Role of Fine Needle Aspiration Cytology in Management of Hepatocellular Carcinoma: A Single Centre Experience

Anna Mrzljak1,4, Ika Kardum-Skelin2,4, Vesna Ćolić Cvrlje1,4, Tajana Filipić-Kanižaj1,4, Dunja Šušterčić2 and Dinko Škrgro3

1 Department of Gastroenterology, »Merkur« University Hospital, Zagreb, Croatia
2 Department of Cytology, »Merkur« University Hospital, Zagreb, Croatia
3 Department of Nephrology, »Merkur« University Hospital, Zagreb, Croatia
4 Zagreb University, School of Medicine, Zagreb, Croatia

ABSTRACT

Hepatocellular carcinoma (HCC) mostly occurs in chronic liver disease and cirrhosis. Liver resection and liver transplantation (LT) represent potentially curative treatments of choice and if not feasible, palliative strategies such as percutaneous interventional techniques (PITs) and chemotherapy (ChT) are considered. Elevated alpha-fetoprotein, typical imaging pattern, needle core biopsy (NCB) and fine needle aspiration cytology (FNAC) complement diagnostic assessment of HCC. We have retrospectively analyzed all patients with contraindications for NCB in which HCC was diagnosed by FNAC during consecutive 5 years in our hospital. Ultrasound guided FNAC provided a safe method of approach and, except for mild transitory discomfort at the site of puncture, no complications were documented. The diagnosis was established on May-Grünwald-Giemsa (MGG) stained aspirates and additional immunocytochemistry. Of our 62 patients, HCC developed in 61.3% cirrhotic and 38.7% non-cirrhotic livers. In the setting of cirrhosis 18.4% of patients underwent LT, 15.8% PITs, 26.3% ChT and 39.5% symptomatic therapy. In non-cirrhotic setting 46% of patients underwent liver resection, and PIT, ChT, and symptomatic therapy were applied in 4%, 25%, 25% of cases, respectively. Pathohistology of resected and explanted livers (18 cases) confirmed the initial diagnosis made on FNAC. Since only early stage of HCC has a better prognosis, every effort should be made to establish prompt and accurate diagnosis. Our observations demonstrate that FNAC offers minimally invasive, rapid and uncomplicated diagnostic approach, with sensitivity from 67% to 93% and specificity from 96% to 100%. FNAC, is of utmost importance in the setting of abnormal coagulation tests and ascites commonly seen in advanced liver disease, facilitating diagnostic workup and treatment decisions.

Key words: hepatocellular carcinoma (HCC), fine needle aspiration cytology (FNAC), treatment

Introduction

Hepatocellular carcinoma (HCC) is the fourth most common cancer in the world and its incidence is still rising. It accounts for about 90% of all primary liver cancers and mostly occurs in the setting of chronic liver disease and cirrhosis. Several important risk factors for HCC have been identified: male gender (3.7:1 men to women)1, higher age (mean age at presentation between 50 and 60 years)2, chronic liver disease (chronic hepatitis or cirrhosis) of any cause3–5, hepatitis B virus (HBV) infection – (chronic HBV infection is the predominant global environmental cause of chronic hepatitis or cirrhosis, whereas 25% of infected or more will develop HCC)6,7, hepatitis C virus (HCV) infection (80% of acutely infected become chronic carriers, and about 60% develop chronic hepatitis of which 20% progress to cirrhosis over a period of 20–25 years, and a proportion of these develop HCC)8,9, iron overload: hereditary hemochromatosis (HH) and dietary iron overload (with a relative risk of greater than 200)10, aflatoxin (mycotoxin produced by the fungi Aspergillus flavus), nonalcoholic fatty liver disease (NASH) and diabetes mellitus (DM)11, inherited metabolic disorders: alpha-1 antitrypsin (α1AT) deficien-
cy, hereditary tyrosinemia type 1 (HT1), glycogen storage
diseases and Wilson’s disease12–15.

Although elevated level of serum alpha-fetoprotein
and a typical imaging pattern (demonstrated by ultra-
sound, computer tomography – CT, magnetic resonance –
MR and angiography) may be sufficient for diagnosis, in
inconclusive cases tissue diagnosis is mandatory. Needle
core biopsy (NCB) and fine needle aspiration cytology
(FNAC) both can provide definitive morphological as-
ssessment. HCC is typically diagnosed late in its course,
and the median survival following diagnosis is approxi-
mately 6 to 20 months16. Treatment strategies, depend-
ing on tumor extent and underlying liver dysfunction, in-
clude liver resection or transplantation, percutaneous
interventional techniques (PITs), chemotherapy (ChT)
and symptomatic therapy.

Materials and Methods

This study was carried out by the multidisciplinary
team of tertiary Gastroenterology centre from »Merkur«
University Hospital. We retrospectively identified all
HCC patients diagnosed only by FNAC at our institution
in the period from January 2004 to July 2009. Data col-
lected from patients’ medical records specified clinical,
laboratory, imaging and cytological results. Diagnostic
assessment included imaging studies (ultrasound, CT or
MR imaging) and serum alpha-fetoprotein level. Ultra-
sound guided FNAC with the CHIBA needle of 0.7 mm
(22 gauge) was performed in patients with clinical sug-
gestion of HCC with contraindications for NCB; abnor-
amal coagulation parameters, refractory ascites or lack of
a safe access. The cytological smears were stained by the
standard May-Grünwald-Giemsa method (MGG) (Figure
1a and b). Immunocytochemical staining was performed
by LSAB method with monoclonal antibodies (DAKO),
applying a panel of α-fetoprotein (Figure 1c), BerEP4,
CD31, CK8, CK18 (Figure 1d), and in selected cases, CK
5/6, CK7, CK 19, CK 20, CD117, desmin, SMA, chromogranin, synaptophysin, thyroid transcription factor-1
(TTF-1), Ca19,9, CEA etc. for the diagnosis of HCC and
distinction of HCC from metastatic tumors (MC) or to
identify the primary tumor site of MC. Statistical analy-
ses were performed using MedCalc for Windows, version
9.5.0.0 (MedCalc Software, Mariakerke, Belgium). Data
were analyzed with Mann Whitney test, χ²-test and Fi-
sher’s exact test. Differences were considered significant
when two sided p<0.05.

Results

In period from 2004 to 2009, all patients with contra-
indications for NCB in which HCC was diagnosed by
FNAC are analyzed. FNAC was performed under the
guidance of ultrasound. No serious post-procedural com-
plications were documented, except for mild transitory
discomfort at the site of puncture. Summarized data of
all our patients are presented in Table 1. 62 patients
were included, mean age of 63 years (range 47–86, SD
13). The majority of patients (61.3%) developed HCC in
cirrhotic liver, whereas 38.7% in non-cirrhotic form of
parenchymal liver disease. Dominant gender in both
groups was men: 81.6% in cirrhotic group (CG) and
83.3% in non-cirrhotic group (NCG). HCC developed in
significantly earlier age in CG (mean age 62±9.1) than in
NCG (mean age 70±9.3, Mann Whitney, p<0.02). In the
majority of patients in CG (63.2%) the etiology of under-
lying liver disease was known; alcohol in 47.4% and viral
diseases (HBV and HCV) in 15.8% of cases. In compari-
on, in 83.3% of cases in NCG, the etiology of the liver
disease remained unknown and in only 8.3% it was alco-

Figure 1. Fine needle aspiration cytology of hepatocellular carcinoma: a) well-differentiated (MGG, 1000x), b) poorly differentiated
(MGG, x1000), c) α-feto protein positive cells (immunocytochemistry, LSAB, 1000x), d) Cytokeratin 18 positive cells (immunocytoche-
mistry, LSAB, x1000).
hol and 8.3% viral infection. There was no statistically significant difference between the groups regarding tumor presentation (multifocal or solitary) at the time of diagnosis (χ², p=0.56).

In the setting of decompensated cirrhosis, 18.4% of patients which met Milan criteria, underwent liver transplantation, 15.8% underwent PIT, 26.3% were treated with chemotherapy and 39.5% with symptomatic therapy. In non-cirrhotic group HCC was treated in 45.8% of cases by liver resection, in 25% with chemotherapy, in 25% with symptomatic therapy and in 4% by PIT. Pathohistological analysis of resected (11 cases) and explanted livers (seven cases) confirmed the initial diagnosis made on FNA specimens.

Discussion

There is a general consensus that periodic abdominal ultrasound (every six months) and the measurement of serum AFP level in surveillance of high risk groups can detect HCC in its early stage, which is associated with better prognosis17. However, significant proportion of HCC lesions are not associated with elevated AFP and although imaging methods show a typical imaging pattern, that finding is not always pathognomonic, especially for small and hypovascular tumors18–20. Therefore, additional morphological assessment should be made either by FNAC or NCB. Although NCB is considered a gold standard for identification of HCC, in the clinical practice, especially in the setting of advanced parenchymal liver disease, it is not always a feasible method. Major obstacles for its use are: low platelet count, abnormal coagulation parameters, refractory ascites or localisation of lesion with no safe access route. Limited by aforementioned parameters, diagnostic workup may be delayed or interrupted enabling further progression of malignant disease and restraining treatment options. FNAC results in a little liver damage and has a high safety. The occurrence rate of complications ranges from 0.05% to 0.16% and includes bleeding, bile leakage, infection and death21. In theory, pulling out the needle after aspiration procedure may result in further seeding of the tumor cells. None of these complications were observed in our survey. The specificity and positive predictive value of FNAC is ranges from 96% to 100% and its sensitivity ranges from 67 to 93%22–24. These results are comparable to the accuracy of NCB (sensitivity range 61 to 94%)25,26.

Liver resection is the treatment of choice for HCC in non-cirrhotic and Child A cirrhotic patients, but in the setting of decompensated liver disease, liver transplantation (LT) represents an effective treatment as it simultaneously treats the tumor and the underlying liver disease. If not feasible, percutaneous interventional techniques (PITs); percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), selected transcatheter arterial chemoembolization (TACE) provide optional choices of treatment. Liver resection or LT are considered best treatment options for HCC because of potential complete recovery. However these procedures are mainly limited by the size of tumor and the possibility of disease dissemination. Therefore every effort should be made to enable the accurate diagnosis in the early stage of the disease.

**Table 1: Data for Patients with Hepatocellular Carcinoma**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cirrhotic group % (n)</th>
<th>Non-cirrhotic group % (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>61.3% (38)</td>
<td>38.7% (24)</td>
<td>0.89</td>
</tr>
<tr>
<td>Female</td>
<td>18.4% (7)</td>
<td>16.7% (4)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>81.6% (31)</td>
<td>83.3% (20)</td>
<td></td>
</tr>
<tr>
<td>Age, mean±SD,</td>
<td>62±9.1</td>
<td>70±9.3</td>
<td>&lt;0.002*</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>47.4% (18)</td>
<td>8.3% (2)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Viruses (HBV+HCV)</td>
<td>15.8% (6)</td>
<td>8.3% (2)</td>
<td>0.64</td>
</tr>
<tr>
<td>Undetermined</td>
<td>36.8% (14)</td>
<td>83.3% (20)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Tumor presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multifocal</td>
<td>52.6% (20)</td>
<td>41.7% (10)</td>
<td>0.56</td>
</tr>
<tr>
<td>Solitary</td>
<td>47.4% (18)</td>
<td>58.3% (14)</td>
<td></td>
</tr>
<tr>
<td>Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic resection</td>
<td>0</td>
<td>45.8% (11)</td>
<td></td>
</tr>
<tr>
<td>OLT</td>
<td>18.4% (7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PITs</td>
<td>15.8% (6)</td>
<td>4.2% (1)</td>
<td>0.32</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>26.3% (10)</td>
<td>25% (6)</td>
<td>0.86</td>
</tr>
<tr>
<td>Symptomatic therapy</td>
<td>39.5% (15)</td>
<td>25% (6)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*statistically significant, OLT – orthotopic liver transplantation, PIT – percutaneous interventional techniques
Out of 62 HCC patients with contraindications for NCB, detection of HCC by FNAC in 64.2% (18.4% CG and 45.8% NGC) of patients still in early stage of the malignant disease allowed application of potentially curative treatment options (LT and resection). Our observations demonstrate that in spite of obstacles in advanced liver disease, FNAC offers safe and effective diagnostic approach steering the treatment to more beneficial prognosis.

Therefore since only early stage of the disease offers potentially curative treatment options it is imperative, especially in high risk group patients, to perform intensive screening for HCC and, if suspected, every effort should be made to establish early and accurate diagnosis.

REFERENCES


A. Mrzljak

Department of Medicine, Merkur University Hospital, Zajčeva 19, 10 000 Zagreb, Croatia

e-mail: anna.mrzljak@gmail.com

ULOGA CITOLOŠKE PUNKCIJE U STRATEGIJI HEPATOCELULARNOG KARCINOMA – ISKUSTVO JEDNOG CENTRA

SAŽETAK

Hepatocelularni karcinom (HCC) se razvija uglavnom u kroničnoj bolesti jetre ili cirozi. Resekcija i transplantacija jetre (LT) su potencijalne metode izlječenja HCC, no ukoliko nisu primjenjive, dolaze u obzir palijativne strategije poput potencijalno curativih medija (LT i resekcija) i citoloških punkcija (fine needle aspiration cytology – FNAC) komplementarne su postavljanjem dijagnostičkih i terapeutičkih odluka. U skupini 62 pacijenata sa contraindicacijama za NCB, detekcija HCC pomoću FNAC je omogućila potencijalno curativne terapije, ukoliko su pacijenti u ranom (srednjim) stadiju bolesti. FNAC je omogućila early diagnosis i planiranje navedenih terapija, čime se osigurava bolja prognosno-terapeutička strategija.

A. Mrzljak

Department of Medicine, Merkur University Hospital, Zajčeva 19, 10 000 Zagreb, Croatia

e-mail: anna.mrzljak@gmail.com

384
ciroze, kod 46% bolesnika je izvršena resekcija, u 4% primjenjen je PITs, u 25% ChT i u 25% simptomatska terapija. Patohistološka analiza reseciranih i eksplantiranih jetri (18 slučajeva) potvrdila je citološku dijagnozu. Budući rani stadiji bolesti imaju bolju prognozu, potrebna je brza i točna dijagnostika. Naša zapažanja ukazuju da je FNAC minimalno invazivna, brza i jednostavna dijanostička metoda sa osjetljivošću od 67% do 93% i specifičnošću od 96% do 100. Citološka punkcija, naročito u slučajevima uzapređene bolesti jetre s komplikacijama (loša koagulacija i ascites) omogućuje rano postavljanje dijagnoze, te time i raniji početak liječenja.