INTRODUCTION

Hepatocellular carcinoma (HCC), a highly prevalent and lethal cancer, is the sixth most common cancer and the third leading cause of cancer-related death worldwide ***(Ferlay et al., 2010).***

Resection is currently indicated in patients with single asymptomatic HCC and extremely well-preserved liver function who have neither clinically substantial portal hypertension or abnormal bilirubin levels. Less than 5% of cirrhotic patients with HCC, however, meet these criteria. Image-guided tumor ablation has a major role in the therapeutic management of HCC. Several methods of therapeutic management have been developed, including intratumoral injection of ethanol or acetic acid and thermal ablation with radiofrequency, laser, microwave, or cryosurgery ***(Tateishi, et al., 2005)***.

Percutaneous ethanol injection has been the most widely used technique. Results from several series have provided indirect evidence that the long-term survival of patients with early-stage HCC who were treated with ethanol injection is substantially greater than the long-term survival of patients who received the best supportive care. The major limitation of ethanol injection is the high local recurrence rate, which may reach 33%–43%. Radiofrequency (RF) ablation is the method receiving the greatest attention to date for percutaneous treatment of focal hepatic malignancies. Findings from previous studies have shown that RF ablation can achieve more

effective local tumor control with fewer treatment sessions than ethanol injection. Radiofrequency ablation (RFA) has emerged as a new treatment modality and has become a main modality of locoregional therapy because of its effectiveness and safety for small HCC (<5.0 cm), with a 3-year survival rate of 62–77% ***(Lencioni et al., 2005).***

Extensive clinical researches have indicated that RFA is an effective treatment for small HCC and has an outcome equal to that of surgical resection, but has the advantage in being less invasive over surgical resection ***(Lupo, et al., 2007).***

Prognostic factors affecting the survival after RFA mainly included tumor size, tumor number, safety margin, liver function reserved, and so on ***(Peng, et al., 2008)***

Recently, there are increasing evidences that the presence of systemic inflammation correlates with poorer cancer-specific survival in certain cancers. Various markers of systemic inflammatory response, including cytokines, C-reactive protein (CRP), and absolute blood neutrophil or lymphocyte count as well as their ratio such as neutrophil-to-lymphocyte ratio (NLR) have been investigated for their prognostic roles in certain cancer populations ***(Jung et al., 2011).***

Studies had demonstrated that an elevated NLR may correlate with a poor prognosis in patients with HCC who underwent transcatheter arterial chemoembolization (TACE) ***(Huang et al., 2011),*** curative resection ***(Gomez et al., 2008)*** and orthotopic liver transplantation (OLT) ***(Wang, et al., 2011).***

In the “NLR”, “N” represents the number of circulating neutrophils, and it could represent the levels of circulating angiogenesis-regulating chemokines, growth factors and proteases which are major contributors to tumor related angiogenesis ***(Hung, et al., 2011).***

On the other hand, “L” represents the number of circulating lymphocyte, which have pivotal roles in cytotoxic cell death and cytokines production that inhibit proliferation and metastatic activity of tumor cells ***(Ding, et al., 2010).***

Aim of the Work

The aim of this study is assessment of prognostic Value of Blood Neutrophils to Lymphocytes Ratio in patient with HCC treated with Radiofrequency ablation.

Chapter 1

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma is a primary cancer of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. The cell(s) of origin are believed to be the hepatic stem cells, although this remains the subject of investigation. Tumors progress with local expansion, intrahepatic spread, and distant metastases. In general, the tumors are discovered either during routine screening or when symptomatic because of their size or location. Tumors may present as a single mass lesion or as diffuse growth, which can be difficult to differentiate from the surrounding cirrhotic liver tissue and the regenerating liver nodules on imaging studies. The presentation may be caused in part by mass effect that can lead to obstruction of the biliary system or anywhere affecting the liver vasculature. Without aggressive surgical resection, ablative therapy, or liver transplantation, hepatocellular carcinoma results in liver failure and eventual death ***(Alison, 2005).***

Etiology and risk factors:

The most frequent factors include chronic viral hepatitis (types B and C), alcohol intake and aflatoxin exposure. In Africa and East Asia, the largest attributable fraction is due to hepatitis B (60%) whereas in the developed Western world, only 20% of cases can be attributed to HBV infection, while

chronic hepatitis C appears to be the major risk factor ***(Parkin et al., 2005).***

Approximately 90% of HCCs are associated with a known underlying risk factor (Table 1).

Table (1): Geographical distribution of main risk factors for HCC worldwide.

Geographic area	AAIR	Risk factors		Alcohol	Others
	M/F	HCV (%)	HBV (%)	(%)	(%)
Europe	6.7/2.3	60-70	10-15	20	10
Southern	10.5/3.3				
Northern	4.1/1.8				
North America	6.8/2.3	50-60	20	20	10 (NASH)
Asia and Africa		20	70	10	10 (Aflatoxin)
Asia	21.6/8.2				
China	23/9.6				
Japan	20.5/7.8	70	10-20	10	10
Africa	1.6/5.3				
WORLD	16/6	31	54	15	

Updated from ***(Llovet et al., 2003),*** *according to IARC data (IARC. <http://www-dep.iarc.fr/>; 2011). AAIR, age-adjusted incidence rate.*

Worldwide, approximately 54% of cases can be attributed to HBV infection (which affects 400 million people globally) while 31% can be attributed to HCV infection (which

affects 170 million people), leaving approximately 15% associated with other causes. Cirrhosis is an important risk factor for HCC, and may be caused by chronic viral hepatitis, alcohol, inherited metabolic diseases such as hemochromatosis or alpha-1-antitrypsin deficiency, and non-alcoholic fatty liver disease. All etiologic forms of cirrhosis may be complicated by tumor formation, but the risk is higher in patients with hepatitis infection. Overall, one-third of cirrhotic patients will develop HCC during their lifetime ***(Sangiovanni et al., 2006).***

Long-term follow-up studies have demonstrated that approximately 1–8% per year of patients with cirrhosis develop HCC (e.g. 2% in HBV-infected cirrhotic patients and 3–8% in HCVinfected cirrhotic patients) ***(Ioannou et al., 2007).***

In general, features of liver disease severity (low platelet count of less than 100 _ 103, presence of esophageal varices), in addition to older age and male gender, correlate with HCC development among patients with cirrhosis ***(Lok et al., 2009).***

Recent studies have shown that liver cancer incidence increases in parallel to portal pressure as directly measured ***(Ripoll et al., 2009),*** or in parallel to the degree of liver stiffness as measured by transient elastography ***(Masuzaki et al., 2009).***

Several studies have identified HBV-related factors as key predictors of HCC development in patients with chronic hepatitis B infection ***(Lok, 2004).***

Hepatitis B virus e antigen (HBeAg) seropositivity ***(Yang et al., 2002)***, high viral load ***(Chen et al., 2006)***, and genotype C ***(Yu et al., 2005)*** are independent predictors of HCC development. In addition, hepatitis B viral load correlates with the risk of progression to cirrhosis ***(Iloeje et al., 2006).***

Similarly, in a recent meta-analysis, HCV genotype 1b is claimed to increase the risk of HCC development ***(Raimondi et al., 2009).***

Dietary exposure to aflatoxin B1, derived from the fungi Aspergillus flavus and A. parasiticus, is an important co-factor for HCC development in some parts of Africa and Asia. These molds are ubiquitous in nature and contaminate a number of staple foodstuffs in tropical and subtropical regions. Epidemiologic studies have shown a strong correlation between the dietary intake of aflatoxin B1, TP53 mutations and incidence of HCC, specifically in HBV-infected individuals ***(Hsu et al., 1991).***

Regarding other risk factors, patients with hemochromatosis develop HCC in up to 45% of cases ***(Deugnier et al., 1993)***, most often with a background of cirrhosis, and HCC is well documented as a complication of cirrhosis associated with alpha-1-antitrypsin deficiency ***(Perlmutter, 2006).***

HCC develops occasionally in patients with Wilson's disease, but only in the presence of cirrhosis ***(Polio et al., 1989).***

Obesity, diabetes and fatty liver disease have come to be recognized as a cause of HCC ***(Marrero et al., 2005)***, although the mechanisms by which these overlapping conditions contribute to cancer development remain elusive. Cirrhosis due to non-alcoholic steatohepatitis may give rise to HCC but it appears that these factors may also be additive to chronic viral hepatitis ***(Marrero et al., 2005).***

Epidemiologic evidence of a link between cigarette smoking and the occurrence of HCC was traditionally conflicting ***(El-Serag et al., 2001),*** but recent evidence support that smoking is a clear co-factor, Heavy smokers have a higher risk than non-smokers ***(Trichopoulos et al., 2001).***

In the general population, the incidence of HCC is increased among patients with HIV infection compared to controls, and HIV appears to be an additive co-factor, exacerbating the risk of HCC in patients with chronic viral hepatitis ***(Marcellin et al., 2008).***

Pathophysiology:

The pathophysiology of hepatocellular carcinoma has not been definitively elucidated and is clearly a multifactorial event. In 1981, after Beasley linked HBV infection to hepatocellular carcinoma development, the cause of hepatocellular carcinoma was thought to have been identified ***(Beasley et al., 1981).***

Inflammation, necrosis, fibrosis, and ongoing regeneration characterize the cirrhotic liver and contribute to hepatocellular carcinoma development. In patients with HBV, in whom hepatocellular carcinoma can develop in livers that are not frankly cirrhotic, underlying fibrosis is usually present, with the suggestion of regeneration. By contrast, in patients with HCV, hepatocellular carcinoma invariably presents, more or less, in the setting of cirrhosis. This difference may relate to the fact that HBV is a DNA virus that integrates in the host genome and produces HBV X protein that may play a key regulatory role in hepatocellular carcinoma development ***(Zhang et al., 2006)***.

Hepatocellular carcinoma: pathobiology:

Recent analysis has sought to elucidate the genetic pathways that are altered during hepatocarcinogenesis. Among the candidate genes involved, the *p53, PIKCA,* and *ß-catenin* genes appear to be the most frequently mutated in patients with hepatocellular carcinoma. Additional investigations are needed to identify the signal pathways that are disrupted, leading to uncontrolled division in hepatocellular carcinoma. Two pathways involved in cellular differentiation (ie, *Wnt-ß-catenin*, *Hedgehog*) appear to be frequently altered in hepatocellular carcinoma. Up-regulated WNT signaling appears to be associated with preneoplastic adenomas with a higher rate of malignant transformation ***(McKillop et al., 2006).***

Additionally, studies of inactivated mutations of the chromatin remodeling gene *ARID2* in 4 major subtypes of

hepatocellular carcinoma are being performed. A total of 18.2% of individuals with hepatitis C virus–associated hepatocellular carcinoma in the United States and Europe harbored *ARID2* inactivation mutations. These findings suggest that *ARID2* is a tumor suppressor gene commonly mutated in this tumor subtype ***(Li et al., 2011).***

While various nodules are frequently found in cirrhotic livers, including dysplastic and regenerative nodules, no clear progression from these lesions to hepatocellular carcinoma occurs. Prospective studies suggest that the presence of small-cell dysplastic nodules conveyed an increased risk of hepatocellular carcinoma, while large-cell dysplastic nodules were not associated with an increased risk of hepatocellular carcinoma. Evidence linking small-cell dysplastic nodules to hepatocellular carcinoma includes the presence of conserved proliferation markers and the presence of nodule-in-nodule on pathologic evaluation. This term describes the presence of a focus of hepatocellular carcinoma in a larger nodule of small dysplastic cells ***(Cameron et al., 1993).***

Recent work speculated that hepatocellular carcinoma develops from hepatic stem cells that proliferate in response to chronic regeneration caused by viral injury. The cells in small dysplastic nodules appear to carry markers consistent with progenitor or stem cells **(Alison, 2005).**

Diagnosis:

Nowadays, early HCC diagnosis is feasible in 30–60% of cases in developed countries and this enables the application of curative treatments. In fact, while tumors less than 2 cm in diameter represented <5% of the cases in the early nineties in Europe, currently they represent up to 30% of cases in Japan. This trend is expected to continue growing in parallel to the wider implementation of surveillance policies in developed countries ***(Llovet & Bruix, 2008).***

However, detection of these minute nodules of < 2 cm poses a diagnostic challenge as they are difficult to characterize by radiological or pathological examination ***(Bolondi et al., 2005).***

Proper definition of nodules as pre-neoplastic lesions or early HCC has critical implications. Dysplastic lesions should be followed by regular imaging studies, since at least one-third of them develop a malignant phenotype ***(Terasaki et al., 1998).***

Non-invasive diagnosis:

Accurate diagnosis of small liver nodules is of paramount importance. Until 2000, diagnosis was based on biopsy. This approach had some limitations related to feasibility due to location and risk of complications, such as bleeding or needle-track seeding ***(Stigliano et al., 2007).***

In addition, achieving accuracy in differentiating between highgrade dysplastic nodules and early HCCs was complex, since stromal invasion, the most relevant criteria, is

difficult to recognize even for an expert pathologist ***(Roskams & Kojiro, 2010).***

In 2001, a panel of experts on HCC convened in Barcelona *by European Association for the Study of the Liver* **(EASL)** reported for the first time non-invasive criteria for HCC based on a combination of imaging and laboratory findings. In principle, a unique dynamic radiological behavior (contrast up-take in the arterial phase by CT, MRI, angiography or US) represented the backbone of radiological diagnosis of early HCC. In cirrhotic patients with nodules >2 cm, coincidental findings by two imaging techniques were considered diagnostic, or alternatively, one imaging technique alongwith AFP levels above 400 ng/ml. In all other circumstances biopsy was mandatory ***(Bruix et al., 2001).***

In 2005, the EASL panel of experts and the American Association for the Study of Liver Diseases (AASLD) guidelines adopted a new HCC radiological hallmark, i.e. contrast uptake in the arterial phase and washout in the venous/late phase. Non-invasive diagnosis was established by one imaging technique in nodules above 2 cm showing the HCC radiological hallmark and two coincidental techniques with nodules of 1–2 cm in diameter (CT, MRI and US-contrast). AFP levels were dropped from the diagnostic scheme ***(Bruix & Sherman, 2005).***

Recent updated AASLD guidelines have proposed that one imaging technique (CT or MRI) showing the HCC

radiological hallmark suffices for diagnosing tumors of 1–2 cm in diameter ***(Bruix & Sherman, 2011).***

Finally, a recent prospective study, testing the accuracy of imaging techniques in nodules between 1 and 2 cm detected by ultrasound, showed false positive diagnosis – mostly due to high grade dysplastic nodules – above 10% with either 1 or 2 imaging techniques, with a specificity of 81% and 85%, respectively ***(Sersté et al., 2011).***

Hence, the non-invasive diagnosis of 1–2 cm lesions remains a challenging issue, with no unequivocal data in prospective validation studies. While the panel considers incorporating the 1 technique rule in order to have a consistent approach in the field, a more cautious application of this rule is recommended in suboptimal settings, where the technology at disposal or the local skills are not at the high-end level. In these circumstances, we recommended to use two coincidental techniques, since the negative consequences of high rates of false-positive diagnosis off set the benefit ***(Rimola et al., 2009)***.

Additional prospective studies to confirm the accuracy of this approach are recommended in order to support a more strong recommendation at the 1A level. Regarding which imaging techniques should be used, it has to be pointed out the fact that the HCC radiological hallmark is based on the tumor vascular dynamic performance. This limits the usage of US-contrast – since US microbubbles are confined to the intravascular space – as opposed to iodinated contrast-CT or

gadolinium based MR imaging, in which standard contrast agents are rapidly cleared from the blood pool into the extracellular space. A recent study showed that lesions other than HCC, i.e. cholangiocarcinoma, displayed homogeneous contrast uptake at US-contrast followed by washout, i.e. the vascularpattern assumed to represent the hallmark of HCC ***(Rimola et al., 2009).***

Thus, latest generation CT and/or MRI following reported protocols are recommended for non-invasive diagnosis of *HCC* ***(Lencioni et al., 2005).***

On the other hand, recent advances in the use of perfusion CT or MRI with liver-specific contrast agents have not so far provided solid data to support their use as alternate criteria. It is important to point out that the HCC radiological hallmark only occurs in a small proportion of patients with tiny tumors (1–2 cm) ***(Bolondi et al., 2005),*** and thus biopsy or tissue biomarkers will be required in most instances. Delaying diagnosis beyond 2 cm leads to increased levels of treatment failure or recurrence, since it is known that satellites and microscopic vascular invasion rise exponentially beyond this size cut-off ***(Roskam, 2011).***