

# Neutrophil Lymphocyte Ratio in Prediction of Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C

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## 1. Introduction

In Egypt, hepatocellular carcinoma (HCC) is a national health problem. It is now the first cause of cancer related mortality (1). This is mainly attributed to the heavy burden of chronic hepatitis C (CHC) virus infection (14.7%), which leads to liver cirrhosis in approximately 20% of patients within 20 years of infection. Among cirrhotic patients, 1-4% will develop HCC every year (2).

It is well known that the diagnosis of HCC at early stages allows the application of curative therapies like surgery and thermal ablation. This can be reached through the application of the surveillance program to high risk populations.

In recent years, accumulating evidence shows that increased systemic inflammation is associated with poor cancer-specific survival in a variety of cancers (3-4). These studies revealed that the host's inflammatory response to cancer and/or the systemic effects exerted by the cancer cells leads to upregulation of the inflammatory process (5-6). The presence of a systemic inflammatory response can be detected by both the elevation of the C-reactive protein (CRP) level (7) and neutrophil-lymphocyte ratio (NLR) (8). Neutrophils and other cells such as macrophages have been reported to secrete tumor growth promoting factors, including vascular endothelial growth factor (9-10), hepatocyte growth factor (11), IL-6 (12), IL-8 (13), matrix metalloproteinases (14) and elastases (15), and thus likely contribute to a stimulating tumor microenvironment. Neutrophilia as an inflammatory response inhibits the immune system by suppressing the cytolytic activity of immune cells such as lymphocytes, activated T cells, and natural killer cells (16-17). Thus, NLR reflects an immune microenvironment that favors tumor vascular invasion and suppresses the host immune surveillance.

## 2. Aim of the Work

The aim of this work is to examine the utility of neutrophil lymphocyte ratio in predicting the occurrence of HCC in chronic HCV Egyptian patients.

## 3. Patients and Method

This study was conducted in Specialized Medical Hospital, Mansoura University in the period from January 2014 to December 2015. It included 111 adult patients (100 males, 11 females) with CHC and untreated HCC (group I) and 222 adult patients (128 males, 94 females) with CHC without HCC (group II). CHC was diagnosed by ELISA for HCV antibody and confirmed by quantitative serum HCV RNA. HCC diagnosis was based on EASL criteria (18) i.e. focal hepatic lesion characterized by arterial phase enhancement and washout in portal and delayed phases, obtained by contrast enhanced abdominal CT and or MRI. NLR is derived from absolute neutrophil and absolute lymphocyte counts of the full blood count, provided that there was no ongoing bacterial infection at the time of blood sampling. Routine work up was done for all patients including liver and renal biochemical tests. We computed receiver operating characteristic (ROC) curve for NLR concerning HCC occurrence. We also calculated stratum specific likelihood ratios (SSLR) of NLR in relation to HCC. SSLR was calculated as the proportion of diseased subjects with a test result in a given range (group I) divided by the proportion of non-diseased subjects (group II) with a test result in the same range. We used the percentiles method for calculation of the SSLR as follows (19):

Step 1. Establish the strata and tabulate the stratum specific test results.

Step 2. Compute proportion of patients with the disease with that results.

Step 3. Compute proportion of patients without the disease with that results.

Step 4. Divide the fractions with the disease by the fractions without the disease.

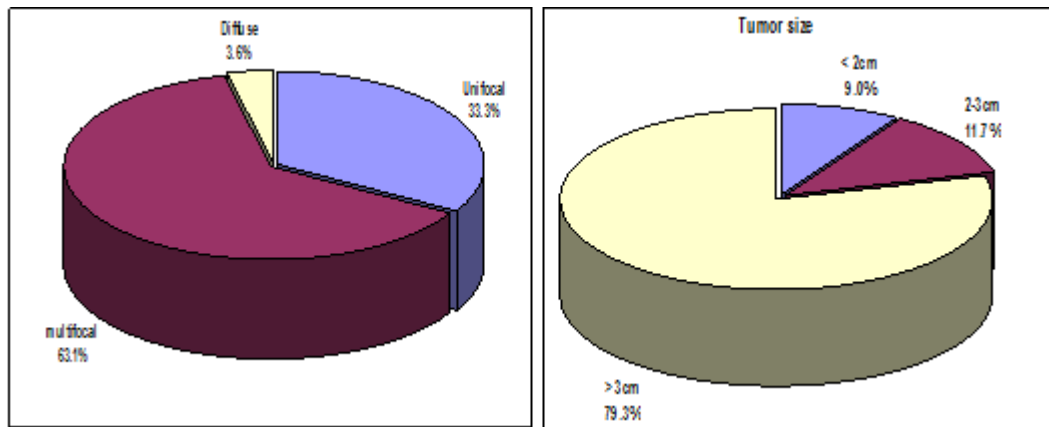
Step 5. Calculate confidence intervals.

## 4. Results

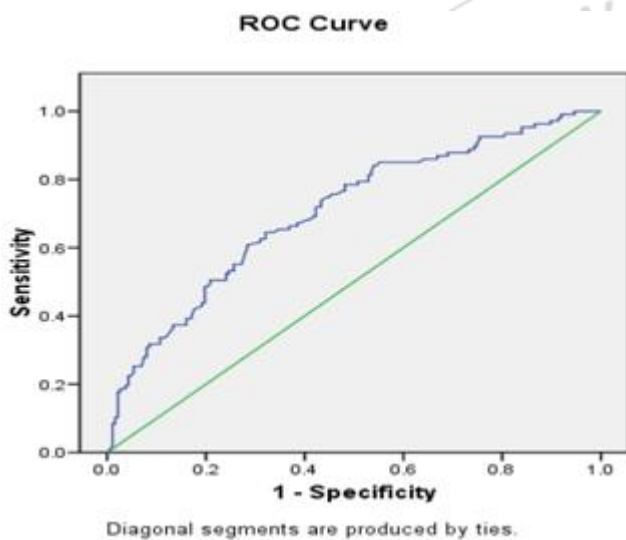
Baseline tumor and patients characteristics are shown in figure (1) and table (1). NLR was significantly higher in group I than group II. It was  $2.56 \pm 1.31$  in group I versus  $1.75 \pm 0.91$  in group II ( $P \leq 0.00$ ). The cut off value of NLR above which there was a high risk of HCC occurrence was  $\geq 2.015$ . Area under the curve (AUC) was 70.5%, sensitivity was 60.7%, specificity was 71.7%, positive predictive value of 55.1%.

negative predictive value of 76.1%, accuracy of 67.7% and positive likelihood ratio of 2.14 (figure2). The SSLR for HCC

presence by NLR was 0.54 if NLR was < 2, 1.74 if NLR was 2 to 4 and 7.14 if NLR was >4.



**Figure 1: Baseline tumor characteristics**



**Figure 2:** The cut off value of NLR above which there was a high risk of HCC development was  $\geq 2.015$ . AUC was 70.5%, sensitivity was 60.7%, specificity was 71.7%, PPV of 55.1%, NPV of 76.1%, accuracy of 67.7% and positive LR of 2.14.

( $\times 103/\mu l$ )	Non-HCC	160.76	69.26	.000
INR	HCC	1.31	.28	.000
	Non-HCC	1.11	.16	
Total bilirubin (up to 1mg/dl)	HCC	1.68	1.07	.000
	Non-HCC	1.01	.57	
Creatinine (up to 1.2 mg/dl)	HCC	.93	.28	.017
	Non-HCC	.87	.20	

$p > 0.05$  (insignificant);  $p < 0.05$  (significant);  $p < 0.01$  (Highly significant)

## 5. Discussion

HCC is a major health problem in Egypt and represents the first cause of cancer related mortality (1). Chronic hepatitis C is the major risk factor of HCC in Egypt. Application of surveillance program to high risk populations is very important to diagnose HCC at early stages. The early diagnosis allows the application of curative therapies and improves patient outcome. The definition of population at highest risk, the surveillance method and frequency of its application is controversial. In EASL clinical practice guidelines, only abdominal ultrasonography (US) is recommended every six months in chronic HCV patients with liver fibrosis stage equal to or more than F3. US is applied every three months if a nodule smaller than one centimeter (cm) is encountered. Contrast enhanced abdominal CT is recommended every three to four months in previously ablated HCC patients. It is also used if abdominal ultrasonography is unreliable as in obese patients (18). In the Japanese clinical practice guidelines, they apply abdominal US and three tumor markers (AFP, PIVKAI, AFP L3) every three months with contrast enhanced abdominal CT or MRI examinations every six to twelve months (as an optional surveillance method) in extremely high risk chronic HCV (cirrhotic) patients. In high risk (chronic HCV) patients, abdominal US plus the three tumor markers every six months are recommended (20).

**Table 1: Baseline Patients Characteristics**

	Group	Mean	Std. Deviation	P value.
Age	HCC	59.34	7.61	.000
	Non-HCC	48.18	8.39	
NLR	HCC	2.56	1.31	.000
	Non-HCC	1.75	.91	
WBCs ( $\times 103$ cells mm <sup>3</sup> )	HCC	5870.37	3480.58	.887
	Non-HCC	5823.92	2232.09	
Child score	HCC	7.36	2.06	.000
	Non-HCC	5.48	1.07	
ALT (0-41 U/L)	HCC	62.00	59.27	.871
	Non-HCC	62.90	38.64	
AST (0-37 U/L)	HCC	88.48	83.07	.007
	Non-HCC	64.99	41.06	
Albumin (3.5-5 g/dl)	HCC	3.23	.62	.000
	Non-HCC	3.94	.57	
Platelet	HCC	123.83	67.45	

In the United States, only 12% of new HCV-related HCC cases are diagnosed through surveillance (21) and less than 20% of patients with cirrhosis who develop HCC have undergone regular surveillance (22). For this reason, it will be crucial to identify those who would benefit most from continued surveillance. The addition of prediction method to surveillance programs may improve their cost-effectiveness by focusing on the extremely high risk groups. Attempts to define patients who need a much closer follow up have been done. In Egypt, Yosry et al concluded that chronic HCV patients expressing fibroscan score  $>25$  k Pa are in need for meticulous follow up by imaging examinations (23). Ethoxibenzyl-magnetic resonance imaging (EOB-MRI) was used to calculate the liver-intervertebral disc ratio (LI) as:  $(\text{post-liver intensity}/\text{post-intervertebral disc intensity})/(\text{pre-liver intensity}/\text{pre-intervertebral disc intensity})$ . Nojiri et al concluded that  $LI < 1.46$  was an independent factor that is associated with the risk of HCC occurrence in chronic hepatitis C patients and that LI may be a substitute for liver biopsy when evaluating this risk and its combined use with Fib-4 is a better predictive method of HCC progression (24).

High NLR significantly predicted poor overall survival (OS) of HCC patients irrespective of the treatment applied. Furthermore, high NLR also significantly correlated with shortened disease free survival (DFS) of patient treated by liver transplantation or surgical resection. So, high NLR is closely associated with more aggressive phenotype of HCC with lower OS and DFS (25).

As regards the epidemiological features of the study patients, males represented 90.1% and females represented 9.9% in group I with strong male predominance, while in group II males represented 57.7% and females represented 42.3%. The mean age of patients in HCC group (group I) was  $59.34 \pm 7.61$  years while it was  $48.18 \pm 8.39$  in group II with highly significant older age in HCC group ( $P \leq 0.000$ ).

As regards the hepatic condition as evaluated by Child-Pugh score, Child class A represented 43.2%, Child class B represented 42.3% while Child class C represented 14.4% in group I. In group II, Child class A represented 89.5%, Child class B represented 8.6% while Child class C represented 1.8%. As regards the tumor burden in group I, the tumor size was smaller than 2cm in 9%, 2-3cm in 11.7% and larger than 3cm in 79.3%. The tumor was unifocal in 33.3%, multifocal in 63.1% and diffuse in 3.6% (figure 1). Mean tumor size was  $5.56 \pm 2.1$  cm. The smallest tumor diagnosed non-invasively was 1.3 cm. The distribution of Seventh edition TNM tumor stage (26) in group I was as follows: stage I represented 19.3%, stage II represented 25.5%, stage IIIa represented 19.3%, stage IIIb represented 18.3%, stage IIIc represented 1%, stage IVa represented 8% and stage IVb represented 7%. These results were obtained after adherence to the surveillance programme as recommended by EASL guidelines and indicate the need for more meticulous follow up in some patients.

NLR was significantly higher in group I than group 2. It was  $2.56 \pm 1.31$  in group I versus  $1.75 \pm 0.91$  in group II ( $P \leq 0.000$ ). Using ROC curve, the cut off value of NLR above which there was a high risk of HCC occurrence was  $\geq 2.015$ . Area under the curve (AUC) was 70.5%, sensitivity was 60.7%, specificity was 71.7%, positive predictive value of 55.1%, negative predictive value of 76.1%, accuracy of 67.7% and positive likelihood ratio of 2.14. In clinical practice, however NLR will not be used as a diagnostic test of HCC, but used as an indicator of the risk of HCC. In this aspect, SSLR is better than a fixed cutoff value (23). The SSLR for HCC presence by NLR was 0.54 if  $NLR < 2$ , 1.74 if  $NLR$  was 2 to 4 and 7.14 if  $NLR > 4$ . This means that  $NLR > 4$  is 7.14 times more likely to occur in CHC patients with HCC than CHC patients without HCC. So, CHC patients expressing  $NLR > 4$  are in need for much closer follow up for earlier detection of HCC.

## 6. Conclusion

Neutrophil lymphocyte ratio (NLR) could be used as a predictor of HCC occurrence in chronic HCV Egyptian patients. Chronic HCV patients expressing  $NLR > 4$  are in need for much closer follow up.

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