ORIGINAL ARTICLE UDC 616.36–004–091.8:616–079.4

Expression of CD34 in cirrhotic liver – reliance to dedifferentiation

Ekspresija CD34 u ciroznoj jetri − zavisnost od dediferencijacije

Jasmina Gligorijević*, Biljana Djordjević*, Aleksandar Petrović†, Aleksandra Radičević*, Simonida Stojanović*

School of Medicine, *Department of Pathology, †Department of Histology, **Niš, Serbia**

Abstract

Background/Aim. The vascular supply of dysplastic nodules (DN) is altered compared with surrounding cirrhotic nodules. Dysplastic nodules contain unpaired arteries which are isolated arteries unaccompained by bille ducts. In adition, capillarization or neovascularization is evident on CD34 and CD31 staining. The investigation of angiogenic profile of regenerative, dysplastic and nodules of hepatocellular carcinoma aimed at assessing whether vascular profile is in reliance to the process of dedifferentiation of hepatocytes during the course of cirrhosis. **Methods.** Thirty four liver nodules from surgical biopsies of 12 patients previously undiagnosed to have cirrhosis, were classified as regenerative, dysplastic and small hepatocellular carcinomas (HCC). The investigation included 8 large regenerative nodules (LRN), 11 low grade dysplastic nodules (LGDN), 12 high grade dysplastic nodules (HGDN) and 3 early HCC. Serial sections of the nodules and surrounding cirrhotic liver tissue were immunostained against CD34. The vascular counting method was performed. The results were analysed using SPSS computer statistical program. **Results.** The number of capillary unites showed significant differences among nodular types, with the largest number of capillaries in hepatocellular carcinoma as well as strong reliance to dedifferentiation. **Conclusion.** There is a significant correlation of sinusoidal capillarization to dediferentiation of the liver tissue during the course of cirrhosis. From diagnostic view, capillary counting may be helpfull to distinguish dysplastic from nondysplastic nodules. The appearance of dysplastic nodules in nonselected surgical biopsies is frequent enough to challenge caution during the follow-up of cirrhotic patients.

Key words:
liver cirrhosis; carcinoma, hepatocellular; antigens, cd34; diagnosis; cell dedifferentiation.

Apstrakt

Uvod/Cilj. Vaskularizacija displastičnih čvorića izmenjena je u poređenju sa okolnim cirotičnim čvorićima. Displastični čvorići imaju neuparene arterije koje su izolovane i koje ne prate žučni kanali. Osim toga, očigledna je kapilarizacija ili neovaskularizacija pri CD34 i CD31 obojenju. Cilj rada bio je istraživanje angiogenog profila regenerativnih, displastičnih i nodula hepatocelularnog karcinoma radi utvrđivanja međuzavisnosti vaskularnog profila i stepena dediferencijacije hepatocita u toku procesa ciroze u jetri. **Metode.** Analizirana su trideset četiri nodula dobijena putem 12 hirurških biopsija jetre osoba kod kojih nije prethodno dijagnostikovana ciroza. Noduli su klasifikovani u regenerativne, displastične i nodule hepatocelularnog karcinoma. Analizom je obuhvaćeno osam velikih regenerativnih nodula, 11 nodula niskog gradusa, 12 nodula visokog gradusa displazije i tri nodula ranog hepatocelularnog karcinoma. Serijski preseci nodula i okolnog ciroznog jetrinog tkiva obojeni su imunohistohemijskom metodom za prikazivanje CD34. Za analizu vaskularnih jedinica primenjen je specifični metod vaskularnog brojanja, a rezultati su statistički analizirani uz upotrebu SPSS kompjuterskog statističkog programa. **Rezultati.** Broj kapilarnih struktura bio je značajno različit između nodularnih tipova i kod najvećeg broja nodula hepatocelularnog karcinoma, ujedno sa značajnom korelacijom prema stepenu dediferencijacije hepatocita. **Zaključak.** Postoji snažna korelacija između sinusoidne kapilarizacije i dediferencijacije tkiva jetre u toku ciroze. Sa stanovišta dijagnostike, brojanje kapilara je od koristi u razlikovanju nedisplaznih od displaznih nodula. Prisustvo displaznih nodula u neselektovanim hirurškim biopsijama dovoljno je često da zahteva pažnju pri praćenju bolesnika sa cirozom jetre.

Ključne reči:
jetra, ciroza; karcinom, hepatocelularni; antigeni, cd34; dijagnoza; ćelija, dediferencijacija.

Introduction

Space occupyng, non malignant liver lesions arising in cirrhosis are currently classified into: large regenerative (LRN), low-grade dysplastic nodules (LGDN) and high-grade dysplastic nodules (HGDN), as recommended by international group of experts [1]. Dysplastic nodules (DN) are evident on gross examination of hepatic spacements as distinct nodular

Correspondence to: Jasmina Gligorijević, School of Medicine, Department of Pathology, Novoprojektovana bb, 18 000 Niš, Serbia. Phone: +381 18 42 34 092. E-mail: jasminag@eunet.rs

lesions that differ from surrounding hepatic parenchyma in terms of size, color, texture or degree of bulging at the cut surface [2]. Dysplastic nodules are characterized by a number of cytoarchitectural and angioarchitectural abnormalities [3–6]. Still, there are a lot of uncertainties in imaging diagnostic procedures that are not precise enough to qualify DN [7–9]. Morphological differentiation between an early stage of well-differentiated hepatocellular carcinomas (HCC) and DN is often difficult and diagnostic confusion concerning those lesions is a controversal issue. Based on clinical and pathological details of early HCC, the pathway for human hepatocarcinogenesis has been well-established during the last decade [6]. It is evident that many HCC develop through a progressive pathway from premalignant lesions to HCC in cirrhotic liver. As HCC show tendency to increasing incidence, the pathologists are, and will be, frequently faced with nodular lesions and small HCC. Diagnostic uncertainity between well-differentiated HCC in the early stage and DN, in particular HGDN, do exist.

The vascular supply of DN is altered compared with surrounding cirrhotic nodules. Dysplastic nodules contain unpaired arteries which are isolated arteries unaccompained by bille ducts [10, 11]. In adition, capillarization or neovascularization is evident on CD34 and CD31 staining [12–14].

We analysed nodular lesions for CD34 expression in surgical biopsies of liver cirrhosis to: 1) compare capillarization of the hyperplastic and dysplastic nodules; 2) investigate the incidence of these changes in nonselected surgical liver biopsies and 3) demonstrate if vascular count can make the distinction between premalignant and nonmalignant lesions.

Methods

Thirty four liver nodules from surgical biopsies of 12 patients, 7 women and 5 men, mean age 52.33 years, were analysed. The biopsies were taken during the laparoscopic surgery for: acute cholecystitis (7 patients), obstructive jaundice (2 cases), acute hemorrhagic gastric ulcer (1 case), splenectomy after trauma (1 case) and liver carcinoma (1case). None of patients was previously diagnosed as cirrhotic.

All of examined lesions were detected grossly as expansive growths in surrounding nodular background and measured 0.2–1.2 cm. Microscopically, they were classified as LRNs (those without architectural differences in comparison to adjacent cirrhotic nodules), as LGDN (showing normal architecture and large cell changes) or as HGDN (containing uneven foci of architectural abnormalities, nuclear crowding and small cell changes).

Serial sections of each nodule and surrounding cirrhotic liver and associated HCC – three casses, were immunostained with monoclonal antibody against CD34, a specific and sensitive marker to detect capillary unites.

The assessment of capillary units was performed in all dysplastic, large regenerative, and malignant nodules according to the method proposed for vascular counting [15] as follows: 3 mostly vascularized areas were identified by low magnification (×40) and those "hot spots" were marked by coloured pen to avoid topografical confusion. Vessel counting was performed under the high magnification (×200) within every "hot spot" area; this was performed to avoid topografical bias owing to a random evaluation [14]. Mean values of CD34 positive units were calculated for each single lesion and after that, mean value (± SD) was calculated for each nodule type. The results were statistically analysed using SPSS computer statistical program.

Results

The study included 8 LRN, 11 LGDN, 12 HGDN and 3 nodules of well-differentiated HCC. The distribution of different types of nodules is presented in Table 1. HGDN were more frequently associated to HCC (58,33%) than LGDN (36.36%) or LRN (8.33%).

Table 1

The distribution of different types of nodules

Patients	LRN	LGDN	HGDN	HCC
1			1	
2			1	
3		1		
4		1		
5	2	1	1	
6	2	1	1	
7	2	2	1	
8	1			
9	1	1		
10		1	3	1
11		2	2	1
12		1	2	1

LRN – large regenerative nodules; LGDN – low grade dysplastic nodules; HGDN – high-grade dysplastic nodules; HCC – hepatocellular carcinomas

Immunohistochemical expression of *de novo* formed capillary units is ilustrated in Figure 1. Sinusoidal capillarization and CD34 positive forms were at the periphery of the LRN and LGDN and were more centrally located in HGDN, while randomly in HCC (Figure 2). The number of capillaries was not significantly different among specific nodular types, but the greatest one was in HGDN (Table 2).

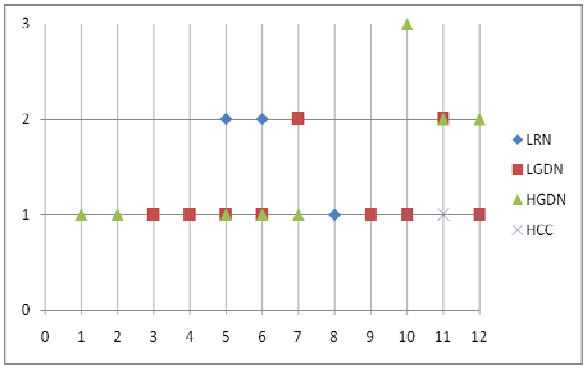

Fig. 1 – Semiquantitative assesment of CD34 positive units in cirrhotic liver

LRN – large regenerative nodules; LGDN – low grade dysplastic nodules; HGDN – high-grade dysplastic nodules; HCC – hepatocellular carcinomas

The Tacmann's test for comparison of mean values showed statistical signifficant differences among tested groups ($p < 0.001$) and it confirmed the results of Anne's

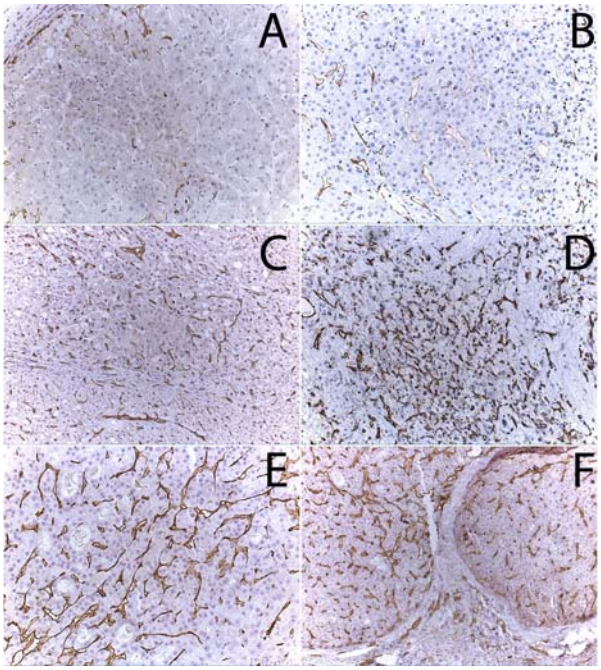

Fig. 2 – Immunostaining for CD34

A – CD34 (APAP ×200)
B – large regenerative module – LRN (APAP ×200)
C – low-grade dysplastic nodule – LGDN (APAP ×200)
D – high-grade dysplastic nodule – HGDN (APAP ×200)
E – hepatocellular carcinoma – HCC (APAP ×200)
F – hepatocellular carcinoma –HCC (in the left nodule)
***Notice: Increasing number of vascular units through HGDN and HCC**

Table 2
The number of capillaries in dependence of nodular types

Types of nodules	N	CD34 positive cappilare units	
		$\bar{x} \pm SD$	95% CI
LRN	24	14.33 ± 4.43	12.46 – 16.20
LGDN	33	20.73 ± 4.67	19.07 – 22.38
HGDN	36	35.42 ± 6.04	33.37 – 37.46
HCC	9	56.78 ± 6.51	51.77 – 61.79

LRN – large regenerative nodules; LGDN – low grade dysplastic nodules; HGDN – high-grade dysplastic nodules; HCC – hepatocellular carcinomas; CI – confidence interval; N – number of tested "hot spots"

analysis ($F = 184.75$; $p < 0.001$). There is a statistically significant difference in vascular units number among the tested groups ($p < 0.001$) with the greatest number of CD34 positive units in HCC and the smallest ones in LRN (Table 2) .

Discussion

We investigated the sinusoidal capillarization in reliance to dedifferentiation in the cirrhotic liver. Nodules were classified according to standardized nomenclature [1] dividing them into hyperplastic (LRN) and dysplastic (low and high-grade). Biological and clinical significance of those nodules is not elucidated. Previous investigations had shown the progression from cirrhosis to HCC to be followed by a shift of vascular supply mainly from venous to arterial type [10–14]. It is obvious that HCC are higly vascularized tumors. The process of neovascularization runs in parallel to the process of dedifferentiation [6]. Putting these facts in connection to the appearance of morphologically different nodules in cirrhosis and the process of dedifferentiation, it was accepted that abnormal vascularization can be of diagnostic help to recognize those lesions with potential to neoplastic transformation [10, 11, 15, 17]. A functional and biological background for these morphological entities is necessary as it was demonstrated that a number of entirely benign looking LGDN are monoclonal growths, as are some HGDN and HCC [18]. It is clear that clonality type together with morphological and biological characteristics are sufficient in concluding about the nature of nodules in cirrhosis.

Previous investigations of unpaired arteries and sinusoidal capillarization demonstrated an increased number of both structures in hyperplastic and dysplastic nodules.

In this study, we investigated the CD34 positive units in hyperplastic and dysplastic nodules of cirrhotic liver as well as in tree small, well-differentiated HCC. The analysis was performed on surgical liver biopsies taken during laparoscopic surgery in previously undiagnosed cirrhotic patients. The incidence of small HCC was 25% and morphologically specific nodules, other than cirrhotic, were found in 13.02% of all the analysed nodules. We found that incidence important as it reflected a native status in the moment of diagnosis, remaining on silent course of both cirrhosis and HCC, although the number of cases included in the study was small.

It was previously shown that there was no difference in angiogenic profile among cirrhotic nodules, LRN and LGDN [5, 10, 11, 16]. The results of investigations of unpaired arteries and sinusoidal capillarisation by Rancolli et al. [16] suggest that the extent of capillarization but not the arterialization is increasingly upregulated in HGDN and fully malignant lesions. Contrary to other investigators [11, 16–18] we found significant differences in CD34 positive units among all tested types of nodules in cirrhotic liver, as well as in HCC. This discrepances could be the result of different selection of LGDN. We adopted very strict histomorphologic criteria and only nodules with a large cell change and expansive growth measuring 0.2–1.2 cm were included as LGDN. We must be very careful in conclusion about diagnostic value of the results. It is necessery to undertake more investigations to establish a cut-off value of vascular units in premalignant and malignant LN.

Finally, we showed the differences between LRN and DN on the basis of vascular profile. As mentioned previously, some of them are in fact monoclonal. Undoubtedly, only the molecular caracterization of individual hepatocelular nodules will contribute to determine which nodules are dysplastic or neoplastic [19–21]. A correlation of basic biological analysis with morphophenotypic features is expected to provide a helpful information of clinical significance.

There is a huge attempt in following cirrhotic patients and tracing the processes of mild- to high-grade dysplastic, or carcinoma. Despite technological advances, imaging cir-

rhotic patients remain a challenging issue because nonmalignant DN mimic a small HCC. Through progression from regenerative nodules to LGDN, HGDN and HCC, it is possible to visualize new arterial vessels. It is neovascularity that allows HCC to be diagnosed and is a key for imaging cirrhotic patients [22, 23]. The analysis of neovascularization in biopsies in combination to imaging results will help in following patients in risk for developing HCC and for early detection and treatment of carcinoma.

Conclusion

There is a strong correlation of sinusoidal capillarization with dedifferentiation of the liver tissue during the course of cirrhosis. From diagnostic view, capillary counting may be helpful to distinguish dysplastic from nondysplastic nodules. The appearance of dysplastic nodules in nonselected surgical biopsies is frequent enough to challenge caution during the follow-up of cirrhotic patients.

R E F E R E N C E S

1. *Wanless I, Callea F, Craig JR, Crawford JM, Desmet VJ, Farber* E et al. Terminology of nodular lesions of the liver: recommendation of the World Congress of Gastroenterology Working Party. Hepatology 1995; 22: 983–93.
2. *Party IW*. Terminology of chronic hepatitis, hepatic allograft rejection, and nodular lesions of the liver: summary of recommendations developed by an international working party, supported by the World Congress of gastroenterology, Los Angeles, 1994. Am J Gastroenterol 1994; 89: S177–81.
3. *Ferrell L, Wright T, Lake J, Roberts J, Asher N*. Incidence and diagnostic features of dysplastic nodules vs small hepatocellular carcinoma in cirrhotic liver. Hepatology 1992; 16: 1372–81.
4. *Bottino A, Nogueira C, Pannain V*. Analysis of morphological variables and arterialization in the differential diagnosis of hepatiac nodules explanted cirrhotic livers. Diagn Pathol 2007; 2: 51.
5. *Morise Z, Sugioka A, Mizoguchi Y, Kato R,Tanahashi Y*. Stepwise carcinogenesis of hepatocellular carcinoma in a nodule surrounded by hyperplastic and hypervascular liver tissue.Can J Surg 2009; 52(1): E5–E7.
6. *Kojiro M, Roskams* T. Early hepatocellular carcinoma and dysplastic nodules. Sem Liver Dis 2005; 25(2): 133–42.
7. *Borzio M, Borzio F, Croce A, Sala M, Salmi A, Leandro G,* et al. Ultrasonography-detected macroregenerative nodules in cirrhosis: a prospective study. Gastroenterology 1997; 112: 656–62.
8. *Erler BS, Hsu L, Truong HM, Petrovic LM, Kim SS, Huh MH,* et al. Image analysis and diagnostic classification of hepatocellular carcinoma using neural network s and multivariante discriminant functions. Lab Inves 1994; 71: 446–51.
9. *Kokudo N, Bandai Y, Imanishi H, Minagawa M, Uedera Y, Harihara Y,* et al. Managemant of new hepatic nodules detected by intraoperative ultrasonography during hepatic resection for hepatocellular carcinoma. Surgery 1996; 119: 634–40.
10. *Terada T, Nakanuma Y*. Arterial elements and perisinusoidal cells in borderline hepatocellular nodules and small hepatocellular carcinomas. Histopathology 1995; 27: 333–9.
11. *Park YN, Yang CP, Fernandez GJ, Cubukcu O, Thung SN, Theise ND*. Neoangyogenesis and sinusoidal "capillarisation" in dysplastic nodules of the liver. Am J Surg Pathol 1998; 22: 656–62.
12. *Messerini L, Novelli L, Comin CE*. Microvessel density and clinicopathological characteristics in hepatitis C virus and hepatitis B virus related hepatocellular carcinoma. J Clin Path 2004; 57(8): 867–71.
13. *Kimura H, Nakajima T, Kagawa K*. Angiogenesis in hepatocellular carcinoma as evaluated by CD34 immunohistochemistry. Liver 1998; 18: 14–9.
14. *NakashimaY, Nakashima O, Hsia CC*. Vascularization of small hepatocellular carcinomas: correlation with differentiation. Liver 1999; 19:12–8.
15. *Bossi P, Viale G, Lee AKC, Alfano RM, Coggi G, Bosari S*. Angiogenesis in colorectal tumors: microvessel quantitation in adenomas and carcinomas with clinico-pathological correlations. Cancer Res 1995; 55: 5049–53.
16. *Roncalli M, Roz E, Coggi G* . The vascular profile of regenerative and dysplastic nodules of the cirrhotic liver: implication for diagnosis and classification. Hepatology 1999; 30(5): 1174–8.
17. *Matsui O, Kadoya M, Kameyama T*. Benign and malignant nodules in cirrhotic liver: distinction based on blood suply. Radiology 1991;178: 493–7.
18. *Paradis V, Laurendeau I, Vidaud M, Bedossa P*. Clonal analysis of macronodules in cirrhosis. Hepatology 1998; 28: 953–8.
19. *Lee JS, Thorgeirsson SS*. Genetic profiling of human hepatocellular carcinoma. Sem Liv Dis 2005; 25(2): 125–7.
20. *Yamada TY, De Souza AT, Finkelstein S, Jirtle RL*. Lose of the gene encoding mannose 6-phosphatase/insulin-like growth factor II receptor is an early event in liver carcinogenesis. Proc Natl Acad Sci 1997; 94: 1051–5.
21. *Roberts LR, Gores GJ*. Hepatocellular carcinoma: molecular pathways and new terapeutic targets. Sem Liv Dis 2005; 25(2): 212–25.
22. *Kondo F*. Histological features of early hepatocellular carcinomas and their developmental process: for daily practical clinical application: hepatocellular carcinoma. Hepatol Int 2009; 3(1): 283–93.
23. *Lencioni R, Cioni D, Crocetti LJ*. Magnetic resonance imaging of liver tumors. J Hepatol 2004; 40: 162–71.

Received on April 6, 2009.
Revised on December 3, 2009.
Accepted on December 16, 2009.