INTRODUCTION

Hepatitis C virus (HCV) is estimated to infect 185 million chronically worldwide, with 3-4 million new infections per year and over 350,000 deaths due to HCV-related liver disease each year ***(Gower et al., 2016)***. HCV infection has variable long term impact ranging from minimal effects to chronic hepatitis, advanced fibrosis, cirrhosis, decompensated cirrhosis, hepatocellular carcinoma and may also induce extra hepatic complication ***(Maasoumy and Wedemeyer 2016).***

The main goal of HCV therapy is to eradicate it in order to prevent hepatic and extra-hepatic complications and to improve overall survival ***(Lavanchy, 2011).*** Advances in the treatment of HCV infection have demonstrated over 90% cure rates, as defined by the sustained virological response (SVR), i.e. undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after the end of therapy. Long-term follow-up studies have shown that an SVR corresponds to a definitive cure of HCV infection in more than 99% of cases ***(Poiteau et al., 2016).***

Direct Antiviral Agents (DAA) can be divided into 3 classes defined by the Non Structural (NS) HCV protein they target: NS3 Protease inhibitors, NS5B Polymerase inhibitors and NS5A protein inhibitors ***(Swain et al., 2015)***.

The high efficacy, combined with the near perfect safety profile of DAAs, has challenged the need for regular on treatment monitoring of efficacy and safety, a feature that was one of the mainstays of pegylated interferon (PegIFN) based regimens ***(Asselah et al., 2016).***

In light of the advances in HCV therapy, simplification of diagnosis confirmation, pre-treatment diagnostic workup and treatment monitoring is required to ensure broad access to these new therapies. Introduction of these highly potent therapies has necessated the need for response-guided therapy and follow up by markers that implicates successful response, one of these markers is the hemoglobin-haptoglobin scavenger receptor CD163, which is located exclusively on the surface of macrophages and monocytes ***(Gronbaek et al., 2012).***

CD163 is shed from the cell surface into the circulation upon macrophage activation, and is thus a highly specific marker of macrophage activation. The soluble form of CD163 (sCD163) has shown promising capacity as a biomarker of the severity and outcome of various liver diseases ***(Gronbaek et al., 2012).***

Aim of the Work

The aim of this study is to assess the accuracy of the biomarker soluble CD 163 in defining the regression of liver fibrosis in patients with hepatitis C treated with direct antiviral agents in comparison to standard methods.

Chapter one
Hepatitis C Virus (HCV)

Viral hepatitis is estimated to be the 7th leading cause of mortality worldwide. About half of this mortality is attributed to hepatitis C virus (HCV), a primary cause of liver fibrosis, cirrhosis and cancer ***(Stanaway et al., 2016).*** The highest prevalence has been reported in Africa and the Middle East, with a lower prevalence in the Americas, Australia and Northern and Western Europe. In Africa, the highest prevalence of HCV infection has been reported in Egypt and Cameroon (>10%) ***(Hajarizadeh et al., 2013).***

HCV is a small single-stranded ribonucleic acid (RNA) of positive polarity, and is an enveloped virus belonging to the Hepacivirus genus within the Flaviviridae family. It consists of approximately 9600 nucleotides in length, which encode three structural proteins (core, E1, and E2), the ion channel protein p7, and six nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) ***(Abdel-Ghaffar et al., 2015).***

Hepatitis C virus (HCV) genotype (GT) 4 represents 12%-15% (15-18 million) of total global HCV infection. It is prevalent in Northern and Equatorial Africa and the Middle East, and is also present in some countries in Europe. GT-4 (and subtype 4a in particular) dominates the HCV epidemic in Egypt ***(Abdel-Ghaffar et al., 2015).***

In developed countries, the most important route of HCV transmission is intravenous drug abuse, whereas in resource-poor countries invasive procedures or injection-based therapies with contaminated instruments are the predominant source of new infections ***(Hauri et al., 2004)***.

It has been postulated that the Egyptian HCV epidemic has been caused by extensive iatrogenic transmission during the era of parenteral-antischistosomal-therapy mass-treatment campaigns before 1985 ***(Mohamoud et al., 2013).***

In 2008, an Egyptian Demographic Health Survey (EDHS) was carried out in Egypt. Results showed that HCV antibody prevalence was 14.7%. Most (>90%) HCV isolates were found to belong to GT-4 with the remaining belonging to GT-1 ***(Guerra et al., 2012)***.

In 2015, a national Egyptian health issue survey (EHIS) was conducted to reevaluate the prevalence of hepatitis C virus (HCV) infection. The prevalence of HCV antibody was found to be 10.0% and that of HCV RNA to be 7.0% ***(Kandeel et al., 2017).***

An estimated 29% reduction in HCV RNA prevalence has been seen since 2008, which is largely attributable to the ageing of the group infected 40–50 years ago during the mass schistosomiasis treatment campaigns.

Prevention efforts may have also contributed to this decline, with an estimated 75% decrease in HCV incidence in the 0–19 year age groups over the past 20 years ***(Kandeel et al., 2017).***

People with chronic HCV are at risk of increased fibrosis progression. Host factors associated with an increased risk of fibrosis progression include male sex, ethnicity (black individuals), age >40 years at infection, immunosuppression for example, human immune deficiency virus (HIV) coinfection, chronic hepatitis B virus (HBV) co-infection, diabetes mellitus, insulin resistance, obesity and hepatic steatosis. Behavioral factors such as heavy alcohol intake are also associated with an increased risk of liver fibrosis progression ***(McCaughan and George, 2004).***

Chronic HCV infection generally progresses slowly, with limited advanced liver disease in the initial 10–15 years of infection (even in individuals with cofactors for fibrosis progression). Thus, the duration of chronic HCV infection and the patient's age are key determinants of morbidity and mortality ***(Hajarizadeh et al., 2013).***

The estimated probability of cirrhosis at 20 years after infection (in the absence of HCV treatment) is 16% and is more than two fold higher (41%) at 30 years ***(Sierra et al., 2013).***

The rate of HCC (hepatocellular carcinoma) development among people with chronic HCV infection has been estimated as 1-3% after 30 years. However, in people with HCV-related cirrhosis, HCC develops at an annual rate of 2-4%. Factors associated with risk of developing HCV-related HCC include age >55 years, high levels of alcohol consumption and male sex ***(Hajarizadeh et al., 2013).***

Identifying patients with cirrhosis or advanced fibrosis is of particular importance, as the choice of treatment regimen and the post-treatment prognosis depend on the stage of fibrosis. Patients with advanced fibrosis and those with cirrhosis need continued post-treatment surveillance for HCC every 6 months ***(Afdhal et al., 2015).***

In chronic hepatitis C, non-invasive methods should be used instead of liver biopsy to assess liver disease severity prior to therapy. Liver stiffness measurement can be used to assess liver fibrosis ***(Chou and Wasson, 2013).***

The Fibroscan device works by measuring shear wave velocity. In this technique, a 50 megahertz (MHz) wave is passed into the liver from a small transducer on the end of an ultrasound probe (Figure 1). The probe also has a transducer on the end that can measure the velocity of the shear wave (in meters per second) as this wave passes through the liver. The shear wave velocity can then be converted into liver stiffness, which is expressed in kilopascals ***(Nezam, 2012).***

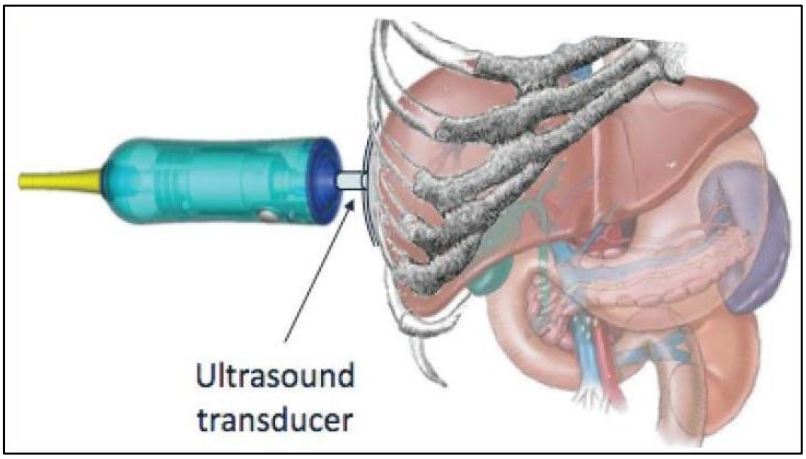

Figure (1): Fibroscan (transient elastography) used to detect liver stiffness

Located on the bottom of the Fibroscan scoring card is Fibroscan results in kilopascal (kPa) measurements 0-75, more accurately Fibroscan results range from 2.5 kPa to 75 kPa. Between 90–95% of healthy people without liver disease will have a liver scarring measurement less than 7.0 kPa (median is 5.3 kPa). A person with chronic hepatitis C and a liver stiffness more than 14 kPa has approximately a 90% probability of having cirrhosis, while patients with liver stiffness more than 7 kPa have around an 85% probability of at least significant fibrosis ***(Hosni, 2015)***.

Well-established panels of fibrosis biomarkers can also be applied. Both liver stiffness measurement and biomarkers perform well in the identification of cirrhosis or no fibrosis, but they perform less well in resolving intermediate degrees of fibrosis ***(Chou and Wasson, 2013).***

In low- and middle-income countries, as well as in settings where treatment expands outside of specialty clinics, aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 (FIB4) are generally available, simple and cheap, and the information they provide is reliable ***(Degos et al., 2010).***

Liver biopsy may be required in cases of known or suspected mixed etiologies (e.g. metabolic syndrome, alcoholism or autoimmunity) ***(Herrmann et al., 2018).***

Among patients with chronic HCV infection and advanced fibrosis, hepatic elastography is predictive of their risk of HCC. People with liver stiffness >25 kPa are at 1.8-2.7 times higher risk of HCC development than those with a liver stiffness of 10–25 kPa ***(Hajarizadeh et al., 2013).***

Because each NS protein is involved in HCV entry, infection, replication, or maturation, they are potential antiviral targets. Hepatitis C virus replication takes place entirely within the cytoplasm, therefore it does not establish latency making it easier to cure ***(Adel-Ghaffar et al., 2015).***

WHO have recently formulated the ‘global health sector strategy on viral hepatitis, 2016–2021’, with service coverage targets to eliminate HCV as a public health threat by 2030 ***(Applegate et al., 2018).***

CHAPTER TWO
DIRECT ANTIVIRAL AGENTS

Since HCV was found to be a major health problem the goal of therapy is to cure HCV infection in order to: (i) prevent the complications of HCV-related liver and extra - hepatic diseases, including hepatic necro-inflammation, fibrosis, cirrhosis, decompensation of cirrhosis, HCC, severe extra-hepatic manifestations and death; (ii) improve quality of life and remove stigma; (iii) prevent onward transmission of HCV ***(Martinot-Peignoux et al., 2015).***

With advances in the molecular understanding of crucial components of the viral life cycle, new direct-acting antiviral agents (DAAs) have been developed at a remarkable pace. DAAs were initially used with PEGIFN and ribavirin, which improved response rates but also increased toxic effects. Combining DAAs targeting different stages in the viral life cycle has proven highly effective and enabled the development of interferon-free and largely ribavirin-free regimens, greatly improving the tolerability of therapy. With well-tolerated oral regimens, cure rates now exceed 90% for most patient populations ***(Afdhal et al., 2014).***

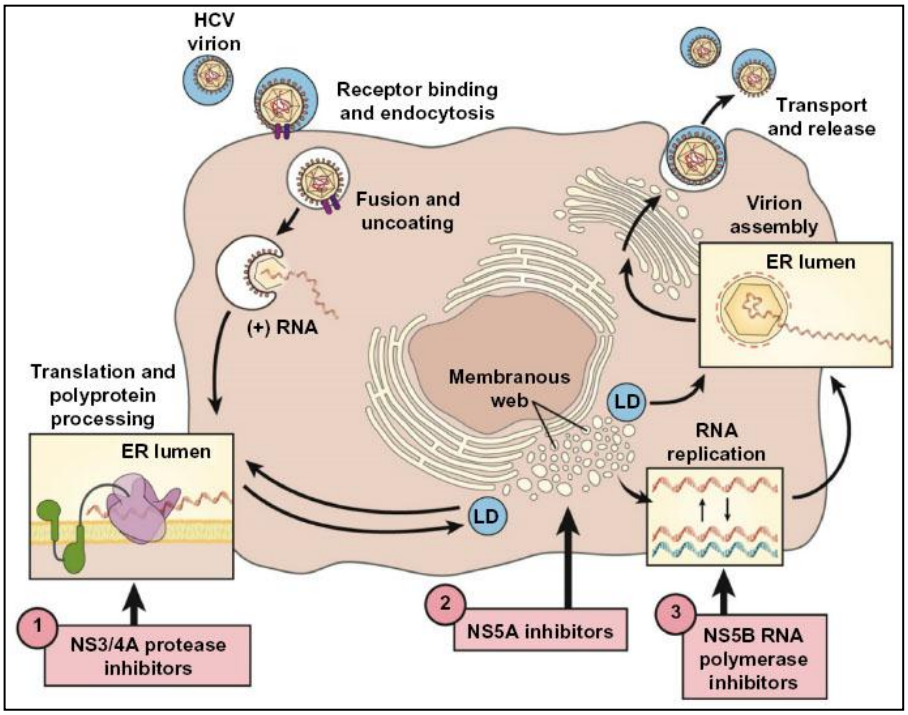

Figure (2): Therapeutic targets of the HCV replication cycle ***(Soriano et al., 2009).***

During the IFN era, it was obvious that future therapies would need to be less complicated, of shorter duration, and much less toxic to be applicable across the wide HCV disease spectrum. Theoretically, drug interference with any of the vital intracellular activities of the viral life cycle could be used to eradicate viral infection ***(Soriano et al., 2009).***

The endpoint of therapy is an SVR, defined by undetectable HCV RNA in serum or plasma 12 weeks (SVR12) or 24 weeks (SVR24) after the end of therapy, as assessed by a sensitive molecular method with a lower limit of detection ≤15 IU/ml. Both SVR12 and SVR24 have been

accepted as endpoints of therapy by regulators in Europe and the United States, given that their concordance is >99% ***(Martinot-Peignoux et al., 2015).***

DAAs of all the major classes were designed to directly inhibit viral enzymes and proteins. The NS proteins NS3/4A protease–helicase and NS5B RdRp and the NS5A protein all perform crucial activities for the viral life cycle and by far have been the favorite targets for development of new DAAs ***(Soriano et al., 2009).***

NS3/4A Protease Inhibitors (PI)

NS3/4A protease inhibitors are inhibitors of the NS3/4A serine protease, an enzyme involved in post-translational processing and replication of HCV. Protease inhibitors disrupt HCV by blocking the NS3 catalytic site or the NS3/NS4A interaction. In addition to its role in viral processing, the NS3/NS4A protease blocks TRIF-mediated Toll-like receptor signaling and Cardif-mediated retinoic acid–inducible gene 1 (RIG-1) signaling, which result in impaired induction of interferons and blocking viral elimination. Thus, inhibition of the NS3/4A protease could contribute to antiviral activity through two mechanisms. The first generation protease inhibitors ***telaprevir*** and ***boceprevir*** were the first direct-acting antivirals available for the treatment of HCV, and were used in conjunction with peginterferon and ribavirin for the treatment of genotype 1 infection ***(Pockros, 2010).***

Although these first-generation PIs represent a major advance in the treatment of HCV, they have several limitations, including narrow genotype specificity and a low barrier to resistance. The appearance of mutations in the NS3 gene during therapy, specifically amino acid substitutions at positions Arg155, Ala156, and Asp168 are viewed as signature mutations for NS3/4A PIs. Compounds that belong to the second wave of the first-generation macrocyclic PIs, including ***simeprevir*** and ***asunaprevir***, have similar limitations. By contrast, the second-generation PI ***grazoprevir*** retains activity against mutated Arg155 (R155K) ***(Götte and Feld, 2016).***

NS5B Inhibitors

NS5B is the RNA-dependent RNA polymerase of HCV, and is another logical therapeutic target. Clinically relevant NS5B inhibitors can be classified into non-nucleotide inhibitors (NNIs) and nucleotide inhibitors (NIs) that act at distinct stages of RNA synthesis. Crystal structures of NS5B reveal a tertiary fold with distinct subdomains is referred to as ‘thumb’, ‘fingers’ and ‘palm’ ***(Götte and Feld, 2016).***

NS5B has a catalytic site for nucleoside binding and at least four other sites at which a non-nucleoside compound can bind and cause allosteric alteration. The enzyme’s structure is highly conserved across all HCV genotypes, giving agents that inhibit NS5B efficacy against all six genotypes ***(Kati et al., 2015).***

NNIs non-nucleotide inhibitors: NNI binding pockets are allosteric sites, which is compatible with non-competitive mechanisms of action. Different chemical classes of NNIs can bind to distinct regions in the thumb (thumb sites I and II) ***(Götte and Feld, 2016).***

Thumb site I inhibitors. Beclabuvir is a potent indole-based thumb site I inhibitor ***(Rigat et al., 2014).***

Thumb site II inhibitors. Radalbuvir is a potent thumb site II inhibitor that has advanced into phase II trials ***(Dvory-Sobol et al., 2014).***

Palm site inhibitors. The palm site, sometimes also subdivided into sites I and II, is a hydrophobic area in close proximity to the binding site of the initiating nucleotide. ***Dasabuvir*** is an approved palm site II inhibitor that is selective for genotype 1 ***(Kati et al., 2015).***

Nucleotide inhibitors: Unlike NNIs, NIs compete with the incoming nucleoside triphosphate for binding and incorporation. NIs are administered as prodrugs that require metabolic activation to the triphosphate form that is eventually accommodated at the nucleotide-binding site of NS5B ***(Appleby et al., 2015).***

Sofosbuvir is a phosphoramidate prodrug that initially yields a monophosphate following intracellular hydrolysis, which is further modified to its diphosphate and ultimately to its active triphosphate form that binds to the active site of NS5B. Sofosbuvir and related 2′-*C*-methylated compounds act as potent chain terminators ***(Appleby et al., 2015).***

Sofosbuvir is a drug of special interest among the directly antiviral drugs due to its high potency, low side effects, oral administration and high barrier to resistance ***(Harmeet et al., 2014).***

Sofosbuvir and ledipasvir are available in a two-drug fixed-dose combination containing 400 mg of sofosbuvir and 90 mg of ledipasvir in a single tablet. Biliary excretion of unchanged ledipasvir is the major route of elimination with renal excretion being a minor pathway (approximately 1%) While no dose adjustment of sofosbuvir and ledipasvir is required for patients with mild or moderate renal insufficiency, the safety of the sofosbuvir-ledipasvir combination has not been assessed in patients with severe renal impairment (eGFR <30 ml/ min/1.73 m2) or ESRD requiring haemodialysis. Relative to patients with normal renal function (eGFR >80 ml/min/1.73 m2). The most frequent side effects reported with this combination were headache and fatigue ***(EASL, 2018 b).***