Expression of CD34 in cirrhotic liver – reliance to dedifferentiation

Ekspresija CD34 u ciroznoj jetri – zavisnost od dediferencijacije

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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 unaccompanied by bile ducts. In addition, 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 dedifferentiation 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.


Ključne reči: jetra, ciroza; karcinom, hepatocelularni; antiženi, cd34; dijagnoza; čelijska, dediferencijacija.

Introduction

Space occupying, 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. Dysplastic nodules (DN) are evident on gross examination of hepatic spacements as distinct nodular

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lesions that differ from surrounding hepatic parenchyma in terms of size, color, texture or degree of bulging at the cut surface. Dysplastic nodules are characterized by a number of cytoarchitectural and angioarchitectural abnormalities. Still, there are a lot of uncertainties in imaging diagnostic procedures that are not precise enough to qualify DN. 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 controversial issue. Based on clinical and pathological details of early HCC, the pathway for human hepatocarcinogenesis has been well-established during the last decade. 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 uncertainty 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 unaccompanied by bile ducts. In addition, capillarization or neovascularization is evident on CD34 and CD31 staining.

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 (1 case). 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 cases, were immunostained with monoclonal antibody against CD34, a specific and sensitive marker to detect capillary units.

The assessment of capillary units was performed in all dysplastic, large regenerative, and malignant nodules according to the method proposed for vascular counting as follows: 3 mostly vascularized areas were identified by low magnification (×40) and those “hot spots” were marked by coloured pen to avoid topographical confusion. Vessel counting was performed under the high magnification (×200) within every “hot spot” area; this was performed to avoid topographical bias owing to a random evaluation. 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**

<table>
<thead>
<tr>
<th>Patients</th>
<th>LRN</th>
<th>LGDN</th>
<th>HGDN</th>
<th>HCC</th>
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<tr>
<td>1</td>
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<td>12</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>1</td>
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</table>

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 illustrated in Figure 1. Sinusoidal capillari-
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 highly 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.

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>
<thead>
<tr>
<th>Types of nodules</th>
<th>N</th>
<th>CD34 positive capillare units</th>
<th>( \bar{x} \pm SD )</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>LRN</td>
<td>24</td>
<td>14.33 ± 4.43</td>
<td>12.46 – 16.20</td>
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<tr>
<td>LGDN</td>
<td>33</td>
<td>20.73 ± 4.67</td>
<td>19.07 – 22.38</td>
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<tr>
<td>HGDN</td>
<td>36</td>
<td>35.42 ± 6.04</td>
<td>33.37 – 37.46</td>
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<tr>
<td>HCC</td>
<td>9</td>
<td>56.78 ± 6.51</td>
<td>51.77 – 61.79</td>
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</tbody>
</table>

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”

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-
rhicotic patients remain a challenging issue because nonma-
lignant DN mimic a small HCC. Through progression from
regenerative nodules to LGDN, HGDN and HCC, it is pos-
sible to visualize new arterial vessels. It is neovascularity
that allows HCC to be diagnosed and is a key for imaging
cirrhotic patients. 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 capillariza-
tion 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.

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