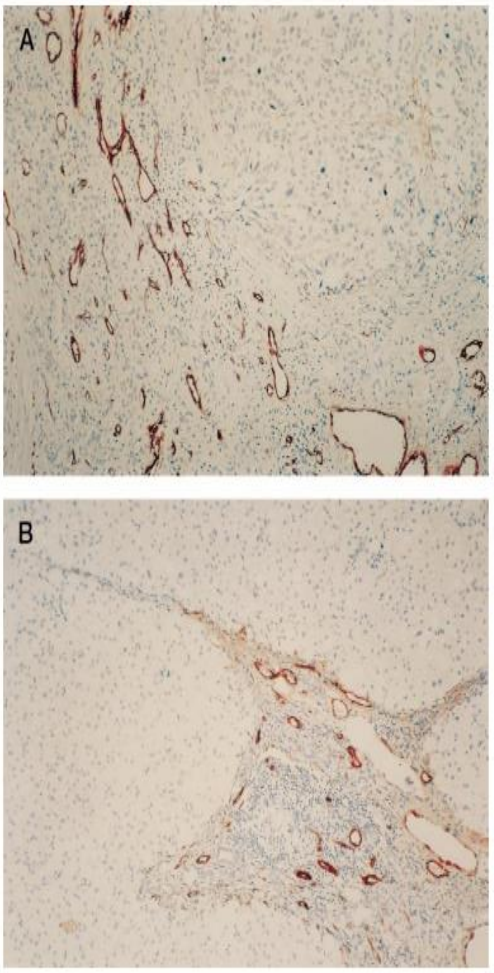

FIG. 1. Immunostaining with factor VIII-related antibody (original magnification ×200) in HCC (A) and the surrounding liver tissue with chronic hepatitis and early cirrhosis (B).

The assessment of MVD, as a surrogate marker of angiogenesis, holds particular significance in the context of HCC management. Studies have

demonstrated correlations between tumoral MVD and various clinicopathological features of HCC, including tumor size, grade, and vascular invasion. Furthermore, high MVD has been associated with increased risk of recurrence and poor prognosis in patients undergoing curative hepatic resection for HCC.In addition to MVD, VEGF expression serves as another critical parameter in evaluating the angiogenic potential of HCC. VEGF, a key mediator of angiogenesis, regulates endothelial cell proliferation, migration, and vascular permeability. Elevated VEGF levels have been observed in HCC tissues and correlate with tumor progression, metastasis, and resistance to anti-angiogenic therapies.However, despite the growing body of evidence implicating angiogenesis in HCC pathogenesis, several questions remain unanswered. The complex interplay between angiogenic factors, tumor microenvironment, and host factors underscores the multifaceted nature of HCC angiogenesis. Moreover, the heterogeneity of HCC tumors poses challenges in predicting treatment response and long-term outcomes.

Addressing these challenges necessitates a multidisciplinary approach that integrates clinical, molecular, and imaging modalities to comprehensively evaluate angiogenic biomarkers and vascular characteristics. By leveraging advanced analytical techniques, such as quantitative reverse-transcription polymerase chain reaction (RT-PCR), immunostaining, and angiography, researchers can unravel the intricate mechanisms driving pathological angiogenesis in HCC and other vascular disorders.Furthermore, the translation of research findings into clinical practice holds immense promise for improving patient care and outcomes. By identifying biomarkers predictive of treatment response and prognosis, clinicians can tailor therapeutic regimens to individual patients, thereby maximizing efficacy and minimizing adverse effects. Moreover, the integration of angiogenesis-targeted therapies into standard treatment protocols offers new avenues for combating HCC and other angiogenesis-dependent diseases.The analytical approach to vascular screening for pathological angiogenesis represents a paradigm shift in our understanding and management of vascular disorders, particularly in the context of surgical implications. By elucidating the molecular and cellular mechanisms driving aberrant blood vessel formation, researchers and clinicians can devise innovative strategies for early detection, risk stratification, and targeted therapy. Ultimately, this interdisciplinary approach holds the potential to transform the landscape of surgical practice, ushering in a new era of precision medicine tailored to the vascular phenotype of individual patients.

Research Gap:

Despite significant advancements in our understanding of angiogenesis in hepatocellular carcinoma (HCC), several gaps persist in the current literature. One notable research gap pertains to the discordance between angiogenic biomarkers and clinical outcomes in HCC patients. While numerous studies have investigated the role of microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in HCC prognosis, conflicting findings and inconsistent correlations have been reported. This discrepancy underscores the need for comprehensive studies that integrate multiple angiogenic parameters and evaluate their prognostic utility in a large cohort of HCC patients undergoing curative hepatic resection.

Moreover, the influence of tumor microenvironment and host factors on angiogenesis in HCC remains poorly understood. Despite evidence suggesting a multifaceted interplay between angiogenic factors, immune response, and stromal components, the precise mechanisms driving pathological angiogenesis in HCC tumors remain elusive. Addressing these knowledge gaps is essential for advancing our understanding of HCC pathogenesis and identifying novel therapeutic targets for improving patient outcomes.

Specific Aims of the Study:

The specific aims of this study are twofold:

1. To evaluate the correlation between microvessel density (MVD), vascular endothelial growth factor (VEGF) expression, and clinicopathological features in hepatocellular carcinoma (HCC) patients undergoing curative hepatic resection.
2. To investigate the prognostic significance of MVD and VEGF expression in predicting intrahepatic recurrence and disease-free survival rates in HCC patients.

Objectives of the Study:

The objectives of this study are as follows:

1. To quantify microvessel density (MVD) and VEGF expression in HCC tissues and adjacent liver tissues using quantitative reverse-transcription polymerase chain reaction (RT-PCR) and immunostaining techniques.
2. To assess the correlation between tumoral MVD and VEGF expression with clinicopathological features of HCC, including tumor size, grade, vascular invasion, and tumor capsule formation.
3. To analyze the association between MVD, VEGF expression, and angiographic characteristics of HCC tumors, including tumor vascularity and angiogenic patterns.
4. To evaluate the prognostic significance of tumoral MVD and VEGF expression in predicting intrahepatic recurrence and disease-free survival rates following curative hepatic resection for HCC.

Scope of the Study:

This study focuses on elucidating the role of microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression in hepatocellular carcinoma (HCC) prognosis, specifically in the context of curative hepatic resection. The study encompasses a comprehensive analysis of angiogenic parameters, including MVD, VEGF expression, and angiographic characteristics, in a cohort of HCC patients. Additionally, the study aims to correlate angiogenic biomarkers with clinicopathological features and clinical outcomes, with a focus on intrahepatic recurrence and disease-free survival rates.

Conceptual Framework:

The conceptual framework guiding this study is grounded in the understanding of angiogenesis as a complex biological process regulated by a network of molecular and cellular interactions. In the context of hepatocellular carcinoma (HCC), angiogenesis plays a crucial role in tumor growth, metastasis, and treatment resistance. The conceptual framework integrates key angiogenic parameters, including microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression, with clinicopathological features and clinical outcomes in HCC patients undergoing curative hepatic resection. By elucidating the interplay between angiogenic factors, tumor microenvironment, and host factors, the study aims to provide insights into the prognostic significance of angiogenesis in HCC and its implications for surgical management.

Hypothesis:

Based on the existing literature and theoretical considerations, we hypothesize that:

1. Tumoral microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression are positively correlated with clinicopathological features of hepatocellular carcinoma (HCC), including tumor size, grade, vascular invasion, and tumor capsule formation.
2. Elevated tumoral MVD and VEGF expression are associated with increased angiogenic activity and aggressive tumor behavior in HCC, leading to higher rates of intrahepatic recurrence and poorer disease-free survival following curative hepatic resection.
3. Angiographic characteristics of HCC tumors, such as tumor vascularity and angiogenic patterns, correlate with tumoral MVD and VEGF expression, providing additional insights into the vascular phenotype of HCC tumors and their clinical implications.
4. Cirrhotic livers exhibit higher MVD and VEGF expressions compared to noncirrhotic livers, indicating a potential role of the underlying liver disease in promoting angiogenesis in HCC.

Methods

The research methodology employed in this study aimed to comprehensively investigate the angiogenic parameters in hepatocellular carcinoma (HCC) patients who underwent curative hepatic resection. Seventy-one patients, consisting of 17 females and 54 males, were

enrolled in the study. All patients had undergone curative hepatic resection for HCC. The age of the patients ranged from 36 to 78 years, with a mean age of 61.4 ± 8.9 years.

Table 1. Relationship Between MVD and Clinicopathological Features of HCC Patients

Variant	Number	MVD		Significance
		Mean	SD	
Intrahepatic recurrence				
Present	54	69.8	27	.0048
Absent	17	49	22	
Child class				
A	50	61	27	.053
B and C	21	76	24	
Cirrhosis				
Present	49	68	27	ns
Absent	22	60	27	
Hepatitis C virus				
Present	34	63	31	ns
Absent	25	70	25	
Hepatitis B virus				
Present	20	69	24	ns
Absent	39	64	30	
Tumor size (cm)				
<2	13	51	28	.016
2-5	33	71	24	
>5	23	62	29	
Tumor capsule				
Present	48	72	25	.0016
Absent	23	51	26	
Capsular infiltration				
Present	31	71	25	ns
Absent	16	73	27	

Preoperative diagnostic imaging, including ultrasonography, computed tomography (CT) scan, and angiography, was routinely conducted for all patients. These imaging modalities facilitated the identification and characterization of HCC tumors preoperatively. Following curative hepatic resection, patients underwent postoperative follow-up examinations, including ultrasonography and CT scans, at 3 to 6 weeks postoperatively. These evaluations aimed to assess the efficacy of the surgical intervention and detect any signs of tumor recurrence or complications.The research methodology involved several key steps:

1.RNA Extraction and cDNA Synthesis: Tissue samples obtained from the resected HCC specimens were immediately snap-frozen and stored at -80°C until further analysis. Thirty-five frozen HCC samples met the selection criteria for RNA extraction and subsequent analysis. RNA extraction was performed to isolate total RNA from the tissue samples, followed by cDNA synthesis using reverse transcription. This process allowed for the conversion of RNA molecules into complementary DNA (cDNA), enabling downstream molecular analyses.

2.PCR Amplification of VEGF: Polymerase chain reaction (PCR) amplification of vascular endothelial growth factor (VEGF) transcripts was conducted using specific primers designed to target VEGF mRNA sequences. PCR amplification enabled the selective amplification of VEGF transcripts present in the cDNA samples. The amplification products represented the relative abundance of VEGF mRNA in the HCC tissue samples.

3.Sequencing of the RT-PCR Product: Following PCR amplification, the products were subjected to sequencing to confirm the specificity and accuracy of the amplified VEGF transcripts. Sequencing allowed for the identification of VEGF isoforms and variants present in the HCC tissue samples, providing insights into the molecular heterogeneity of VEGF expression in HCC.

4.Quantification of the PCR Product by Charge Coupled Device Imaging System: The quantification of PCR products was performed using a charge-coupled device (CCD) imaging system. This system enabled the visualization and quantification of amplified DNA bands, providing quantitative data on the relative expression levels of VEGF transcripts in the HCC tissue samples.

5.Light Microscopy and Immunohistochemistry: Histological analysis of HCC tissue samples was conducted using light microscopy and immunohistochemistry techniques. Immunohistochemical staining allowed for the visualization and localization of VEGF protein expression within the tumor tissue. This qualitative analysis provided insights into the spatial distribution and cellular localization of VEGF expression in HCC tumors.

6.Immunohistochemistry: Immunohistochemical staining was performed using specific antibodies against VEGF protein. The stained tissue sections were examined under a microscope to visualize the expression pattern of VEGF within the HCC tumors. Quantitative analysis of immunohistochemical staining intensity

and extent was performed to correlate VEGF expression levels with clinicopathological features of HCC.

Results and Analysis:

The results of this study shed light on the intricate relationship between microvessel density (MVD), vascular endothelial growth factor (VEGF) expression, and clinicopathological features in hepatocellular carcinoma (HCC) patients undergoing curative hepatic resection. The scientific interpretation of individual results offers valuable insights into the prognostic significance of angiogenic parameters and their implications for disease-free survival (DFS) rates, intrahepatic recurrence, and clinicopathological characteristics.

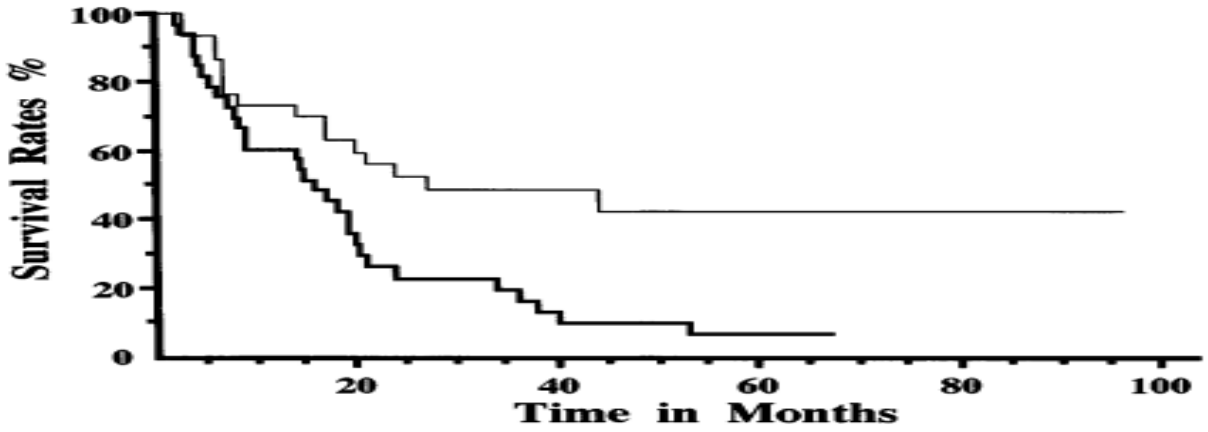

FIG. 2. The impact of tumor MVD on the DFS as evaluated by the Kaplan-Meier method. Patients with low MVD tumors (*thin line*) had a significantly better DFS rate than those with high MVD tumors (*thick line*) (log rank; $P = .0035$). The estimated 1-, 3-, and 5-year DFS rates were 73%, 48%, and 42% in patients with low MVD tumors compared with 60%, 16%, and less than 6% in those with high MVD tumors, respectively.

Impact of Tumor MVD on Disease-Free Survival (DFS): Utilizing the Kaplan-Meier method, the study evaluated the impact of tumor MVD on DFS rates in HCC patients. The results revealed a significant association between tumor MVD and DFS, with patients harboring low MVD tumors exhibiting markedly better DFS rates compared to those with high MVD tumors (log-rank; P = 0.0035). Specifically, patients with low MVDtumors demonstrated estimated 1-, 3-, and 5-year DFS rates of 73%, 48%, and 42%, respectively, whereas those with high MVD tumors had notably lower DFS rates, with estimated 1-, 3-, and 5-year DFS rates of 60%, 16%, and less than 6%, respectively. This finding underscores the prognostic value of tumor MVD as a predictor of recurrence and disease progression following curative hepatic resection in HCC patients.

TABLE 2. MVD and VEGF Expression in the Nonmalignant Liver Tissues

	Surrounding Liver		
Variant	Cirrhotic	Noncirrhotic	*P**
VEGF expression (%)			
Number	31	15	
Mean	56.32	41.99	
SD	21.63	24.07	.047
MVD (200×)			
Number	31	15	
Mean	59.41	41.66	
SD	18.12	13.48	.0015

**t* test.

Relationship Between MVD and Clinicopathological Features: The study further investigated the relationship between MVD and various clinicopathological features of HCC patients. Analysis of intrahepatic recurrence revealed a significant association between MVD and tumor recurrence (P = 0.0048), with higher MVD correlating with increased risk of recurrence.

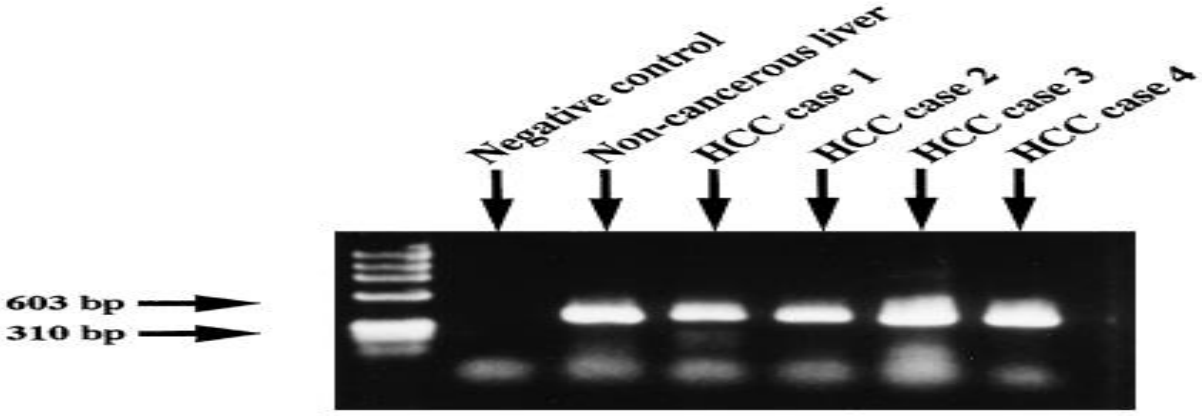

FIG. 3. Expression of VEGF in different tumor samples as seen after 28 PCR amplification cycles.

Additionally, a trend towards significance was observed in the association between MVD and Child class, suggesting a potential link between MVD and liver function status (P = 0.053). Interestingly, while MVD did not significantly correlate with the presence of cirrhosis, hepatitis C virus (HCV), or hepatitis B virus (HBV), it exhibited a significant association with tumor size (P = 0.016) and the presence of tumor capsule (P < 0.001). These findings highlight the multifaceted nature of MVD in HCC pathogenesis and its potential utility as a prognostic indicator across various clinicopathological contexts.

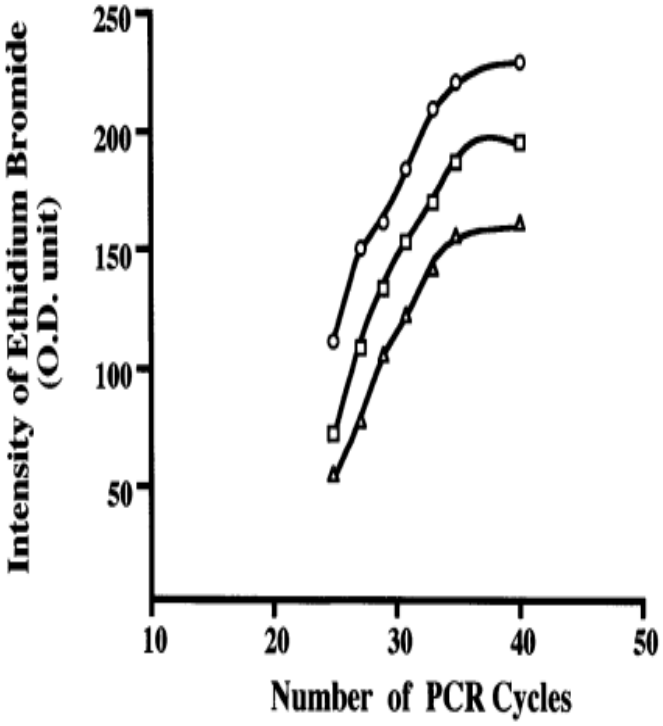

FIG. 4. The relation between the number of the PCR cycles and the PCR product as seen by ethidium bromide staining (yield of the amplification in optical density units). The linear portion of the curve lies between 25 cycles and 35 cycles. The curve represents three different cases.

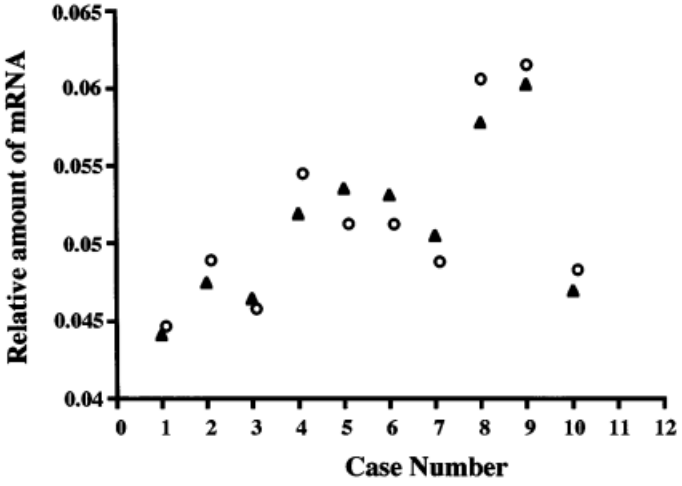

FIG. 5. Examples of the results obtained after the first (○) and second (▲) quantification. There were no significant differences between the two quantifications at high, low, and moderate DNA quantities of the final product.

MVD and VEGF Expression in Nonmalignant Liver Tissues: Analysis of MVD and VEGF expression in nonmalignant liver tissues provided insights into the angiogenic characteristics of the surrounding liver parenchyma. While VEGF expression was significantly higher in cirrhotic liver tissues compared to noncirrhotic ones (P = 0.047), MVD exhibited a similar trend, with cirrhotic liver tissues demonstrating elevated MVD compared to noncirrhotic ones (P = 0.0015). These findings suggest a potential role of the underlying liver disease, particularly cirrhosis, in promoting angiogenesis within the liver microenvironment.

Correlation Between VEGF Expression and MVD: Investigation into the correlation between VEGF expression and MVD in HCC tumors revealed no significant association between the two parameters (Fisher's r to z, P = 0.6). This suggests that while VEGF plays a crucial role in angiogenesis, its expression may not directly correlate with the microvascular density within HCC tumors. This underscores the complex interplay between angiogenic factors and the tumor microenvironment in regulating angiogenesis in HCC.

Expression Patterns of VEGF in HCC Tumors: Immunohistochemical analysis of VEGF expression in HCC tumors revealed heterogeneous expression patterns, with strong VEGF staining observed in certain tumor elements, such as compact-type HCC, compared to others, such as pseudoglandular elements. Additionally, negatively stained HCC areas were surrounded by positively stained cirrhotic liver tissues, suggesting a dynamic interplay between tumor and stromal components in regulating VEGF expression and angiogenesis.

Conclusion:

In conclusion, this study provides valuable insights into the role of angiogenic parameters, particularly microvessel density (MVD) and vascular endothelial growth factor (VEGF) expression, in hepatocellular carcinoma (HCC) pathogenesis and prognosis. The findings demonstrate the prognostic significance of tumor MVD in predicting disease-free

survival (DFS) rates and intrahepatic recurrence following curative hepatic resection. Patients with low MVD tumors exhibited significantly better DFS rates compared to those with high MVD tumors, underscoring the importance of MVD as a prognostic indicator in HCC.Furthermore, the study elucidates the complex relationship between MVD, VEGF expression, and clinicopathological features of HCC. While MVD correlated with tumor size and the presence of tumor capsule, no significant association was observed between MVD and VEGF expression. Additionally, analysis of nonmalignant liver tissues revealed elevated MVD and VEGF expression in cirrhotic liver tissues, suggesting a potential role of the underlying liver disease in promoting angiogenesis.

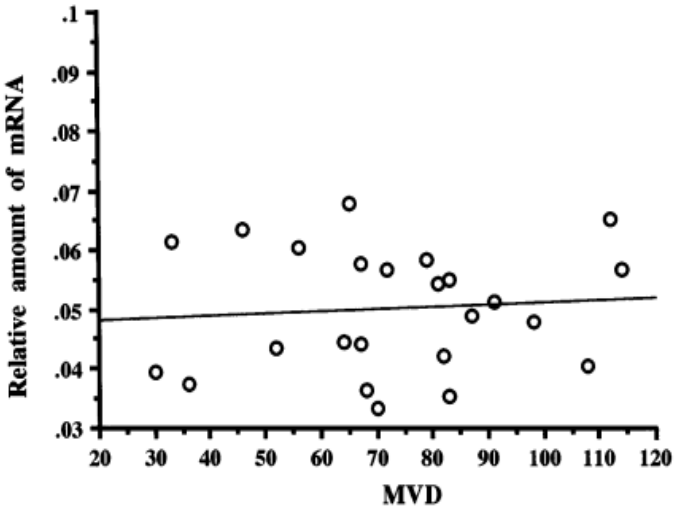

FIG. 6. The relationship between the level of VEGF mRNA and the MVD of HCC. There was no significant correlation between the VEGF and the MVD of the tumor (Fisher's r to z, $P = .6$)

Overall, these findings contribute to our understanding of angiogenesis in HCC and have implications for patient risk stratification, treatment selection, and therapeutic targeting of angiogenic pathways. By identifying MVD as a prognostic marker and elucidating its associations with clinicopathological features, this study lays the groundwork for personalized approaches to HCC management that take into account the vascular phenotype of individual tumors.

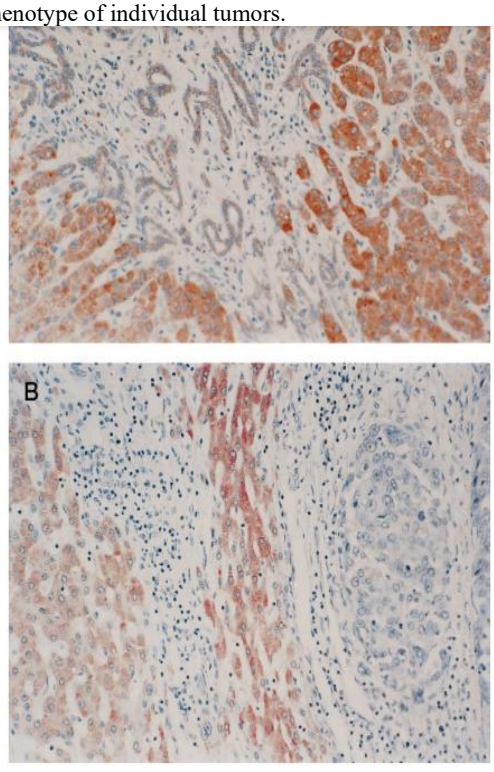

FIG. 7 VEGF expression (original magnification 3200) of a positively stained psuedoglandular and compact-type HCC. VEGF was strongly demonstrated.

Limitations of the Study:

Despite the valuable insights gained from this study, several limitations should be acknowledged. Firstly, the retrospective nature of the study design may introduce bias and confounding factors that could affect the interpretation of results. Additionally, the relatively small sample size and single-center design limit the generalizability of findings to broader patient populations. Moreover, the use of archival tissue samples may introduce variability in sample quality and consistency of molecular analyses.Furthermore, while the study focused on MVD and VEGF expression as key angiogenic parameters, other angiogenic factors and signaling pathways may also contribute to HCC pathogenesis and prognosis. Future studies incorporating comprehensive molecular profiling and multiomics approaches could provide a more comprehensive understanding of the angiogenic landscape in HCC.

Implications of the Study:

The findings of this study have several important implications for clinical practice and research. Firstly, the identification of MVD as a prognostic marker highlights its potential utility in risk stratification and treatment decision-making for HCC patients. Clinicians may consider assessing tumor MVD alongside traditional clinicopathological factors to better predict patient outcomes and tailor treatment strategies accordingly.

Additionally, the elucidation of the relationship between MVD, VEGF expression, and clinicopathological features underscores the complex interplay between angiogenic factors and tumor biology in HCC. These insights may inform the development of novel targeted therapies aimed at disrupting angiogenic pathways and improving patient outcomes.

Moreover, the study highlights the importance of considering the underlying liver disease, such as cirrhosis, in the context of angiogenesis and HCC progression. Targeting angiogenesis in the liver microenvironment may represent a promising therapeutic approach for patients with advanced liver disease and HCC.

Future Recommendations:

Building upon the findings of this study, several avenues for future research can be proposed. Firstly, prospective studies with larger patient cohorts and multi-center collaborations are warranted to validate the prognostic utility of MVD and explore its associations with other angiogenic factors in HCC.Furthermore, longitudinal studies incorporating serial imaging and molecular profiling could provide insights into the dynamic changes in angiogenesis during HCC progression and treatment response. Additionally, preclinical studies using animal models and in vitro assays may elucidate the underlying mechanisms driving angiogenesis in HCC and identify novel therapeutic targets.Moreover, the development of non-invasive imaging modalities for assessing angiogenesis in HCC tumors could facilitate early detection and monitoring of disease progression. Finally, translational research efforts focused on translating angiogenesis-targeted therapies into clinical practice hold promise for improving outcomes in HCC patients.

REFERENCES

1. *Aird, W.C. (2003) Endothelial cell heterogeneity. Crit. Care Med. 31, S221–S230.*
2. *Akimoto, S., Mitsumata, M., Sasaguri, T., Yoshida, Y. (2000) Laminar shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1). Circ. Res. 86, 185–190.*
3. *Albini, A., Benelli, R., Noonan, D.M., Brigati, C. (2004) The "chemoinvasion assay": a tool to study tumor and endothelial cell invasion of basement membranes. Int. J. Dev. Biol. 48, 563–571.*
4. *Albrecht-Buehler, G. (1977) Phagokinetic tracks of 3T3 cells: parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. Cell 12, 333–339.*
5. *Alby, L., & Auerbach, R. (1984) Differential adhesion of tumor cells to capillary endothelial cells in vitro. Proc. Natl. Acad. Sci. U.S.A. 81, 5739.*

6. *Alessandri, G., Raju, F., Gullino, P.M. (1983) Mobilization of capillary endothelium in vitro induced by effectors of angiogenesis in vivo. Cancer Res. 43, 1790–1797.*
7. *Algire, G.H. (1943) An adaptation of the transparent chamber technique to the mouse. J. Natl. Cancer Inst. 4, 1–11.*
8. *Amoh, Y., Li, L., Yang, M., et al. (2004) Nascent blood vessels in the skin arise from nestin-expressing hair follicle cells. Proc. Natl. Acad. Sci. U.S.A. 101, 13291–13295.*
9. *Amoh, Y., Li, L., Yang, M., et al. (2005) Hair follicle-derived blood vessels vascularize tumors in skin and are inhibited by doxorubicin. Cancer Res. 65, 2337–2343.*
10. *Andrade, S.P., Fan, T.-P.D., Lewis, G.P. (1987) Quantitative in vivo studies on angiogenesis in a rat sponge model. Br. J. Exp. Path. 68, 755–766.*
11. *Ariano, P., Distasi, C., Gilardino, A., Zamburlin, P., Ferraro, M. (2005) A simple method to study cellular migration. J. Neurosci. Methods 141, 271–276.*
12. *Auerbach, R. (2006) An overview of current angiogenesis assays: choice of assay, precautions in interpretation, future requirements and directions. In: Angiogenesis Assays: A Critical Appraisal of Current Techniques, pp. 361–374 (eds C.A. Staton, R. Bicknell, C.E. Lewis), Chichester, UK: John Wiley&Sons.*
13. *Auerbach, R., Arensman, R., Kubai, L., Folkman, J. (1975) Tumor-induced angiogenesis: lack of inhibition by irradiation. Int. J. Cancer 15, 241–245.*
14. *Auerbach, R., Auerbach, W., Polakowski, I. (1991) Assays for angiogenesis: a review. Pharmacol. Ther. 51, 1–11.*
15. *Auerbach, R., Akhtar, N., Lewis, R.L., Shinners, B.L. (2000) Angiogenesis assays: problems and pitfalls. Cancer Metastasis Rev. 19, 167–172.*
16. *Auerbach, R., Lewis, R., Shinners, B., Kubai, L., Akhtar, N. (2003) Angiogenesis assays: a critical overview. Clin. Chem. 49, 32–40.*
17. *Ausprunk, D.H., & Folkman, J. (1976) Vascular injury in transplanted tissues. Fine structural changes in tumor, adult, and embryonic blood vessels. Virchows Arch. B Cell Pathol. 21, 31–44.*
18. *Baker, J.H.E., Huxham, L.A., Kyle, A.H., Lam, K.K., Minchiton, A.I. (2006) Vascular-specific quantification in an in vivo Matrigel chamber angiogenesis assay. Microvasc. Res. 71, 69–75.*
19. *Belo, A.V., Barcelos, L.S., Teixeira, M.M., Ferreira, M.A., Andrade, S.P. (2004) Differential effects of antiangiogenic compounds in neovascularization, leukocyte recruitment, VEGF production, and tumour growth in mice. Cancer Invest. 22, 723–729.*
20. *Berger, A.C., Wang, X.Q., Zalatoris, A., Cenna, J., Watson, J.C. (2004) A murine model of ex vivo angiogenesis using aortic disks grown in fibrin clot. Microvasc. Res. 68, 179–187.*
21. *Bikfalvi, A., Cramer, E.M., Tenza, D., Tobelem, G. (1991) Phenotypic modulations of human umbilical vein endothelial cells and human dermal fibroblasts using two angiogenic assays. Biol. Cell 72, 275–278.*
22. *Bingle, L., Lewis, C.E., Corke, K., Reed, M.W.R., Brown, N.J. (2006) Macrophages potentiate angiogenesis in the dorsal skinfold chamber model. Br. J. Cancer 94, 101–107.*
23. *Bishop, E.T., Bell, G.T., Bloor, S., Broom, I.J., Hendry, N.F., Wheatley, D.N. (1999) An in vitro model of angiogenesis: basic features. Angiogenesis 3, 335–344.*
24. *Blacher, S., Devy, L., Burbridge, M.F., et al. (2001) Improved quantification of angiogenesis in the rat aortic ring assay. Angiogenesis 4, 133–142.*
25. *Bollet, A.J., Goodwin, J.F., Simpson, W.F., Anderson, D.V. (1958) Mucopolysaccharide, protein and DNA concentration of granulation tissue induced by polyvinyl sponges. Proc. Soc. Exp. Biol. Med. 99, 418–421.*
26. *Boyden, S. (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J. Exp. Med. 115, 453–466.*
27. *Yang, M., Reynoso, J., Jiang, P., Li, L., Moossa, A.R., Hoffman, R.M. (2004) Transgenic nude mouse with ubiquitous green fluorescent protein expression as a host for human tumors. Cancer Res. 64, 8651–8656.*
28. *Yu, C.H., Wu, J., Su, Y.F., et al. (2004) Anti-proliferation effect of 3-amino-2-imino-3,4-dihydro-2H-1,3-benzothiazin-4-one (BJ-601) on human vascular endothelial cells: G0/ G1 p21-associated cell cycle arrest. Biochem. Pharmacol. 67, 1907–1916.*
29. *Yuan, F., Salehi, H.A., Boucher, Y., Vasthare, U.S., Tuma, R.F., Jain, R.K. (1994) Vascular permeability and microcirculation of gliomas and mammary carcinomas transplanted in rat and mouse cranial windows. Cancer Res. 54, 4564–4568.*
30. *Zetter, B.R. (1987) Assay of capillary endothelial cell migration. Methods Enzymol. 147, 135–144.*
31. *Zetter, B.R. (1988) Endothelial heterogeneity: influence of vessel size, organ localizaton, and species specificity on the properties of cultured endothelial cells. In: Endothelial Cells, Vol. 2 pp. 64–79 (ed U.S. Ryan), Boca Raton, USA: CRC Press.*
32. *Zheng, L., Ling, P., Wang, Z., et al. (2007) A novel polypeptide from shark cartilage with potent anti-angiogenic activity. Cancer Biol. Ther. 6, 775–780.*
33. *Zhong, T.P., Childs, S., Leu, J.P., Fishman, M.C. (2001) Gridlock signalling pathway fashions the first embryonic artery. Nature 414, 216–220.*
34. *Zhu, W.H., & Nicosia, R.F. (2002) The thin prep rat aortic ring assay: a modified method for the characterization of angiogenesis in whole mounts. Angiogenesis 5, 81–86.*
35. *Zijlstra, A., Seandel, M., Kupriyanova, T.A., et al. (2006) Proangiogenic role of neutrophil-like inflammatory heterophils during neovascularization induced by growth factors and human tumor cells. Blood 107, 317–327.*
36. *Zimrin, A.B., Villeponteau, B., Maciag, T. (1995) Models of in vitro angiogenesis: endothelial cell differentiation on fibrin but ot matrigel is transcriptionally dependent. Biochem. Biophys. Res. Commun. 213, 630–638.*