# THE ASSESSMENT OF INTERLEUKIN-6, PROTEIN INDUCED BY VITAMIN K ANTAGONIST II, AND ALPHA-FETOPROTEIN AMONG HEPATOCELLULAR CARCINOMA PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

**LAI THING WEI**

**UNIVERSITI SAINS MALAYSIA**

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**by**

**LAI THING WEI**

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## TABLE OF CONTENTS

## LIST OF TABLES

**Page**

## LIST OF FIGURES

**Page**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AASLD | American Association for the Study of Liver Disease |
| AFP | Alpha-fetoprotein |
| AFP-L3 | lens culinary agglutinin-reactive fraction of Alpha-fetoprotein |
| ALP | Alkaline Phosphatase |
| ALT | Alanine Transaminase |
| APHE | Arterial Phase Hyperenhancement |
| AST | Aspartate Transaminase |
| AUC | Area Under Curve |
| BCLC | Barcelona Clinic Liver Cancer |
| bFGF | Basic Fibroblast Growth Factor |
| CLC | Cardiotrophin-like Cytokine |
| CLD | Chronic Liver Disease |
| CLSI | Clinical and Laboratory Standard Institute |
| CMIA | Chemiluminescent Microparticle Immunoassay |
| CNTF | Ciliary Neurotrophic Factor |
| COVID-19 | Coronavirus Disease 2019 |
| CPG | Clinical Practice Guideline |
| CT | Computed Tomography |
| CT-1 | Cardiotropin-1 |
| CYP2E1 | Cytochrome P450 2E1 |
| DCP | Des-γ-carboxy-prothrombin |
| DMARDs | Disease-Modifying Antirheumatic Drugs |
| DNA | Deoxyribonucleic Acid |
| EASL | European Association for the Study of the Liver |
| EORTC | European Organization for Research and Treatment of Cancer |
| ECM | Extracellular Matrix |
| ECOG | Eastern Cooperative Oncology Group |
| EGF | Epidermal Growth Factor |
| FGF | Fibroblast Growth Factors |
| GGT | Gamma-glutamyltransferase |
| Gla | Gamma-carboxyglutamic |

| | |
|---|---|
| Glu | Glutamine |
| Gp130 | Glycoprotein 130 |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| HIV | Human Immunodeficiency Virus |
| HCC | Hepatocellular Carcinoma |
| HAMA | Human Anti-mouse Antibodies |
| IL-6 | Interleukin 6 |
| IL-10 | Interleukin 10 |
| IL-11 | Interleukin 11 |
| IL-27 | Interleukin 27 |
| IL-31 | Interleukin 31 |
| JAKS | Janus Tyrosine Kinase proteins |
| JSH | Japan Society of Hepatology |
| LIF | Leukemia Inhibitory Factor |
| LoQ | Limit of Quantification |
| MMP | Matrix Metalloproteinase |
| mRECIST | modified Response Evaluation Criteria in Solid Tumors |
| MRI | Magnetic Resonance Imaging |
| NAFLD | Non-alcoholic Fatty Liver Disease |
| NASH | Non-alcoholic Steatohepatitis |
| NP | Neuropoietin |
| OSM | Oncostatin M |
| PEI | Percutaneous Ethanol Ioeinjection |
| PIVKA-II | Protein Induced by Vitamin K Absence or Antagonist-II |
| PPE | Personal Protective Equipment |
| RF | Rheumatoid Factor |
| RFA | Radiofrequency Ablation |
| RLU | Relative Light Units |
| RNA | Ribonucleic Acid |
| ROS | Reactive Oxygen Species |
| SST | Serum Separator Tube |
| STAT3 | Signal Transducer and Activator of Transcription 3 |
| T2DM | Type 2 Diabetes Mellitus |

| | |
|---|---|
| TACE | Transarterial Chemoembolization |
| TARE | Transarterial Radioembolization |
| TGF-α | Transforming Growth Factor-alpha |
| TNF-alpha | Tumour Necrosis Factor-alpha |
| UMMC | University Malaya Medical Centre |
| USM | Universiti Sains Malaysia |
| VEGF | Vascular Endothelial Growth Factor |
| WHO | World Health Organization |
| YLL | Years of Life Lost |

**PENILAIAN PARAS INTERLEUKIN-6, PROTEIN-INDUCED VITAMIN K ANTAGONIST II DAN ALPHA-FETOPROTEIN DALAM KALANGAN PESAKIT BARAH HATI DI HOSPITAL UNIVERSITI SAINS MALAYSIA**

## ABSTRAK

Karcinoma hepatosel (HCC) adalah kanser hati primer yang mempunyai prognosis yang buruk dan prevalens yang tinggi di kalangan rakyat Malaysia. Pada masa ini, ultrasonografi dan serum Alpha-fetoprotein (AFP) adalah satu-satunya ujian untuk pemeriksaan HCC di negara ini. Serum Interleukin-6 (IL-6) dan Protein yang Dicetuskan oleh Ketiadaan Vitamin K atau Antagonis-II (PIVKA-II) ialah dua biomarker serum berpotensi yang ditunjukkan meningkat pada pesakit HCC. Walau bagaimanapun, terdapat kajian yang tidak mencukupi mengenai mereka dalam populasi kita. Kajian ini bertujuan untuk menilai tahap serum IL-6 dan PIVKA-II di kalangan subjek HCC di Hospital Universiti Sains Malaysia (Hospital USM), sebelum dan selepas rawatan, serta membandingkan tahap mereka dengan serum AFP. Dua puluh enam subjek HCC (Kumpulan 1) dan tiga puluh empat subjek sihat (Kumpulan 2) telah diambil. Untuk Kumpulan 1, sampel darah telah dikumpulkan dan penilaian radiologi lesi HCC dilakukan pada lawatan pertama dan 6 minggu selepas rawatan . Bagi Kumpulan 2, sampel darah hanya diambil sekali sahaja. Kajian ini mendapati serum IL-6, PIVKA-II dan AFP mempunyai tahap median yang lebih tinggi dalam subjek HCC berbanding subjek sihat ($p<0.001$). IL-6 tidak berkorelasi dengan rawatan pasca tindak balas radiologi ($p=0.822$). Apabila membandingkan nilai sebelum dan selepas rawatan, paras IL-6 serum tidak berubah dengan ketara ($p=0.328$), manakala paras serum PIVKA-II adalah paling ketara berbeza ($p<0.001$) diikuti oleh paras AFP

serum (p=0.007). Dalam diagnosis HCC, PIVKA-II mempunyai sensitiviti dan kespesifikan yang lebih tinggi (Sn 92.30%, Sp 94.11%) diikuti oleh serum IL-6 (Sn 84.62%, Sp 70.59%) dan AFP (Sn 73.08%, Sp 97.06%). Tambahan pula, gabungan ketiga-tiga biomarker serum menunjukkan prestasi diagnostik terbaik dalam diagnosis HCC (AUC=0.999). Secara konklusif, serum IL-6 dan PIVKA-II mempunyai potensi kegunaan dalam diagnosis HCC serta pemantauan rawatan HCC, terutamanya apabila digunakan dalam kombinasi dengan serum AFP.

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## ABSTRACT

Hepatocellular carcinoma (HCC) is a primary liver cancer that has a poor prognosis and a high prevalence among the Malaysians. At present, ultrasonography and serum Alpha-fetoprotein (AFP) are the only tools for HCC screening in this country. Serum Interleukin-6 (IL-6) and Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II) are two potential serum biomarkers that were shown to be elevated in HCC patients. However, there had been insufficient research on them in our population. This study aimed to assess the serum IL-6 and PIVKA-II levels among HCC subjects in Hospital USM , before and after treatment, as well compare their levels to those of serum AFP. Twenty six HCC subjects (Group 1) and thirty four healthy subjects (Group 2) were recruited. For Group 1, blood samples were collected and radiological assessments of their HCC lesions were done at the first visit and 6-weeks after their local treatment. For Group 2, only blood samples were only collected once. This study found that serum IL-6, PIVKA-II and AFP had higher median levels in HCC subjects than healthy subjects ($p<0.001$). IL-6 was not significantly correlated with radiological response post treatment ($p=0.822$). When comparing pre- and post-treatment values, serum IL-6 levels did not change significantly ($p=0.328$), whereas serum PIVKA-II levels were most significantly different ($p<0.001$) followed by serum AFP levels ($p=0.007$). In HCC diagnosis, PIVKA-II had the higher sensitivity and specificity (Sn 92.30%, Sp 94.11%) followed by serum IL-6 (Sn 84.62%, Sp 70.59%)

and AFP (Sn 73.08%, Sp 97.06%). Furthermore, the combination of all three serum biomarkers demonstrated the best diagnostic performance in HCC diagnosis (AUC=0.999). Conclusively, serum IL-6 and PIVKA-II have potential uses in HCC diagnosis as well as HCC treatment monitoring, especially when used in combination with serum AFP.

# CHAPTER 1

# INTRODUCTION

## 1.1 Preview of The Study

In this introduction chapter, this research study will be summarised. Section 1.2 of this chapter covers the study's background, while section 1.3 will discuss the problem statements. Furthermore, section 1.4 explains the rationale and justification of the study, and section 1.5 clarifies the research questions. Section 1.7 explains about research hypotheses investigated. Section 1.8 focuses on the general and specific objectives related to the research problem. Section 1.9 explains about the study's scope.

## 1.2 Background of The Study

Hepatocellular carcinoma (HCC) is well known as an important primary malignant neoplasm, arising from hepatocytes. Throughout the world, HCC was ranked as the second leading cause of cancer mortality, accounting for almost 9.1% of all deaths in 2012 (Ferlay J. et al, 2014). East and Southeast Asia had the highest incidence of HCC (Alpert and Feller., 1978). In 2015, the Malaysian National Cancer Registry reported that liver cancer was the eighth most common cancer among Malaysians and the sixth most established cancer among males. The Chinese population had a significantly higher incidence rate of HCC than Malays and Indians (Choi et al., 2019).

HCC typically develops in the background of a cirrhotic liver and chronic liver disease, with the primary causes of HCC being hepatitis B (HBV), hepatitis C (HCV), and non-alcoholic fatty liver disease (NAFLD) (Bialecki and Di Bisceglie, 2005). Among these listed causes, HBV infection was the leading cause for HCC worldwide.

The majority of HBV-caused HCC stemmed from developing nations, comprising of 58.8% of the global HCC cases (Nordenstedt, White and El-Serag, 2010). Within the Malaysian population, the leading etiology of HCC was also HBV infection, with a 2006-2009 study demonstrating 57.6% HBV-caused HCC, while another 2011-2016 study found 48.8% of HCC subjects were HBV carriers (Goh et al., 2015; Raja M. et al., 2021). This was followed by idiopathic causes, high alcohol consumption and HCV infection. Unfortunately, the presentation of HCC is generally late, with manifestations of a jaundiced appearance, pruritus, right upper quadrant abdominal pain, portal-systemic anastomosis evidenced by variceal bleeding, and worst of all, hepatic encephalopathy. When patients presented during early HCC, the rate of 5-year overall survival can be as high as 50-70% thanks to the accessibility of various curative techniques, either surgical or ablative treatment (Behne and Copur, 2012). However, because of the typical late presentation of HCC and with approximately 60% of patients diagnosed at its advanced stage, the prognosis of HCC is very poor with some areas having a mortality-to-incidence ratio up to 0.95, meaning the incidence and mortality of HCC are almost similar (Ferlay J. et al, 2014; Ashtari, Mohamad Amin, Afsaneh and Mohamad Reza, 2015). This gruesome fact emphasis how imperative it is to identify HCC patients at an early curable stage, and this can be achieved with an effective surveillance programme.

At present, the latest guidelines from Europe, the United States and Asian regions unanimously agreed for ultrasound as the first-line preferred method of HCC surveillance in high-risk individuals (Kim, Kim, Tang and Lee, 2019). According to a 2009 meta-analysis aiming at evaluating the use of ultrasonography in HCC surveillance programme, ultrasound was found to be relatively accurate in diagnosing HCC at any stage of the disease, with a sensitivity of 94%, but this is juxtaposed by its

poor sensitivity of 63% in detecting early HCC (Singal et al., 2009). A more recent meta-analysis in 2018 found the overall sensitivity of ultrasound surveillance of HCC to be 84%, and for early-stage HCC at only 47% sensitivity (Tzartzeva et al., 2018). The sensitivity of ultrasonography is influenced by the quality of the ultrasound machine used, the ultrasound operator's experience in this imaging technique, body habitus of the patient, and the location of the lesion. When highly suspicious cases of HCC do not display the characteristics features of HCC, all current guidelines recommend liver biopsy for pathological diagnosis of HCC (Galle et al., 2018). The accuracy of diagnosing HCC via a liver biopsy was proportional to the size of the lesion and the number of biopsy attempts, where the more the attempts made, the higher the chance for useful biopsy tissue to be acquired. However, the risk of complications was increased at three or more attempts, with increased risk in older patients and patients with coagulopathy (Neuberger et al., 2020). Among the most concerning complications, the formed biopsy tract may cause HCC tumour seeding along the tract, which significantly increased the risk of HCC upstaging or even recurrence later after treatment (Perkins, 2007).

Serum AFP is traditionally the main tumour biomarker for HCC. Despite this, serum AFP had also been shown to be increased in various benign liver diseases (Liebman et al., 1984). Worse still, previous literature had already demonstrated that 35% of patients with small HCC lesions manifested low to normal serum AFP levels (Llovet, Fuster and Bruix, 2004). For example, one of the earliest studies comparing serum AFP levels in HCC patients and healthy subjects discovered that serum AFP had a sensitivity and specificity of 78.9% and 84.6% respectively, when the cut-off was 10 ng/mL (Marrero et al., 2018). With the realization that serum AFP only delivers a subpar HCC diagnostic performance, researchers had conducted copious studies to

uncover the next best HCC serum biomarker. Among which, two promising candidates, serum Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II) and Interleukin-6 (IL-6), had emerged to challenge serum AFP.

PIVKA-II was initially introduced as a tumour marker for HCC by Liebman et al in year 1984 (Park et al., 2012). It is an abnormal prothrombin with no coagulation activity because it lacks carboxylation of 10 glutamic acid residues in the N-terminus (Park and Park, 2013). PIVKA-II is produced by malignant hepatocytes and results from an acquired defect in posttranslational carboxylation of the prothrombin precursor. A recent study in South Korea suggest that PIVKA-II response may be useful in predicting clinical outcomes following transarterial chemoembolization (TACE) (Park et al, 2012). While Lee et al. discovered that serum AFP level was significantly correlated with radiological response in patients undergoing TACE (Lee et al, 2013). According to He et al., both AFP and PIVKA-II are variably expressed among HCC patients owing to the fact that different risk factors have different mechanisms of carcinogenesis (He et al, 2014). Despite the disease's high prevalence, limited research on PIVKA-II in South-East Asia regions has been conducted to date.

Next, serum IL-6 is a multifunctional cytokine that has roles in regulation of immune system, inflammation, and formation of new red blood cells (Nishimoto and Kishimoto, 2006). IL-6 promotes HCC development by promoting liver cells proliferation, transforming liver cells into HCC progenitor cells, and the progressing those progenitor cells to form HCC nodules and metastases (Loosen et al., 2018). A study done in South Korea in 2012 found out that HCC patients possessed higher serum IL-6 levels than healthy controls (Jang et al, 2012). Moreover, the tumour size, prognosis, and survival of HCC patients were also related to serum IL-6. More studies should be done to determine the potential role of IL-6 as a tumour marker for HCC.

In addition, a Germany study in 2018 serum IL-6 can be a tumour marker for those patients undergoing TACE and identify those patients that benefit most from TACE in term of treatment and survival rate (Loosen et al., 2018). Another study published in China found that serum IL-6 can be used to predict the efficacy of TACE, as well as tumour activity in AFP negative HCC patients after TACE (Wang et al, 2019).

HCC emerges from a dynamic inflammatory environment within diseased liver that predisposes to initiation of cancer, as shown in Figure 1.1 (Hernandez-Gea et al, 2013). In the environment of HCC as well other tumours, the normal cytokine profiles were described in the literature to be significantly altered. Despite this, there is insufficient evidence that these altered cytokine profiles can be used as reliable biomarkers of tumours. Thus, it is imperative that more research is done to validate the role of these two serum biomarkers, PIVKA-II and IL-6, in the management of HCC. As described above, better performing HCC surveillance tools are urgently needed for early diagnosis of this disease, and for detecting tumour recurrence early, so as to lessen the disease burden wrought by HCC.

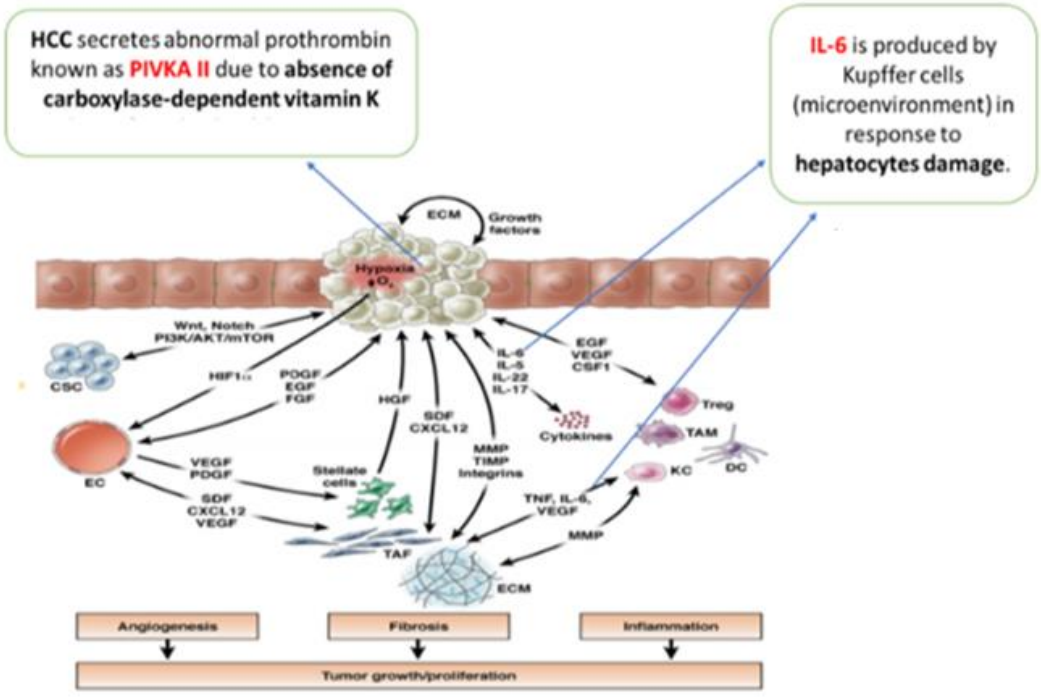


Figure 1.1 Role of the microenvironment in the pathogenesis and treatment of Hepatocellular Carcinoma (Hernandez-Gea et al, 2013)

## 1.3 Problem Statement and Rationale of the Study

This study is carried out because the current literature have shown that different geographical areas, economic status, and ethnic groups have different risk factors for HCC. Therefore, studies done on the local population in Malaysia will likely demonstrate different results compared to previous studies done in East Asian countries and the West. This was the first study in Malaysia that aims to determine the role of IL-6 and PIVKA-II as complementary tools to imaging assessment of HCC lesions and liver biopsy to assist in the early diagnosis, prognosis, treatment response monitoring, prognosticating HCC patients, as well as their use as surveillance tools in high-risk group patients and help detect early recurrence.

Serum biomarkers such as IL-6 and PIVKA-II can be additional tumour markers for HCC as shown in Figure 1.2. Most of the studies on PIVKA-II have been carried out among East Asia and Western populations. To date, most studies comparing the diagnostic performance of PIVKA-II and AFP had concluded that PIVKA-II surpasses AFP in discriminating HCC lesions from other benign liver diseases (Singal et al, 2009; Sharma et al, 2010; Torre et al, 2015; Tateishi et al, 2008). On the other hand, although there were earlier studies assessing the relationship of serum IL-6 levels with the presence of HCC, few have rigorously examined the possible role of this cytokine in HCC progression and overall survival (Haque et al., 2015). Furthermore, these same studies were done in China, Korea, and Bangladesh, but none in a Southeast Asian country.

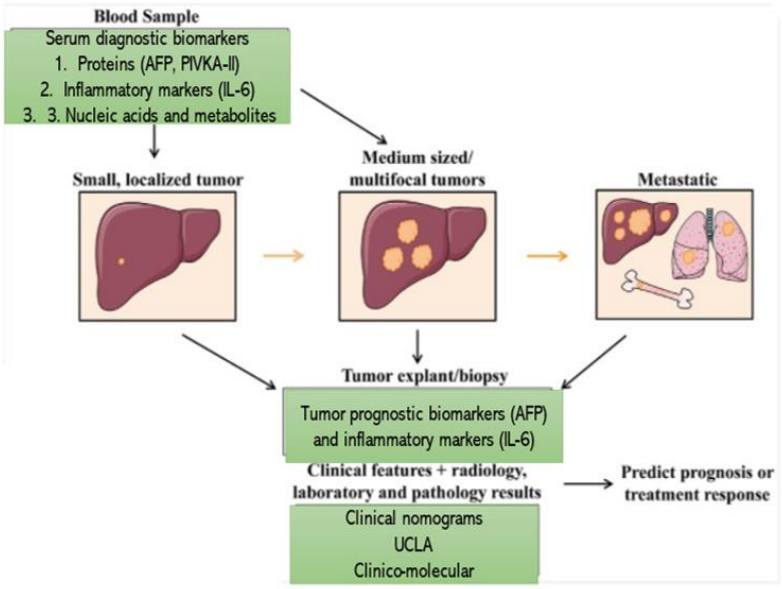


Figure 1.2 Serum biomarkers (IL-6 and PIVKA-II) as potential diagnostic biomarkers in term of HCC diagnosis (Modified from Faltermeier, C., Busuttil, R. W., & Zarrinpar, A., 2015)

## 1.4 Research Questions

1. What is the comparison measurement in the serum level of IL-6, PIVKA-II and AFP among healthy subjects and HCC patients at the baseline?

2. Is there any association between IL-6 response with radiological response pre and post local treatment among HCC subjects?

3. Are there any significant differences in the mean differences of IL-6, AFP & PIVKA II levels pre- and post-treatment of HCC subjects?

## 1.5 Research Hypothesis

1. HCC patients have higher serum levels of IL-6, PIVKA-II and AFP levels compared to healthy subjects.

2. There is an association of IL-6 response with radiological response pre and post local treatment among HCC subjects.

3. There is a significant difference in the mean differences of IL-6, AFP & PIVKA II levels pre- and post-treatment of HCC subjects.

## 1.6 Research Objectives

### 1.6.1 General Objective

To assess and compare IL-6, PIVKA-II levels, pre- and post-local treatment and its association with radiological response among hepatocellular carcinoma patients at Hospital Universiti Sains Malaysia (Hospital USM).

### 1.6.2 Specific Objectives

1. To determine and compare the concentration of IL-6, PIVKA II and AFP levels among hepatocellular carcinoma patients and healthy subjects.

2. To determine the association between IL-6 response with radiological response pre- and post-local treatment among HCC patients.

3. To compare the mean differences of IL-6, PIVKA II and AFP levels in relation to pre & post local treatment among HCC patients.

4. To determine the diagnostic performance of IL-6, PIVKA-II, and AFP as single serum biomarkers or in combination among HCC patients.

## 1.7 Scope of The Study

This study was conducted at the Gastroenterology Medical Specialist Clinic Klinik Perubatan Pakar (KPP) and Radiology Clinic at Hospital USM. All HCC patients and healthy subjects were recruited according to the inclusion and exclusion criteria. During their first visit, subjects underwent a comprehensive interview regarding their background medical history, as well as a baseline blood sample for serum biomarkers such as IL-6, PIVKA-II and AFP, and after 6 weeks of treatment as determined by their gastroenterologist, the patients were followed up for post treatment blood sample collection.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Hepatocellular Carcinoma (HCC)

### 2.1.1 Epidemiology of HCC

HCC was known as one of the deadliest carcinomas in the world in this 21st century. The prevalence of HCC varies globally depending on the prevalence of risk factors for its development. The highest incidence of HCC was seen in Asian and African regions, more specifically Southeast Asia, East Asia, Western Africa, and Northern Africa (more than 20 cases per 100 000 population) due to the higher incidence of HBV and HCV infection in those regions. As a comparison to Asian and African regions, developed countries in Southern Europe, Western Europe, and North America had slightly lower incidences of HCC (10-15 cases per 100 000 population) while Northern, Eastern, and Central Europe, as well as South-Central and Western Asia, had the lowest incidence of HCC (less than 10 cases per 100 000 population) (Ferlay J. et al, 2014). According to the World Health Organization's (WHO) latest report on Research on Cancer 2020, HCC was categorized as the sixth most diagnosed cancer and third principal cause of cancer related death with 905,677 new cases of HCC and 830,180 HCC-related deaths in 2020, accounting for 4.7% of all cancers worldwide (WHO, 2014; WHO, 2020). HCC often develops on top of liver cirrhosis, and the age of presentation was usually after 40 to 50 years old. According to a study by El-Serag HB on the epidemiology of viral hepatitis and HCC, the age of HCC diagnosis varies by geographical regions, for example in China the age of HCC diagnosis was mostly at 55 to 59 years old, while in Europe & North America it was more commonly diagnosed at 63 to 65 years old (El-Serag H, 2012). Overall, the incidence of HCC increased after

the age of 45 for men, and 60 for women worldwide. Furthermore, men were diagnosed with HCC at higher rate than female with an incidence rate 2 to 4 times higher among men (Singal AG and El-Serag HB, 2015). The exact mechanism underlying this fact is still unknown. However, differences sex hormones and immune response to infection and inflammation may contribute to this gender disparity (McGlynn et al, 2015).

In Malaysia, HCC is also recognised as one of the serious cancers because it is always diagnosed late and has a poor prognosis (Azizah AM, 2019). According to the National Cancer Registry 2012-2016 report, HCC was the eighth most diagnosed cancer among Malaysians and the sixth most diagnosed cancer among men population in Malaysia (Azizah AM, 2019). It was one of the prime causes of premature death in Malaysia, apart from cardiovascular diseases. Malaysia, as we all know, is a country distinguished by its multi-ethnicity and diverse cultures. The Malay ethnic population accounts for 69.4% of the Malaysian population, the Chinese ethnic population accounts for 23.2% and the Indian ethnicity constituted for 6.7% (Department of Statistics of Malaysia, 2020). A study carried out by Goh et al at University Malaya Medical Centre (UMMC) from 2006 to 2009 on 384 HCC patients revealed that the Chinese population had the highest incidence of HCC (68.7%) followed by Malay population (20.4%) and the Indian population (10.9%) (Goh KL et al, 2015). The study stated that the average age of HCC diagnosis among Malaysians is 62.5 years old with the male to female ratio at 3.4 to 1. HBV infection (59.2%), cryptogenic causes (16.4%) and HCV (10.6%) were three of the most common causal factors of HCC among Malaysians. Due to lack of national health programs in the past, a high prevalence of HBV infection had led to an increased numbers of HCC cases in recent years as HBV infection takes years to progress into liver cirrhosis and subsequently HCC (Ng K et al, 2004).

It has been predicted that there will be a shift in the context of HCC's risk factors in Malaysia in the future. NAFLD and Non-alcoholic steatohepatitis (NASH) will eventually supplant HBV infection as the leading cause of HCC (Mohamed et al, 2018). With the implementation of national HBV vaccination programmes for children and health-care workers, the introduction of more affordable drugs and advancement in term of management, HBV infection is on the decline as is HCC secondary to HBV infection. However, due to the increased prevalence of NASH and NAFLD in our country, the incidence of HCC among Malaysians will remain high (22.7%), with Indians standing at the highest percentage at 68.2% and Malays at 64.1% (Goh KL et al, 2015). As we know, Malaysia was ranked the most obese country in Asia (Statistica Research Department, 2021) with increasing trends of metabolic syndrome related illnesses, such as obesity and Type 2 diabetes mellitus. Metabolic syndrome is the primary cause of many chronic diseases such as NAFLD, acute coronary syndrome and cerebrovascular accident. Hence, metabolic syndrome remains one of the most important health issues for us in the management of HCC.

HCC continues to be a significant burden. Since 1990, the annual years of life lost (YLLs) due to HCC have increased by 31.5% (Mohamed et al, 2018). Due to lack of effective surveillance guidelines and late presentation leading to advance stage diagnosis of HCC, the prognosis of these patients was poor even with the increment of cost and time of treatment.

### 2.1.2 Pathogenesis of Hepatocellular Carcinoma

Hepatocellular carcinoma develops in an injured liver that created a complex inflammatory environment, predisposing an individual to cancer formation and progression. Multiple aetiologies had been identified as culprits that can chronically

induce liver injury leading to carcinogenesis, including HBV and HCV infection, binge-alcohol drinking, NAFLD, aflatoxin exposure and others (Dhanasekaran, Bandoh and Roberts, 2016).

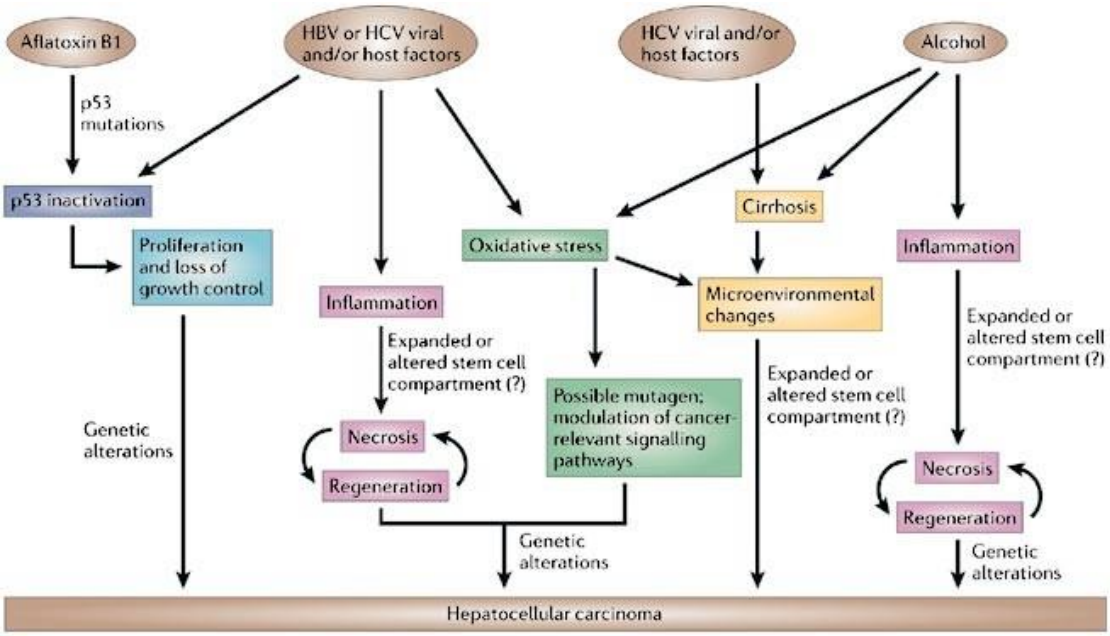


Figure 2.1 Proposed mechanisms of hepatocarcinogenesis for each risk factor (adapted from Farazi and DePinho, 2006)

Persistent affront of liver cells by these risk factors will induce reactive oxygen species (ROS) production, inducing stress to the endoplasmic reticulum in the hepatocytes, and eventually lead to cellular DNA damage and liver cells necrosis. Subsequently, there will be activation of hepatic stellate cells and macrophages in the microenvironment of the liver as a response to liver injury. The activation of these cells will release growth factors and components of extracellular matrix (ECM) that promote the migration of endothelial cells toward the injured site, induce new vessels formation and subsequently fibrosis of the liver (Hernandez–Gea V et al, 2013).

Repeated cycles of liver injury and fibrosis formation will lead to liver parenchyma and vascular architecture damage, inflammation and oxidative DNA damage, as well as the accumulation of mutations and epigenetic anomalies that will predispose to neoplastic transformation of hepatocytes. Moreover, liver injury and

fibrosis are coupled with abundant cytokines such as IL-6, Tumour Necrosis Factor-alpha (TNF-alpha), Interleukin-10 (IL-10) and others that make the condition worse as all these were associated with the increased risk of hepatocellular carcinoma formation (Hernandez–Gea V et al, 2013).

### 2.1.3 Risk Factors Predisposing to Hepatocellular Carcinoma

#### 2.1.3(a) Hepatitis B Virus (HBV) Infection

According to the World Health Organization (WHO), it was estimated that 3.2% of world population has chronic HBV infection. HBV infection is more prevalent in developing and under-developed countries as compared to developed countries, with only <1% of the population in North America and Western Europe infected with HBV (WHO, 2016). According to a systemic review done by Schweitzer A et al there were approximately 95 million HBV-infected individuals in the Western Pacific countries, 75 million in Africa, and only 7 million in the Americas (Schweitzer et al, 2013). The age of diagnosis of HCC among individuals in HBV endemic region was also noted to be ten years earlier than those in non-endemic regions. This fact could be explained through the route of transmission of HBV infection that varies with geographical regions. The majority of those from endemic areas acquired HBV infection at birth via vertical transmission whereas those from non-endemic areas usually acquired HBV infection during adulthood via sexual transmission or accidental exposure to HBV infected blood products (Mittal S and El-Serag H, 2013).

About 90% of those individuals infected by HBV will have their diseases resolve spontaneously, with only 10% developing chronic HBV infection. One-quarter of those 10%, will be found to have HCC. 70% of HBV-related HCC had underlying liver cirrhosis. The percentage of liver cells that undergo neoplastic transformation is

higher among those with HBV infections complicated by liver cirrhosis, but it can also occur in HBV infections without liver cirrhosis, especially in those with a high HBV viral load (Ganem D and Prince AM, 2004).

HBV is a double-stranded DNA virus that possesses the ability to integrate its own genome into a host cell's genome and produces viral proteins that promotes carcinogenesis. This special characteristic enabled the HBV to activate oncogenes and supress tumour suppressor genes, resulting in DNA repair mechanism mismatch with mutation occurrence. In addition, chronic inflammatory damage and hepatocyte regeneration cycles secondary to HBV infection also contribute toward liver carcinogenesis. All these events will lead to malignant transformation of liver cells (Hernandez–Gea et al, 2013). The risk of HBV infection-related HCC is increased further if the patient is male, elderly, having high viral loads and longer duration of infection, has a family history of HCC, infected with HBV genotype C, co-infection with HCV or hepatitis D or chronic alcoholic and tobacco usage (El-Serag H, 2011).

Based on the literature as discussed above, HBV infection has a significant impact on Malaysia's healthcare system. However, its prevalence has decreased over the years with the introduction of a national HBV vaccination programme for all Malaysian during childhood and for healthcare workers (Ng, K. et al, 2004). Routine screening of high-risk individuals for HBV infection and treatment with advanced drugs, nucleoside/nucleotide analogues such as Lamivudine or Entecavir before the development of liver cirrhosis had significantly reduce the risk HCC formation among HBV-infected person. The HCC cases we have now were caused by HBV infection during its endemic period. We believed that the number of HBV-related HCC cases will decrease in the future, with the advancement of new management protocols and techniques.

### 2.1.3(b) Hepatitis C Virus (HCV) infection

HBV infection is the second most common risk factor (30%) for HCC development (WHO, 2016). About 1.9% (140 million) of the world's population were infected with HCV, with Japan having the highest percentage (80-90%), followed by Europe (44-75%) and America (30-50%) (WHO, 2016). According to Donato F et al in 2002, HCV-infected individuals are 17-fold more likely than healthy individuals to develop HCC and the risk of HCC development increases at the rate of 5% per year (Donato F et al, 2002). The risk increases further if the patient is older than 65 years old, infected with HCV genotype Ib, co-infected with HBV or Human Immunodeficiency Virus (HIV), consume alcohol or cigarettes and has diabetes mellitus as comorbid condition (Donato F et al, 2002; Lok AS et al, 2009).

As in HBV infection, HCC often occurs decades after an individual has been infected with HCV. However, HCV related HCC must always occur on the basis of liver cirrhosis, which does not always occur in those with HBV infection (Asahina et al, 2010). The current wave of HCV related HCC was mostly caused by transfusion of unscreened blood products and needle sharing among drug abusers which occurred decades ago (Alter, 2007).

Unlike HBV, HCV is a single-stranded RNA virus that cannot integrate its genome into host cells. Hence, it induces hepatocarcinogenesis differently compared to HBV. HCV infection triggers our body's immune response to fight against these invaders and subsequently induces inflammation and fibrosis that predisposes us to hepatocarcinogenesis. Apart from that, it produces HCV viral proteins that can potentiates DNA mutation and malignancy (Hernandez–Gea et al, 2013).

There is currently no effective vaccination against this bloodborne virus. Thus, the only effective way to avoid HCC was to take preventive measures to avoid HCV

infection. As primary prevention, healthcare personnel must be well-trained, in terms of handling HCV-infected patients, sharps, and clinical waste, and equipped themselves with the appropriate personal protective equipment (PPE) prior to any procedures that pose a risk for HCV infection. Furthermore, all blood products must be screened for HBV and HCV prior to administration. Also, secondary prevention such as early treatment with antiviral therapy and regular screening for early diagnosis of HCC must be practiced widely before it is too late. As we know, the options of curative treatment of HCC reduced drastically with advancement of the disease.

### 2.1.3(c) Non-alcoholic Fatty Liver Disease (NAFLD)

Metabolic syndromes such as Type 2 diabetes mellitus (T2DM) and obesity had emerged as a major health issue globally over the past ten years. Sedentary lifestyles with the emerging of fast-food outlets had increased the prevalence of metabolic syndrome among the global population dramatically, especially those in well-developed western countries. According to global obesity statistics, Malaysia is the most obese Asian country, with the prevalence of obesity increasing from 43.1% in 2006 to 60% in 2014, with an increment of 16.9% in just eight years (Lim K. G., 2016). In addition, obesity has been linked to diabetes mellitus, increasing the risk of insulin resistance in obese individuals. As a result, the prevalence of diabetes mellitus among Malaysian is on the rise, increase from 8.3% in 1996 to 18.3% in 2019 drastically (H. Ismail et al, 2019).

In conjunction with the increment of metabolic syndrome, NAFLD has become the main cause of chronic liver disease (CLD) among the worldwide general adult population, with an estimated global prevalence of 24% (Younossi et al., 2016). On the other hand, the prevalence of NAFLD among Malaysians is also increased over the past

few decades, with an estimated 22.7% of Malaysians having NAFLD (Goh, Ho and Goh, 2012). NAFLD is a spectrum of liver diseases, manifesting as simple cases of steatosis, to non-alcoholic steatohepatitis that will eventually lead to liver fibrosis and cirrhosis. NASH-associated liver cirrhosis may increase the chance of HCC development. In a population-based database by United States, it was clearly stated that NAFLD or NASH had been known as the most common causal factor of HCC, in which it stands for 59% of HCC cases (Sanyal, Poklepovic, Moyneur and Barghout, 2010).

The pathogenesis of NASH-induced HCC may be linked to the oxidative stress, high cell turnover and cellular hyperplasia that occurred in those injured hepatocytes. The ongoing inflammation of liver cells increases the release of tumour prone inflammatory cytokines such as TNF-alpha and IL-6, which then activate the oncogenic pathway via STAT3 (Kutlu, Kaleli and Ozer, 2018).

A study done by Chen Hp et al in 2012 discovered the potential clinical usage of metformin as a drug to reduce the incidence of HCC secondary to NAFLD. However, there is still no proof of any treatments or strategies that can cure NAFLD and reduce risk of HCC formation (Chen H et al, 2012).

### 2.1.3(d) Alcohol, Tobacco Usage and Others

Both chronic alcohol consumption and tobacco usage are well-known risk factors for the development of HCC apart from those mentioned above. According to a nested case-control study published in the Journal of National Cancer Institute in 2011, alcoholic liver disease accounted for 10% of all HCC cases, with 50% of HCC cases also being chronic smoker (Trichopoulos et al, 2011). The pathogenesis of HCC secondary to alcoholic liver disease can be explained by the metabolism of ethanol by hepatocytes. Metabolism of ethanol involves the induction CYP2E1 enzymes in the

liver, which subsequently generate reactive oxygen species (ROS) that will cause oxidative stress to the liver (Seitz and Stickel, 2006). In addition, the product of ethanol, acetaldehyde, is also known to be a carcinogen that will worsen the condition. All these events will eventually lead to malfunction of DNA repair mechanism, more DNA damage and then induce carcinogenic sequence direct or indirectly (Seitz and Wang, 2013). Cigarette's smoking will also worsen the condition by the production of pro-carcinogens such as nitrosamines. Heavy drinkers (more or equal to 3 drinks per day, equivalent to >37.5g of alcohol) possesses higher risk of getting HCC as compared to moderate or non-alcohol drinkers (Turati et al., 2014; Petrick et al., 2018). The summary of causes of non-cirrhotic hepatocellular carcinoma is shown in Figure 2.2.

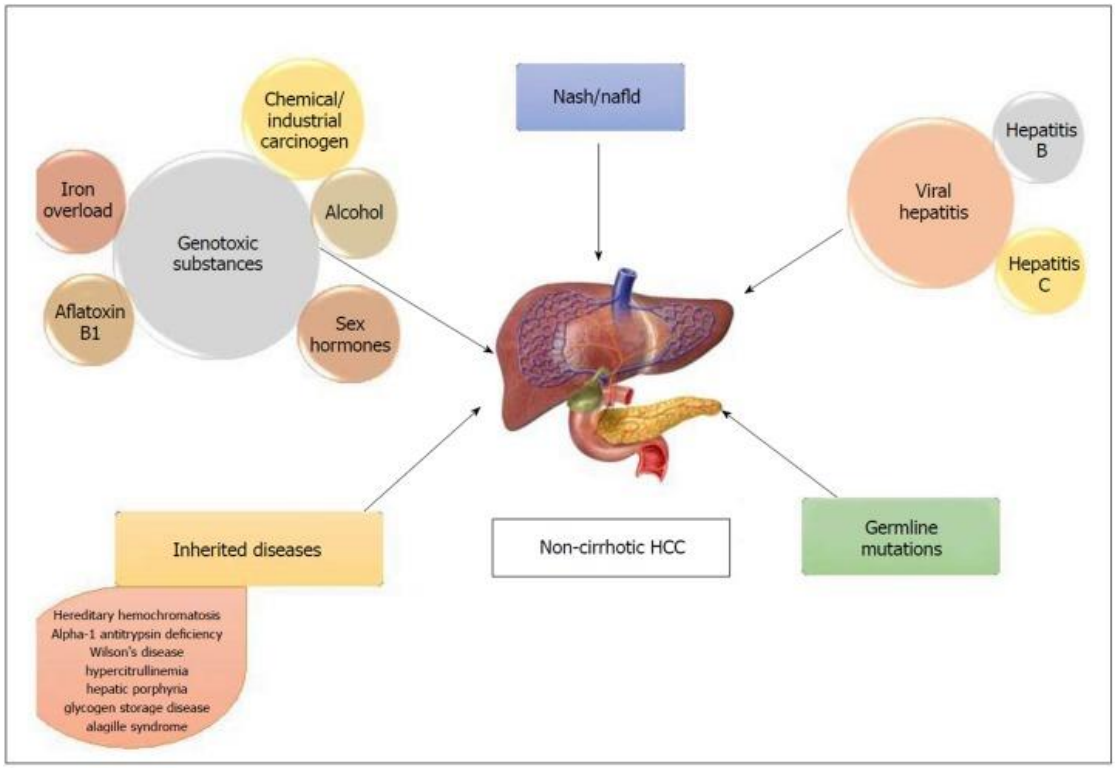


Figure 2.2 Causes of non-cirrhotic hepatocellular carcinoma (Desai, Sandhu, Lai and Sandhu, 2019)

### 2.1.4 Surveillance of Hepatocellular Carcinoma

HCC is the second most common cause of cancer-related death worldwide, with almost all cases of HCC having a poor prognosis. Because of its late presentation, HCC is frequently diagnosed in advanced stages (Tejeda-Maldonado et al, 2015). With the advances in our knowledge on the pathogenesis and pathophysiology of HCC, multiple options of treatment of fighting HCC have been developed. However, almost all HCC cases present late to healthcare centres at incurable states when the well-proven curative method of surgical ablation is no longer applicable for them. Hence, HCC surveillance is important in identifying at-risk populations in early curable states.

Disease surveillance is defined as a screening procedure that is provided on a regular basis to people who are at high risk of contracting a particular illness. The goal of surveillance is to reduce the number of deaths associated with the respective disease. There are a lot of factors that determine the worthiness and suitability of a surveillance programme. The number of cases of the disease endemic to a target population, the accessibility of efficient diagnostic investigation techniques, the implementation of cost-effective treatment modalities, and acceptability of the target population towards the surveillance programme itself, will all have a significant impact on the efficacy of the respective surveillance programme (Prorok, 1992).

After developing a surveillance programme, it is also imperative to define a target population precisely to maximise the effectiveness of the surveillance programme. Utilizing cost-effectiveness models and decision analysis, a surveillance is deemed cost-effective if it improves the life expectancy of a specific population, while costing less than a predefined level. Over the last ten years, most centres use $50,000 per year of life savings as the set threshold for a cost-effective surveillance programme (Centre for Disease Control and Prevention, 2021). In the setting of HCC, our target

population include cirrhotic patients without advanced liver failure or decompensation (Child-Pugh stage A or B), cirrhotic patients with liver failure or decompensation (Child-Pugh C) but already listed for liver transplantation surgery, non-cirrhotic patients with liver failure secondary to HBV or HCV or any aetiology, and any individual at high or intermediate risk of developing HCC. Liver cirrhosis patients with advanced liver failure but are not in the waiting list for liver transplant procedure, are not suitable for the surveillance programme as there is no more effective treatment for them even though the incidence of HCC is higher in more advanced liver disease. Thus, in the above case, we can apply the concept of incidence threshold to achieve maximum cost-effectiveness of treatment towards the respective target population.

#### 2.1.4(a) Imaging-based Tests

Liver ultrasound is widely used as a surveillance or monitoring tool for HCC in Asia, Europe, and United States. It is widely accepted by healthcare centres world-widely as it is a non-invasive tool with minimal or to no risk to patients. Apart from that, liver ultrasound also possesses the capability to identify other serious complications of liver cirrhosis, for example portal hypertension, subclinical ascites, and portal vein thrombosis. This advantage of ultrasound enables all these fretful conditions to be treated earlier. The relatively low cost of ultrasound also makes it more affordable for patients as compared to other more advanced imaging modalities. As a surveillance tool of HCC, ultrasound achieved the sensitivity of 58-89% and specificity of more than 90% (Bolondi et al, 2001). A meta-analysis by Singal A et al showed that ultrasound was efficient in identifying HCC before they were clinically detectable (Singal et al., 2009). However, it only had the sensitivity of 63% in identifying small HCC lesions less than 20mm in size in the early stage of the disease. It is even more

difficult to identify these small HCC tumours if other factors directly obscure the lesion, such as obesity, chest wall deformity, location of lesion and liver cirrhosis. Furthermore, ultrasound as an imaging technique is highly operator dependent, with the ability to accurately screen for HCC being fully dependent on the capability and expertise of the person in charge, not to mention the quality of the ultrasound machine used also playing an important role in HCC diagnosis.

Other imaging modalities such as Computed Tomography (CT) imaging and Magnetic Resonance Imaging (MRI) can only be used as HCC surveillance tools if patients have conditions that make ultrasound assessment difficult such as obesity, chest wall deformity, and abundant intestinal gas (Atiq et al., 2016). Both CT imaging and MRI are not practical as surveillance tools as they are not cost effective, and they also pose risks of radiation and allergic reactions due to contrast used in these imaging modalities.

### 2.1.5 Diagnosis of Hepatocellular Carcinoma

The diagnosis of HCC in those with underlying liver cirrhosis is primarily based on imaging, which is widely accepted since 2001 after dynamic imaging exploration by CT and MRI demonstrated a typical diagnostic pattern of HCC (Bruix et al., 2001). Unique features in imaging that can point to HCC include peculiar vascular derangement resulting from hepatic cell carcinogenesis, as well as the high suspicion of HCC in cirrhotic patients. There are two unique diagnostic characteristics of HCC that can be delineated from contrast-enhanced imaging: i) hypervascularity during the late arterial phase, also known as arterial phase hyperenhancement (APHE), and ii) washout during porto-venous and delayed phase (Ricke, Seidensticker and Mohnike, 2012). These 2 imaging-based features of HCC make it distinct from other malignancies.

Tissue biopsy diagnosis is not necessary, as these imaging features alone are sufficient to diagnose HCC. The two most common HCC imaging modalities are multiphasic enhanced CT scan, and MRI. Both possess different diagnostic performances varying with the size of the lesion, but a recent meta-analysis concluded that MRI is more sensitive than CT imaging in terms of HCC lesions <20mm in size (Lee et al., 2015). The sensitivity of MRI for HCC diagnosis was 62%, while CT imaging sensitivity was only 48%. If the lesion is more or equal to 20mm, there is a minimal difference in sensitivity between MRI and CT imaging (95% vs 92%).

With regards to the non-cirrhotic liver, unlike cirrhotic liver-derived HCC, histopathological diagnosis is necessary for confirmatory diagnosis of HCC. The size of HCC lesions in these types of patients tends to be larger when first discovered or diagnosed, as these patients are not usually among the target population for HCC surveillance, and lack the typical clinical symptoms seen in patients with pre-HCC cirrhosis (Giannini et al., 2013; Schütte et al., 2014). Worse still, the imaging specificity for the non-cirrhotic HCC liver is lower than in a cirrhotic HCC liver, due to the higher clinical likelihood of other alternative diagnoses, such as hepatocellular adenoma and secondary metastases to the liver (Tommaso et al., 2019). This is the reason why some guidelines such as the European Association for the Study of the Liver (EASL) recommended liver biopsy for confirmation of non-cirrhotic HCC (Galle et al., 2018). The specificity of liver biopsy of non-cirrhotic HCC is nearly 100%. However, the sensitivity of liver biopsy diagnosis is highly variable at 66-93%, due to certain factors such as the site of the lesion, the size of the lesion, and the expertise of both the surgeon and the pathologist (Desai, Sandhu, Lai and Sandhu, 2019; Tommaso et al., 2019).

### 2.1.6 Staging of Hepatocellular Carcinoma

Staging is the next step after HCC's diagnosis, to guide subsequent management and predict the prognosis of the patients. According to the latest Clinical Practice Guideline (CPG) by the European Association for the Study of Liver (EASL), it advocates the use of the Barcelona Clinic Liver Cancer (BCLC) staging system for HCC (Galle et al., 2018). BCLC staging incorporates both prognostic factors (liver function, tumour status, and health-performance status) and treatment-dependent factors acquired from randomised control trials (Llovet, Brú, Bruix, 1999). The number and size of lesions, the presence of vascular invasion by the tumour, and liver metastases are the factors that determine tumour status. Baseline liver function is classified using Child-Pugh classification and health performance status is based on Eastern Cooperative Oncology Group (ECOG) classification. BCLC subdivides HCC into 5 categories:

#### 2.1.6(a) BCLC Stage 0 (Very Early HCC)

Single lesion <20mm without evidence of vascular invasion, with Child-Pugh A and ECOG 0. This group of patients is often treated with liver resection or radioactive frequency ablation which offer almost similar survival benefit. The median survival rate is around 80-90% at 5 years.

#### 2.1.6(b) BCLC Stage A (Early HCC)

Single lesion >20mm, or 3 nodules seen each <30mmm size, with Child-Pugh A and ECOG 0. This group of patients is often treated with liver resection, liver transplant or local radioactive frequency ablation. The median survival rate is approximately 50-70% at 36 months.