INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE EGAS MONIZ

MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

THE ROLE OF HUMAN PARAOXONASE 1 (PON-1) IN VIRAL INFECTIONS

Trabalho submetido por
Nuno de Sousa Paulino Neves
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Novembro de 2016

INSTITUTO SUPERIOR DE CIÊNCIAS DA SAÚDE EGAS MONIZ

MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

THE ROLE OF HUMAN PARAOXONASE 1 (PON-1) IN VIRAL INFECTIONS

Trabalho submetido por
Nuno de Sousa Paulino Neves
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Trabalho orientado por
Doutora Maria Gabriela Almeida

e coorientado por
Doutora Sofia Azeredo Pereira

Novembro de 2016

ACKNOWLEDGEMENTS

A todos os que contribuíram para a realização deste trabalho e cujo apoio e dedicação foi indispensável à sua concretização, nomeadamente a toda a equipa do GB^2 do CIIEM-Egas Moniz, especialmente à Doutora Célia Silveira pela preciosa ajuda.

Um agradecimento à equipa de Translational Pharmacology do CEDOC da Universidade Nova de Lisboa, por toda a informação prestada, especialmente à Doutora Sofia Azeredo Pereira e à Doutora Aline Teixeira Marinho pelas revisões e opiniões.

À Doutora Maria Gabriela Almeida, por me ter aceite como orientando e por me ter apoiado e incentivado ao longo destes últimos anos.

E por fim à minha família, que me apoiaram nos bons e nos maus momentos e sem os quais não teria alcançado esta etapa.

RESUMO

De acordo com a Organização Mundial da Saúde, o Vírus da Imunodeficiência Humana, o vírus da Hepatite B e o vírus da Hepatite C são uma das causas mais comuns de doença crônica com origem viral em todo o mundo.

A enzima Paraoxonase-1 é uma enzima dependente do cálcio associada às lipoproteínas de alta densidade, com várias atividades, incluindo paraxaonase, arilesterase e lactonase. A capacidade de hidrolisar vários substratos parece dar à enzima propriedades antioxidantes e sensibilidade ao estresse oxidativo.

Este trabalho faz uma revisão dos estudos mais recentes e relevantes na área e tenta demonstrar as interligações e importância da enzima Paraoxonase-1, que pode fornecer informações clínicas significativas.

Vários estudos apontam para alterações significativas que ocorrem na atividade enzimática da Paraxonase-1 em pacientes infetados com vírus da Imunodeficiência Humana, vírus da Hepatite B e vírus da Hepatite C. Estas modificações parecem ocorrer devido as alterações das atividades da enzima causados pelas infeções no organismo, conduzindo a várias perturbações nas vias anti-oxidantes e anti-inflamatórias, que parecem envolver a Paraoxonase-1.

Sendo as alterações na atividade da Paraoxonase-1 aparentemente motivadas por causas malignas, podem ser retiradas informações clínicas úteis através da avaliação de tais alterações. A enzima Paraoxonase-1 possivelmente poderá ser usada, com mais pesquisas no futuro, como biomarcador para as infeções virais.

Palavras-chave: Paraoxonase-1; Vírus da Imunodeficiência Humana; Hepatite B; Hepatite C

ABSTRACT

According to the World Health Organization, Human Immunodeficiency Virus, Hepatitis B virus and Hepatitis C virus are one of the most common causes of chronic viral related disease.

The Paraoxonase-1enzyme is a calcium-dependent enzyme associated to high density lipoprotein, with various activities, including paraxaonase, arylesterase and lactonase. This ability to hydrolase various subtracts seems to give the enzyme anti-oxidant properties and sensibility to oxidative stress.

This review cross references the information of most of the recent and available studies done in the area and tries to demonstrate the connections and the importance of the Paraoxonase-1 enzyme that could provide invaluable clinical information.

Several studies conclude that significant alterations occur on the enzymatic activity of Paraoxonase-1 in Human Immunodeficiency Virus, Hepatitis B virus and Hepatitis C virus infected patients. This modifications seem to occur due to the increased levels caused by the infections on the organism, leading to multiple disruption on antioxidant and anti-inflammatory pathways that were demonstrated to involve Paraoxonase-1.

Although Paraoxonase-1 activity alterations seem to be caused by malignant causes, positive and useful clinical information might be extracted by measuring such alterations. Paraoxonase-1 possibly will be used with more research in future as a biomarker for some viral infections.

Key Words: Paraoxonase-1; Human Immunodeficiency Virus; Hepatitis B; Hepatitis C

INDEX

	Page(s)
ACKNOWLEDGEMENTS	
RESUMO	2
ABSTRACT	3
INDEX	4
PICTURE INDEX	5
TABLE INDEX	6
ABREVIATIONS LIST	7
1. INTRODUCTION	10
1.1 – HUMAN IMMUNODEFIECIENCY VIRUS (HIV) INFECTIONS	11
1.2 – HEPATITIS B INFECTIONS	15
1.3 – HEPATITIS C INFECTIONS	18
1.4 – BIOMARKERS FOR VIRAL DISEASE	20
1.5 – PARAOXONASE-1	21
2. DEVELOPMENT	26
2.1 – PON-1 AND HIV INFECTIONS	26
2.1.1 – PON-1 ENZYME AND HDL	26
2.1.2 – PON-1 IN HIV INFECTED PATIENTS	27
2.1.3 – PON-1 ACTIVITY, $CD4^+$ AND $CD8^+$ T-CELLS IN HIV INFECTED PATIENTS	32
2.1.4 THE ANTIOXIDANT AND ANTI-INFLAMATORY ROLE OF PON-1 IN HIV INFECTED PATIENTS	33
2.1.5 PON-1 HAS A BIOMARKER FOR HIV INFECTION	34
2.2 – PON-1 AND VIRAL HEPATITIS (HBV AND HCV)	35
2.2.1 – PON-1 ACTIVITY AS A BIOMARKER FOR LIVER DISEASE	35
2.2.2 – PON-1 ACTIVITY AND OXIDATIVE STRESS IN THE LIVER	39
2.2.3 – PON-1 ACTIVITY AND HBV INFECTIONS	42
3 CONCLUSIONS	44
4 REFERENCES	45

PICTURE INDEX

		Page(s)
Fig. 1 -	Adult HIV prevalence (15-49 years) in 2015 by WHO region.	11
Fig. 2 -	Diagram of HIV-1 mature virion.	12
Fig. 3 -	HIV-1 replicative cycle and main antiretroviral therapeutical targets.	13
Fig. 4 -	Diagram of HBV virion.	15
Fig. 5 -	HBV replicative cycle and targets for antiviral therapy.	16
Fig. 6 -	Diagram of HCV virion.	18
Fig. 7 -	Schematic representation of the HCV replicative cycle and targets for antiviral therapy.	19
Fig. 8 -	Schematic representation of overall structure of PON-1.	21
Fig. 9 -	Schematic representation of PON-1 anchored to HDL.	22
Fig. 10 -	Hydrolysis reaction of paraoxon by PON-1.	22
Fig. 11 -	Lactonase activity of PON-1 in the homocysteine metabolism.	23
Fig. 12 -	PON-1 activity HIV in infected individuals.	30
Fig. 13 -	Diagnostic accuracy of serum biomarkers in the diagnosis of chronic hepatitis.	37

TABLE INDEX

		Page(s)
Table 1 -	Antiretroviral drugs used against HIV.	14
Table 2 -	Clinical study comparing HDL levels in human serum between healthy subjects, infected HIV patients with or without ART and between the most used ART's.	28
Table 3 -	Comparison between biochemical variables between Caucasian HIV-infected patients without treatment and HIV-infected patients treated with efavirenz.	28
Table 4 -	Comparison between biochemical variables in healthy subjects and treated and non-treated HIV^+ patients.	29
Table 5 -	Variations of PON-1 activity and concentration, HDL cholesterol ApoA-1 and oxidized LDL with gender, presence of HCV co-infection, lipodystrophy, CD4+ cell count and viral load.	29
Table 6 -	Comparison between PON-1 activity and HIV markers of infection.	32
Table 7 -	Comparison of demographic variables, standard liver function tests and basal PON-1 activity in chronic hepatitis cases and healthy controls.	36

ABREVIATIONS LIST

AE	Serum Arylesterase
AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphate
ALT	Alanine Aminotransferase
Apo A-1	Apolipoprotein A-1
APPs	Acute Phase Proteins
ART	Antiretroviral Therapy
ARV	Antiretroviral
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BHMT	Betaine-Hcy Methyltransferase
cART	combined Antiretroviral Therapy
CBS	Cystathionineβ- synthase
cccDNA	Covalently Closed Circular Deoxyribonucleic acid
CRP	C reactive protein
CSE	Cystathio- nine γ-lyase
Cys	Cysteine
Cyst	Cystathionine
DEP	Dietil-fosfate
EI's	Entry Inhibitors
EMA	European Medicines Agency
FDA	Food and Drug Administration
FI's	Fusion Inhibitors
HBV	Hepatitis B Virus
HCTL	Homocysteine-thiolactone
HCV	Hepatitis C Virus
Hcy	Homocysteine
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus type 1

HIV-2	Human Immunodeficiency Virus type 2
HT	Hcy-thiolactone
HTase	Hcy-thiolactonase
IFN-α	Interferon-alfa
II's	Integrase Inhibitors
LDL	Low Density Lipoproteins
LPS	Lipopolysaccharide
MA	Matrix Protein
MAT	Methionine Adenosyltransferase
Met	Methionine
MetRS	Methionyl-tRNA Synthase
MDA	Malonaldehyde
MPO	Myeloperoxidase
MS	Methionine Synthase
MT	Methyltransferase
NC	Nucleocapsid Protein
NNRTI's	Non-Nucleoside Reverse Transcriptase Inhibitors
NO	Nitric Oxide
NPV	Negative Predictive Value
NUC's	Nucleotide/nucleoside analogues
NRTI's	Nucleoside Reverse Transcriptase Inhibitors
PAF-AH	Platelet Activating Factor Acetyl-hydrolase
pegIFN-α	Polyethyleno Glycol interferon-alfa
PI's	Protease Inhibitors
PNP	*p*-nitrofenol
PON´s	Paraoxonases
PON-1	Paraoxonase 1
PON-2	Paraoxonase 2
PON-3	Paraoxonase 3
PPV	Positive Predictive Value
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic

ROS	Reactive Oxygen Species
RT	Reverse Transcriptase
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction Technique
SAA	Serum Amyloid A
SAHH	S-Adenosyl-Hcy hydrolase
S-Adenosyl-Hcy	S-adenosyl-homocysteine
S-Adenosyl-Met	S-adenosyl-methionine
TNF	Tumor Necrosis Factor
VL	Viral Load
VLDL	Very Low Density Lipoproteins
WHO	World Health Organization

1. INTRODUCTION

Several clinical studies and authors have stated that some viral infections, namely the Human Immunodeficiency Virus (HIV), Hepatitis B (HBV) and Hepatitis C (HCV) viruses, are somehow related with the paraxonase-1 (PON-1) enzyme that could provide invaluable clinical information.

The purpose of this review is to make an assessment of the most recent data on the subject to provide a broader depiction of the field and examine all the available information.

1.1 – HUMAN IMMUNODEFIECIENCY VIRUS (HIV) INFECTIONS

According to World Health Organization (WHO), over 35 million people have globally died from HIV-related causes to date. Almost 1.1million of those deaths were in 2015 alone (WHO, 2016). According to the report of the Joint United Nations Program on HIV and AIDS (UNAIDS) of 2016, there are still 36,7 million people infected around the globe (figure 1) with only 17 million of then been able to access combined antiretroviral therapy (cART) (UNAIDS, 2016). It is considered a major pandemic with no cure to date and the only way to keep the disease controlled is throughout the chronic use of cART which is expensive and not absent of adverse reactions. (Carr & Cooper, 2000; Cost Considerations and Antiretroviral Therapy | Adult and Adolescent ARV Guidelines | AIDSinfo, 2016).

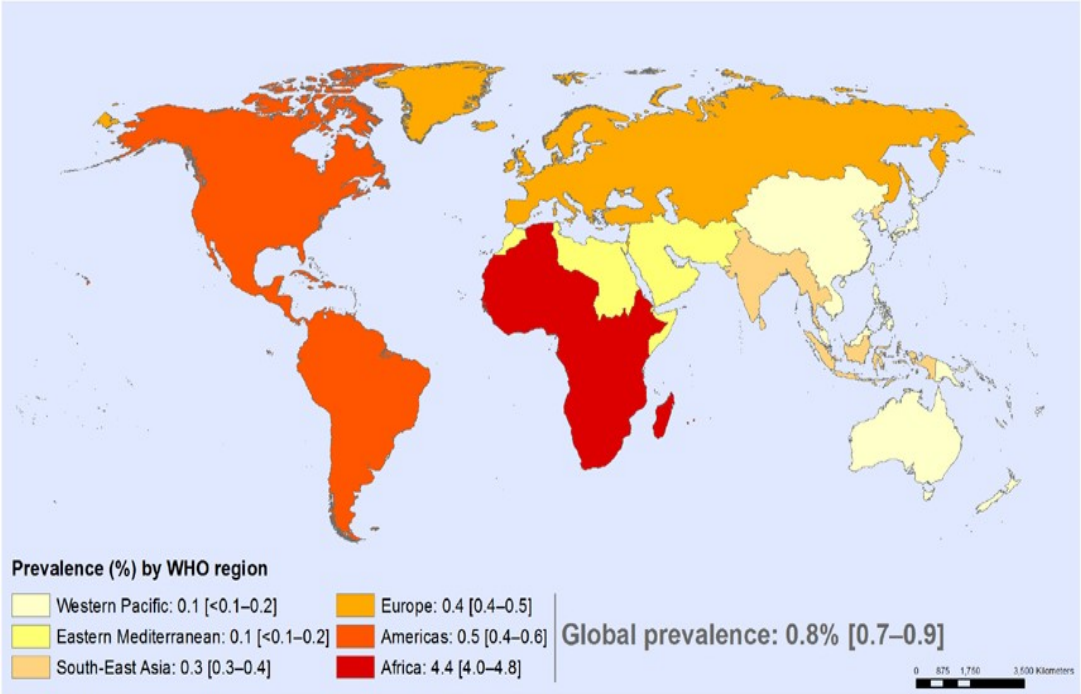

Fig. 1 - Adult HIV prevalence (15-49 years) in 2015 by WHO region. Adapted from Global Health Observatory – WHO (2016).

The known causing agents of Acquired Immune Deficiency Syndrome (AIDS) are HIV-1 (figure 2) and HIV-2. Though they share some similarities (in key proteins like Gag or Pol), they differ in almost 50% of their genome. The differences in the viral genotype translate in several symptomatic disparities such as the latency period, which is increased in the HIV-2 strain, or the increased infection rate observed in HIV-1 strains, which remains to date the major culprit of HIV infections all over the world. (Simon, Ho & Karim, 2010).

In Portugal, HIV infections are caused not only by the HIV-1 variant but by the HIV-2 genotype due to the geopolitical and historical ties to Guinea-Bissau and Cape Verde, were the majority of HIV-2 infections have been recorded (Fernández-Hidalgo, Almirante & Pahissa, 2009).

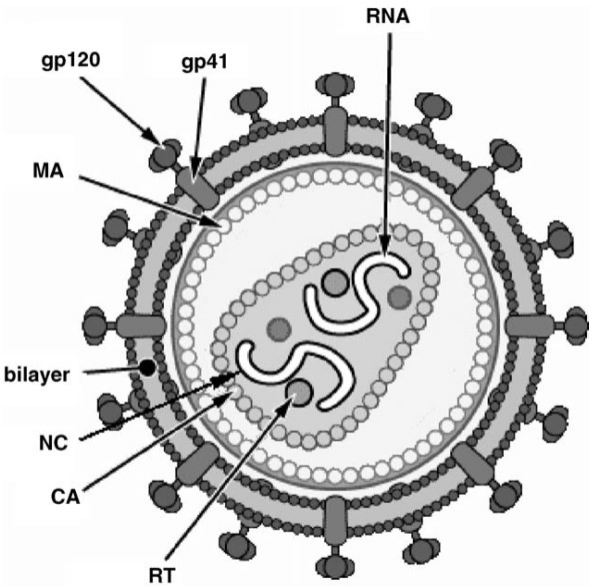

Fig. 2 – Diagram of HIV-1 mature virion. MA (matrix protein); CA (capsid protein); NC (nucleocapsid protein); RT (reverse transcriptase); RNA (ribonucleic acid); gp120 and gp41 (glycoprotein receptors). Adapted from Sierra et al. (2005).

The worldwide spread of HIV indicates that the virus has one of the most effective infection mechanisms, taking advantage of cellular pathways while neutralizing and hiding from the immune system. The primary target are $CD4^+$ T lymphocyte cells, which are crucible for induction of specific humoral and cell-mediated immune response. In the long term, an untreated host will develop opportunist diseases that otherwise would remain at bay with an uncompromised immune response and eventually lead to death (Sierra, Kupfer & Kaiser, 2005). With access to ART, it is possible to extend the life expectancy but with severe complications such as dyslipidemias, atherosclerosis, neurodegenerative diseases, to name a few (Depairon et al., 2001; Marsillach, Parra, Coll, Joven, & Camps, 2008; Pereira et al., 2009).

The HIV replicative cycle (figure 3) is very complex and its duration and outcome varies according to the target cell and cell activation (Coffin, Hughes & Varmus, 1997).

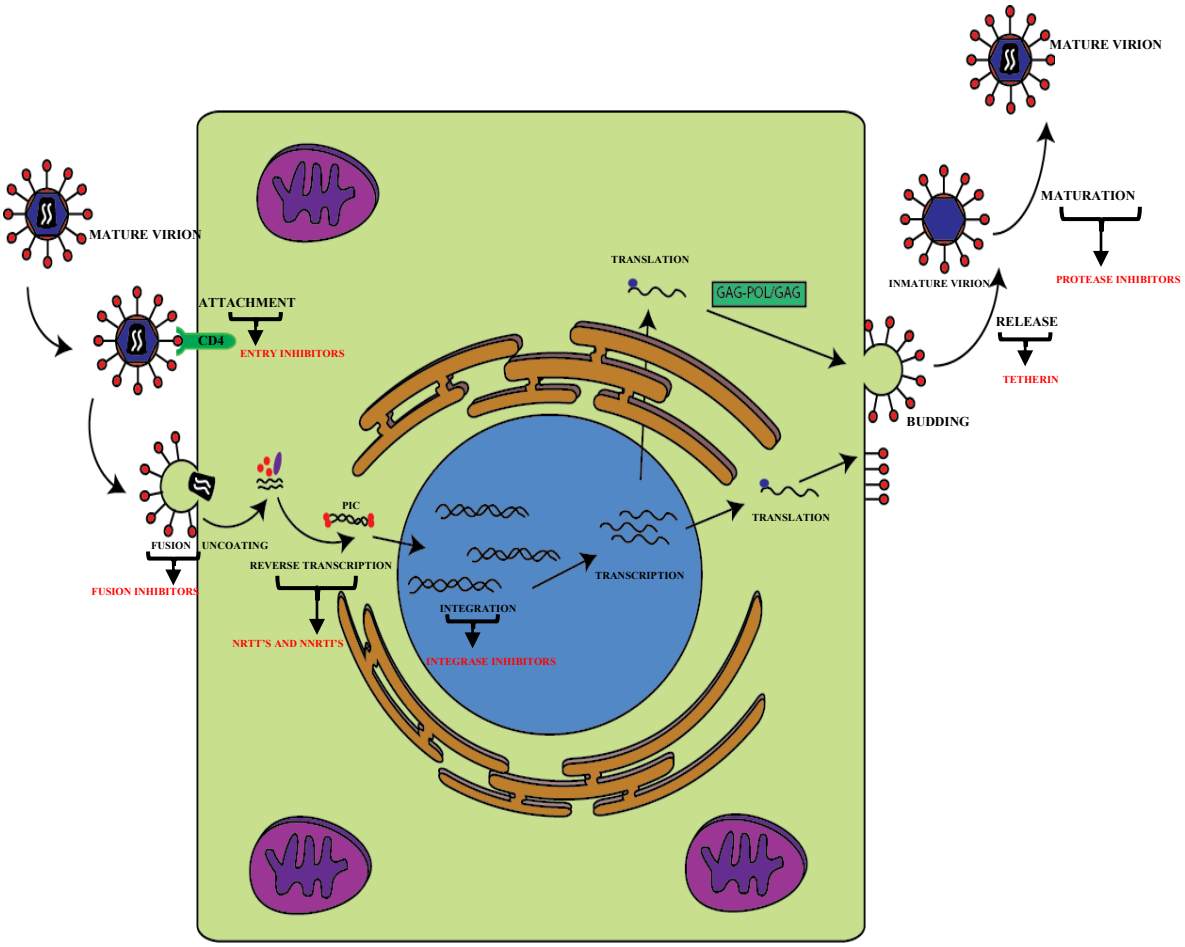

Fig. 3 – HIV-1 replicative cycle and main antiretroviral therapeutical targets. Once a mature virion attaches itself to the CD4 membrane receptor of the lymphocyte T cell, it begins a fusion process in with it releases all the viral machinery into the cell, including the viral RNA and reverse transcriptase. Then it starts to reverse transcript the RNA into the pre-integration complex, which is incorporated in the cell's DNA via integrase. Once integrated in the nuclear DNA, it begins the transcription and translation process, taking advantage of the cells own machinery. Once all the viral component are fully formed, they start to assembly and release themselves from the cell creating a new immature virion. For a time this virion undergoes a maturation process by the viral protease to produce all the mature viral particles. Adapted from Sierra et al. (2005).

Over the years several strategies were established to decrease the viral load hoping to increase the quality of life of infected patients and even develop a possible cure. Several antiretroviral drugs have been created that exploited numerous key stages in the replicative cycle of the virus, such as fusion inhibitors, entry inhibitors, reverse transcriptase inhibitors (nucleotide based and non-nucleotide based), protease inhibitors and integrase inhibitors (table 1) (FDA, 2016).

Table 1 - Antiretroviral drugs used against HIV.

Drug Class	Generic Name
Nucleos(t)ide Reverse Transcriptase Inhibitors (NRTI's)	Abacavir
	Didanosine
	Emtricitabine
	Lamivudine
	Stavudine
	Tenofovir
	Zidovudine
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI's)	Efavirenz
	Etravirine
	Nevirapine
	Rilpivirine
Protease Inhibitors (PI's)	Atazanavir
	Darunavir
	Fosamprenavir
	Indinavir
	Nelfinavir
	Ritonavir
	Saquinavir
	Tipranavir
Fusion Inhibitors (FI's)	Enfuvirtide
Entry Inhibitors (EI's)	Maraviroc
Integrase Inhibitors (II's)	Dolutegravir
	Elvitegravir
	Raltegravir

Adapted from FDA (2016).

HIV infections are prone to cause lipid and acute phase protein (APP) alterations (Treitinger et al., 2001), especially during the initial stages of infection. Some lipid modifications might be related to the host's reaction to infection intermediated by cytokines, seen as an increase in serum Tumor Necrosis Factor (TNF), interleukins and decrease in High Density Lipoprotein (HDL) cholesterol (Feingold & Grunfeld, 1992), which can be linked to viral replication and consequent cholesterol membrane metabolism (Ono & Freed, 2001; Rose et al., 2008). The acute phase response has been shown to be correlated to the increase in plasma triglyceride concentrations and the increased turn-over and loss of protein mass (Treitinger et al., 2001).

1.2 - HEPATITIS B INFECTIONS

Hepatitis B is also a disease caused by a viral infection. The causing agent is the Hepatitis B Virus (HBV) which is a small, enveloped DNA virus that belongs to the *Hepadnaviridae* family. It targets liver cells and can cause acute or chronic liver disease. According to the WHO, in 2016, more than 240 million people were chronically infected with HBV, with 686,000 people dying every year from complications related to the disease (WHO, 2016a). This infection can be easily prevented by vaccination, however high costs and lack of awareness of HBV infection risks hamper the successful outcome of immunization. Also, the high prevalence in some regions (e.g. Asia) is due to vertical transmission of HBV virus, from chronically infected mothers (Grimm, Thimme & Blum 2011).

HBV is a small, enveloped DNA virus (figure 4). There are currently eight known different genotypes (A-H), being the A and D the most common in Europe. The virus itself does not seem to be cytopathic. The cytotoxicity in HBV infection comes from immune mediated responses (Grimm et al., 2011).

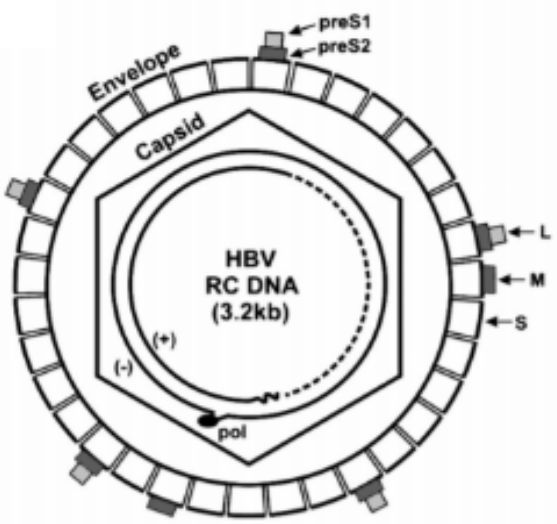

Fig. 4 – Diagram of HBV virion. Envelope proteins, S, M, L, with their S, preS2 and preS1 domains. The HBV genome (HBV RC DNA), in the capsid, is a partly double stranded, incomplete circle, with the polymerase (pol) attached to the "minus" strand. Adapted from Locarnini (2004).

Currently, there are seven drugs approved by both the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for hepatitis B infections (figure 5).

The conventional interferon-alfa (IFN-α), the PEGylated interferon-alfa (pegIFN-α) and the nucleotide/nucleoside analogues (NUC's), such as adefovir, entecavir, telbivudine and tenofovir and lamivudine, also both used in HIV infections (EASL, 2012; Stein & Loomba, 2009).

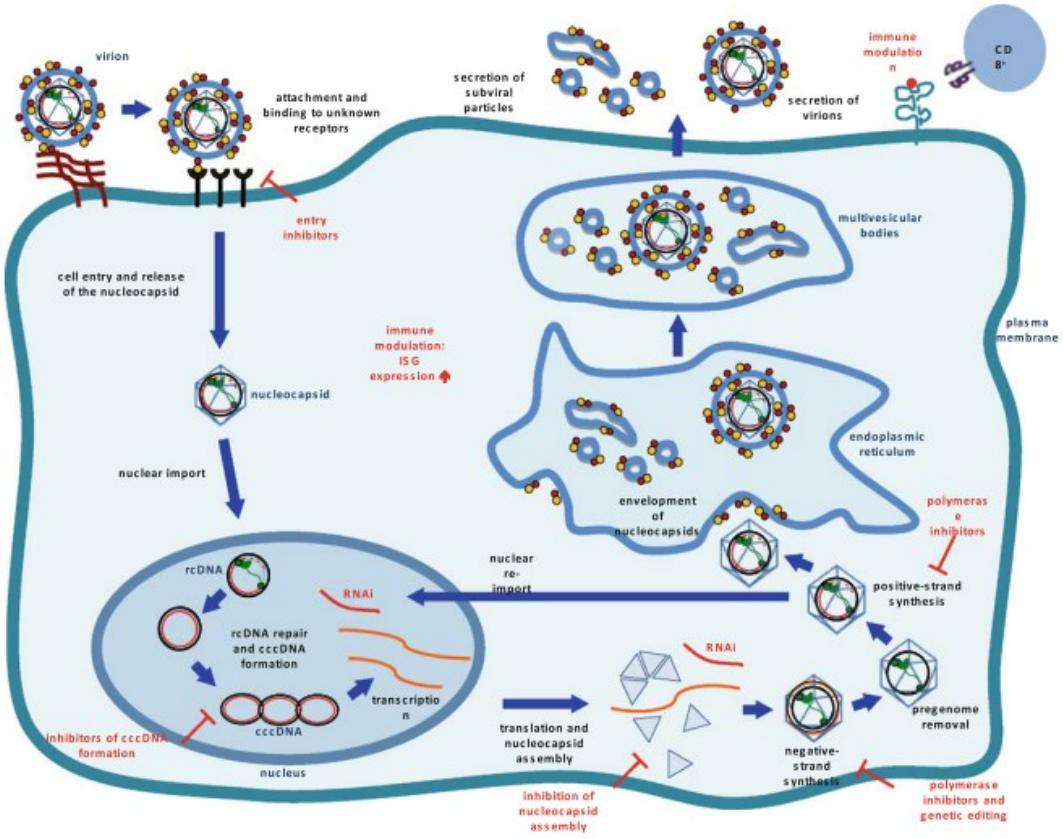

Fig. 5 – HBV replicative cycle and targets for antiviral therapy. HBV uses reverse transcription despite not being a retrovirus. In its replication process the virus gains entry into the cell by binding to the sodium taurocholate co-transporting polypeptide receptor on the surface is endocytosed. Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones. The partially double stranded viral DNA is then made fully double stranded by viral polymerase and transformed into covalently closed circular DNA (cccDNA). This cccDNA serves as a template for transcription of four viral mRNAs by host RNA polymerase. The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions that are released from the cell or returned to the nucleus and re-cycled to produce even more copies. Adapted from Grimm, Thimme & Blum (2011) and Stein & Loomba (2009).

The aims of cART are preventing disease progression to cirrhosis, end-stage liver disease or death and to improve the quality of life of chronically HBV-infected patients (Wang, 2012).

More than 95% of patients with acute HBV infection recover spontaneously and without the necessity for ART (Tassopoulos et al., 1987). However, patients with fulminant or severe hepatitis must be evaluated for liver transplantation (Castaldo & Chari, 2006).

Occasionally, the division amongst true acute hepatitis B and reactivation of HBV is problematic and often requires a liver biopsy. Although nucleotide analogues therapy is first line treatment in each case (Tillmann et al., 2006; Garg et al., 2011;).

1.3 – HEPATITIS C INFECTION

Hepatitis C is also a disease caused by a RNA virus (HCV) (from the *Flaviridae* family), with more than 86% chance of developing chronic infection. By the WHO assessments, it is estimated to affect at least 150 million people chronically worldwide, a significant proportion prone to develop complications like cirrhosis or liver cancer (WHO, 2016b). Although drugs can cure approximately 90% of people, the access to diagnosis and treatment is highly ineffective. This is due mainly to the high cost of treatment (WHO, 2016b).

Hepatitis C can be found worldwide but the most affected regions are Africa and Central Asia (WHO, 2016b).

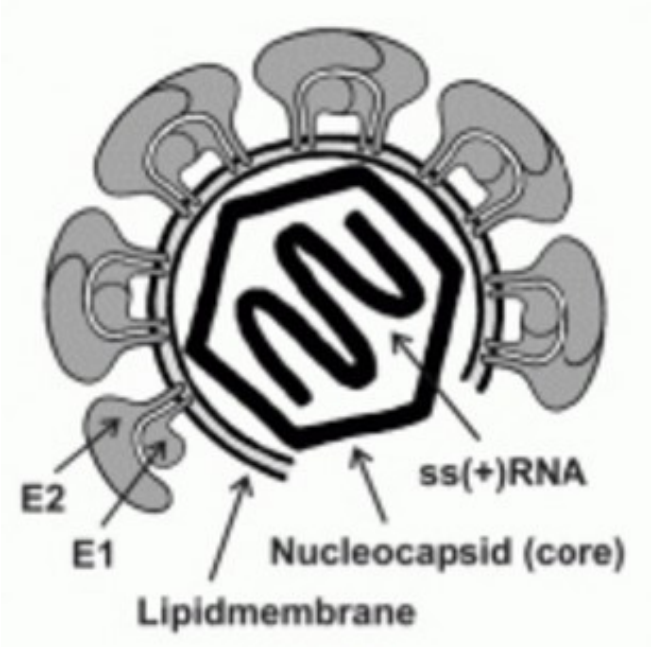

Fig. 6 – Diagram of HCV virion. HCV adopts an icosahedral scaffold; glycoproteins E1 and E2 are attached to the lipid envelope. Inside the envelope is the nucleocapsid or core, forming an internal icosahedral viral coat that encapsulates the viral genomic positive-strand RNA. Adapted from Chevaliez & Pawlotsky (2006).

The diagnosis of hepatitis C is divided in two steps, the first constitutes the screening for anti-HCV antibodies with a serological test. The second part is a confirmation test which screens for RNA. This is because some people are able to fight the initial infection successfully and no longer have the virus but still test positive for the anti-bodies of HCV (WHO, 2016b).

Nowadays, the available ART options are very effective (figure 7). However, as above mentioned, they can be extremely expensive. To date, there are no vaccines available has for preventing HCV infection ("Hepatitis B and C - Hepatitis B and C Treatments," 2016.; "WHO | Hepatitis C," 2016).

HBC and HCV are both prone to cause chronic liver disease and increase the occurrence of reactive oxygen species and hepatic lipid peroxidation. This causes an accumulated oxidative stress that leads to liver cell apoptosis (Zeisel, Crouchet, Baumert & Schuster, 2015).

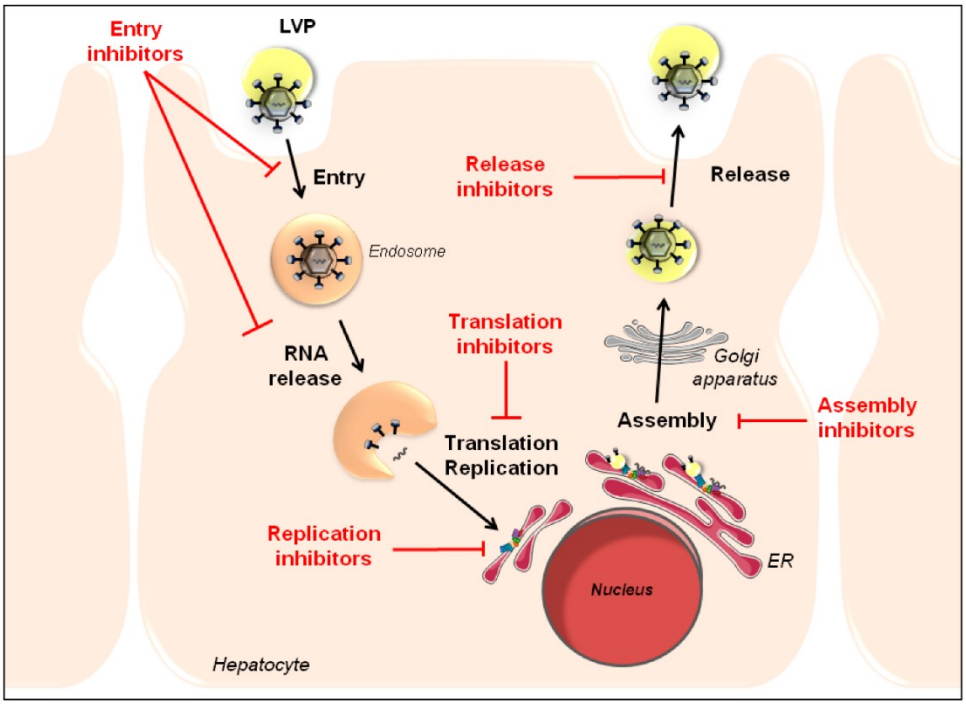

Fig. 7 – Schematic representation of the HCV replicative cycle and targets for antiviral therapy. HCV interacts with the basolateral membrane of hepatocytes, resulting in viral entry into the host cell. The virus is internalized *via* endocytosis and translation of the HCV RNA occurs in the cytoplasm following viral fusion and uncoating. Viral replication takes place within the cytoplasm in perinuclear endoplasmic reticulum (ER)-derived membranes called the "membranous web". Progeny virions are assembled on cytosolic lipid droplets and subsequently transported along the secretory pathway and maturated in the Golgi before their release through microtubular transport and endocytic recycling compartment. Targets for antiviral therapy are highlighted in red. Adapted from Zeisel et al. (2015)

1.4 – BIOMARKERS FOR VIRAL DISEASE

A biomarker is a quantifiable biological or biochemical variable which indicates, when measured objectively, the functioning of biological processes, pathogenic or a pharmacological response to a given therapy (Strimbu & Tavel, 2010). It is normally found in blood, urine, tissue biopsy, saliva or other biological samples. Continued research into a number of urinary, serologic and genetic biomarkers, will guide clinicians with diagnosis, prognosis, and treatment. (Vasan, 2006; Downes & Shah, 2012). Ideally, the means of analysis of a clinical biomarker of interest must meet certain requirements such as high reproducibility, precision and specificity (Laborde et al., 2012).

In viral infections such as HIV, HBV or HCV infections, early diagnosis is critical to improve not only the therapeutic outcomes, but also the survival rate and the prognosis. PON-1 enzyme appears to be sensitive in early stages of some viral infections and to the course of their progression due to a close association of the enzyme with oxidative stress (Rose et al., 2006; Gangadharan, Antrobus, Dwek & Zitzmann, 2007; Marsillach, Parra, Coll, Joven & Camps, 2008; Ali, Shehata, Ali-Labib & Zahra, 2009; Downes & Shah, 2012). If a relation can be establish, a new biomarker to better evaluate patients and the progression of disease may be developed.

1.5 – PARAOXONASE-1

Paraoxonases (PON´s) are a family of enzymes, called PON-1, PON-2 and PON-3. Their genes are located in humans at the chromosome 7q21-22 (Kulka, 2016; Primo-Parmo, Sorenson, Teiber & La Du, 1996).

PON-1 was first described by Abraham Mazur, in 1946, who reported the existence of an enzyme capable of hydrolyzing organophosphates in animal tissues (Mazur, 1946). This eventually led to the discovery of PON-1 in the early 1950's (Aldridge, 1953). Described as a calcium dependent serum HDL-associated serum aryldialkylphosphatase (Aldridge, 1953), the structure of PON-1 (figure 8) consists of a six-bladed β-propeller, each blade consisting of four β-sheets. In the active center of the PON-1 enzyme there is a tunnel containing two calcium atoms that is crucial for structural stability and catalytic activity. The enzyme also has an anchoring portion for the High Density Lipoprotein (HDL) particle that is located at the top of the propeller which has three α-helices (figure 9) (Harel et al., 2004).

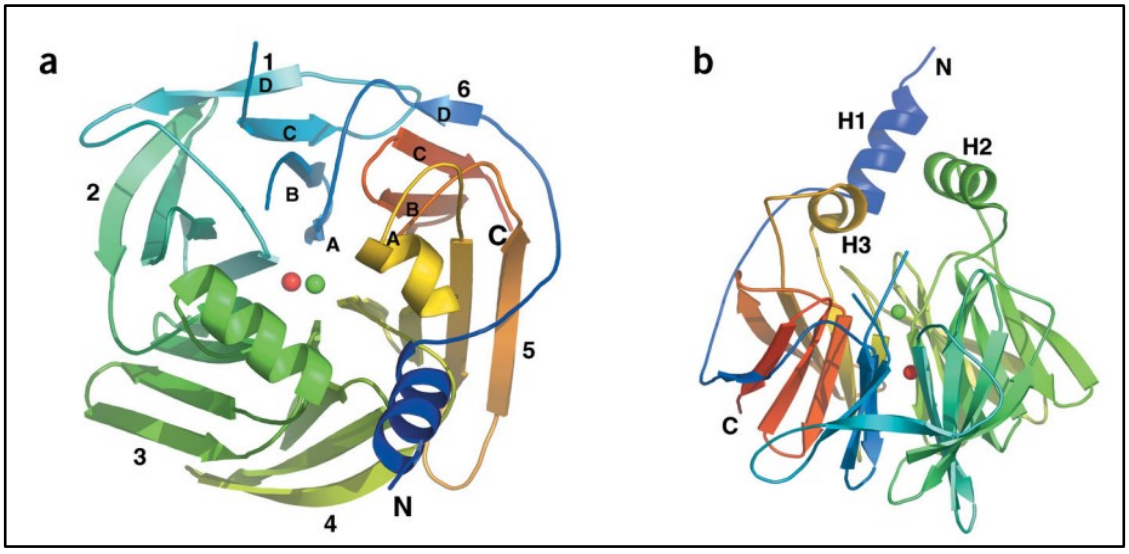

Fig. 8 – Schematic representation of overall structure of PON-1. **a)** View of the six-bladed *β*-propeller from above. The top of the propeller is, by convention, the face carrying the loops connecting the outer *β*-strand of each blade (strand D) with the inner strand (A) of the next blade. Shown are the N and C termini, and the calcium atoms in the central tunnel of the propeller (Ca^1, **green**; Ca^2, **red**); **b)** A side view of propeller, including the three helices at the top of the propeller (H1–H3). N-terminal residues 1–15 and a surface loop connecting strands 1B and 1C (residues 72–79) are not visible in the structure. Adapted from Harel et al. (2004).

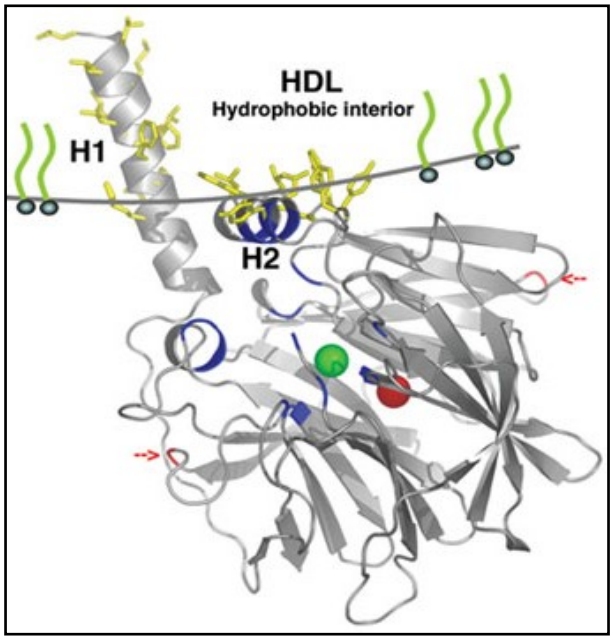

Fig. 9 – Schematic representation of PON-1 anchored to HDL. Adapted from Harel et al. (2004).

The enzyme was named after studying its first known subtract, paraoxon, an active metabolite of the organophosphate parathion (Lessire et al., 1996; van Himbergen, van Tits, Roest & Stalenhoef, 2006). The ability of PON1 to hydrolyze paraoxon has been largely employed over the years for quantifying the activity of different enzyme variants and their tissue concentrations (figure 10) (Shih *et al.*, 1998; Camps, Marsillach & Joven, 2009). Over the years it became clear that the activity of PON-1 was highly variable according to the genetic background of the individual, making possible to associate certain alleles with the activity of the enzyme. It was also showed that the presence of low-activity alleles varied considerably between each ethical group, with elevated frequency being perceived in Caucasian populations (Eckerson, Wyte & La Du, 1983).

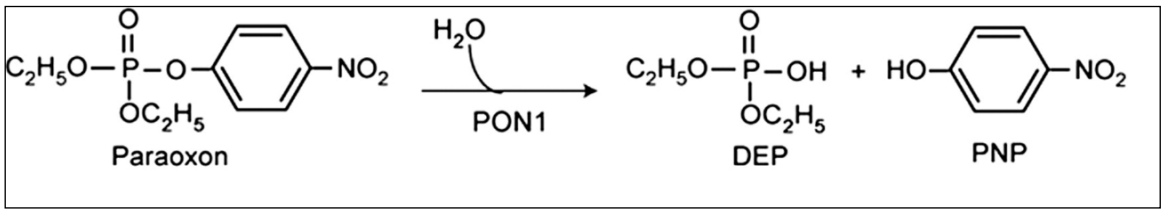

Fig. 10 – Hydrolysis reaction of paraoxon by PON-1. DEP – dietil-fosfate; PNP – *p*-nitrofenol. Adapted from Chambers (2008)

PON-1 has demonstrated a strong lactonase activity for homocysteine-thiolactone and other xenobiotic lactones (Jakubowski, 2000; Ng et al., 2005). Being evolved in the homocysteine metabolism via homocysteine-thiolactone (figure 11), the impact of the study of PON-1 increases, specially due to its possible role in the prevention of cardiovascular diseases (mainly atherosclerosis) (Shih *et al.*, 1998) and involvement in Alzheimer's disease (Jakubowski, 1997; Cervellati *et al.*, 2015).

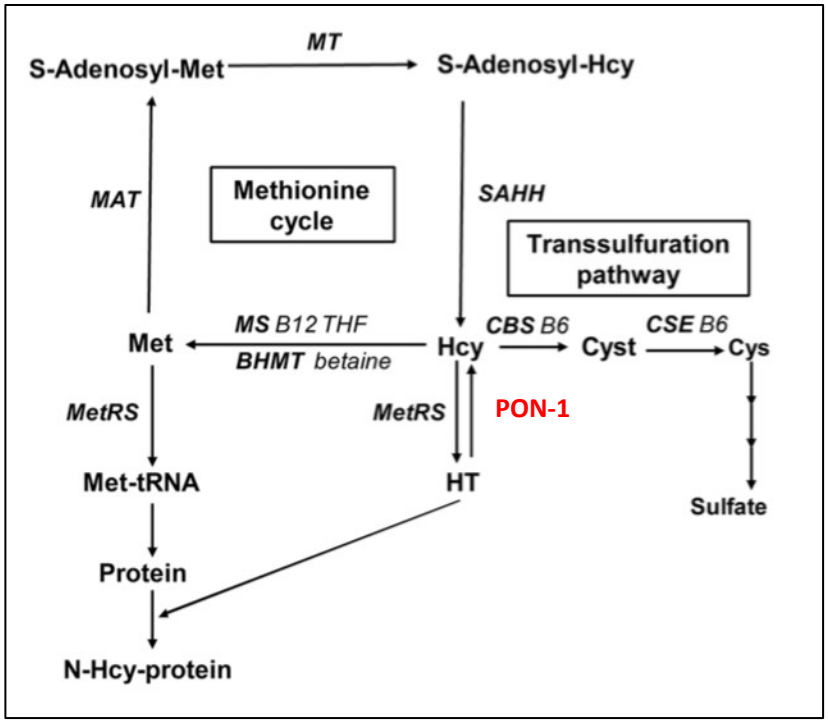

Fig. 11 – Lactonase activity of PON-1 in the homocysteine methabolism. Homocysteine (Hcy) is maintained at very low levels in serum due to the methionine cycle, which leads to a rapid methyl transfer reaction using the following enzyme substrates: methionine (Met), S-adenosyl-methionine (S-Adenosyl-Met), and S-adenosyl-homocysteine (S-Adenosyl-Hcy). The methionine cycle is controlled by methionine synthase (MS), betaine-Hcy methyltransferase (BHMT), methionine adenosyltransferase (MAT), methyltransferase (MT), and S-Adenosyl-Hcy hydrolase (SAHH). Met and Hcy are also metabolized by methionyl-tRNA synthase (MetRS) to become Met- tRNA and Hcy-thiolactone (HT). Under normal conditions, Hcy-thiolactone is rapidly converted to Hcy by Hcy-thiolactonase (HTase), in this case PON-1. HT reacts spontaneously with protein ε-lysine residues, leading to formation of N-Hcy-protein. Alternatively, Hcy can enter the transsulfuration pathway. It is then converted to cystathionine (Cyst) by cystathionine β - synthase (CBS), which is further converted to cysteine (Cys) by cystathio- nine γ-lyase (CSE). The transsulfuration pathway ends with sulfate. Adapted from Usuki (2012)

According to Aviram et al., (1998), PON-1 might have two different active sites that give it asterolytic and hydroxyperoxide activities - the first one metabolizes substrates such as esterified lipids, while the lather is involved in the reduction of hydroperoxides. It has been suggested that both catalytic activities may act in sequence, as seen with *p*-nithophenol 13-hydroperoxylinoleate when used as PON-1 subtract. The reduction mechanism paralleled hydrolysis, indicating an esterolytic activity requirement before the esterified fatty acid hydroperoxide is reduced (Karabina, Lehner, Frank, Parthasarathy & Santanam, 2005).

Human PON-1 is thought to be mainly synthetized and secreted by the liver. In fact, Northern-blot analysis of hepatic tissue samples presented only positive results for PON-1 mRNA (Hassett et al., 1991). However, recent evidences suggested that other tissues such as lungs, brain, pancreas, small-intestine and kidney have the same capacity; the presence of PON-1 mRNA in such tissues was detected, using reverse transcriptase polymerase chain reaction technique (RT-PCR) (Eckerson et al., 1983). Nevertheless, the liver is, the most likely source of PON-1 due to the high PON-1 gene expression; besides, it is here that HDL is synthesized and secreted into the circulation.

Human HDLs particles form a heterogeneous group of lipoproteins, classified according to their increasing size and lipid and protein composition: HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, HDL_{2b} (Rosenson et al., 2011). In bovine serum, 85% of the total PON-1 activity has been associated with HDL fractions. Less than 5% of the total PON-1 activity was identified in very low density lipoproteins (VLDL) and low density lipoprotein (LDL) (Miyamoto, Takahashi, Oohashi, Sato, & Oikawa, 2005).

There is no consensus about the major role of PON-1 in the human organism. The first hypothesis was proposed by Mackness, Arrol & Durrington (1991). This research work highlighted the safeguards against copper-induced LDL oxidation provided by HDL and PON-1. It was observed that PON-1 prevented lipoperoxide generation during the process of LDL oxidation, which indicated that this enzyme might be involved in a protective function of HDL. Of notice is that a PON-1 free sulfhydryl group in cysteine 284 was shown to be essential for the enzyme's activity against lipid peroxides but not for its activity against paraoxon or other xenobiotics. This suggests that the mechanisms of hydrolysis are somewhat different (Aviram et al., 1998).

PON-1 is also regarded as a negative acute phase protein and its concentration seams to decrease during inflammatory conditions. In humans and rabbits, during acute phase response, apolipoprotein A-I (ApoA-1) concentration and PON-1 activity were

simultaneously depleted (Lenten et al., 1995). It appears that under inflammatory conditions, a displacement of the Apo A-1 by the positive acute phase proteins (APPs) serum amyloid A (SAA) in HDL might occur, thus leading to a decrease in PON-1 activity. However, in mice with acute infection a decrease in PON-1 and platelet activating factor acetyl-hydrolase (PAF-AH) activities was concomitant with unchanged Apo A-1 levels (Lenten et al., 2001). Acute phase response induced by endotoxin, lipopolysaccharide (LPS), caused a decrease in PON-1 serum activity (over 60%) within 48 hours in Syrian Hamsters and initiated a rapid decrease in PON-1 mRNA concentrations, persisting for at least 48 hours, in hamster hepatic cell cultures. Moreover, it was observed that mediators such as TNF and IL-1 diminished PON-1 activity in a similar manner (Feingold, Memon, Moser & Grunfeld, 1998).

The enzyme PON-1 shows a similar behavior to other APPs during the inflammatory process. PON-1 negative acute phase response changes with the elevation of APP positive, when measured in severe conditions in human sepsis and animal parasitic infections. People in critical states during infection had decreased PON-1 activity and a noteworthy negative correlation with C reactive protein (CRP) (Novak et al., 2010), which is a well-studied positive acute phase protein (Jain, Gautam, & Naseem, 2011).

2. DEVELOPMENT

2.1 – PON-1 AND HIV INFECTIONS

2.1.1 – PON-1 ENZYME AND HDL

As mentioned before PON-1 is an esterase/lactonase enzyme, present in HDL, with a broad spectrum of substrates. Yet, its physiological function is not completely understood. However, a considerable amount of evidence suggests that, among other roles, PON-1 may hydrolyze oxidized lipid peroxides from LDL and HDL and, thus, possess antioxidant and anti-inflammatory properties (Parra et al., 2007).

Several studies have shown significant alterations on the enzymatic activity of PON-1 in HIV infected patients. Severe dyslipidemias and low levels of HDL (Riddler et al., 2007; Stein et al., 2008) cause long-term infected HIV patients to develop increased risk of cardiovascular diseases (Lang et al., 2010).

Low levels of HDL cholesterol is widely prevalent in Western countries, and is an independently predictive risk of cardiovascular disease, even in individuals with low concentrations of LDL. However, low concentrations of HDL are frequently concomitant with increased concentrations of small, cholesterol-depleted LDL particles and increased concentrations of cholesterol enriched triglyceride remnants. Thus, the cardiovascular risk associated with low HDL values is difficult to distinct from that of other associated lipoprotein abnormalities (Assmann, Schulte, Von Eckardstein & Huang, 1996; Otvos, Jeyarajah & Cromwell, 2002).

The decrease of lipid transfer to HDL, including free cholesterol, triglycerides and phospholipids, seems to severely disturb the protective antioxidant role provided by PON-1 HDL, disrupting the protection provided against the peroxidation of LDL and also in inflammatory processes, mainly the protection against free-radicals generated by homocysteine-thiolactone (HCTL) (Olszewski & McCully, 1993; Jakubowski & Gtowacki, 2011).

2.1.2 – PON-1 IN HIV INFECTED PATIENTS

Viral replication and some clinical manifestations of HIV infection involve an imbalance in the reduction–oxidation status and free radical production (Schwarz, 1996). It is likely that the HIV-induced oxidized environment could result in an increased binding of free radicals to PON-1, resulting in a less active enzyme in circulation; in agreement to the reports of oxidized lipids reacting with a free (–SH) group at PON's cysteine-284 site leading to inactivation of the enzyme. Another reason for this decrease could be the lower concentrations of HDL cholesterol and Apo A-1 (Aviram et al., 1999).

HIV infected patients frequently develop long-term pro-atherogenic metabolic modifications. These problems may be explained by the infection itself or by secondary effects of the ART, mainly protease inhibitors (Périard et al., 1999). These findings gained additional clinical relevance since the introduction of effective therapeutic measures, which have gradually changed HIV infections from a life threatening to a chronic disease. Furthermore, the combination of classical cardiovascular risk factors with some genetic polymorphisms appears to predispose these individuals to a higher risk of premature arteriosclerosis (Alonso-Villaverde et al., 2005; Coll et al., 2005; Coll et al., 2006). There are changes in lipoprotein metabolism in the course of HIV infection, including increased lipid peroxidation, hypocholesterolemia, hypertriglyceridemia and low HDL concentration (Rose et al., 2006). Also, patients with higher HDL concentrations appear to have a better disease course than patients with lower HDL concentrations (Alonso-Villaverde et al., 2003).

When PON-1´s activity is compared between HIV infected patients and healthy subjects, it is possible to conclude that the activity is decreased in HIV infected patients (44.3 $nmol.min^{-1}.mL^{-1}$ ± 38.6 vs 71.8 $nmol.min^{-1}.mL^{-1}$ ± 37.4 ; p=0,001) (Daminelli et al., 2008). Regarding to HDL levels, it is dependent on the cART and the viral load of the patient (table 2) (Siegel et al., 2015). It has also been observed that the ratio of PON-1 activity/HDL is increased in treated patients with cART containing the non-nucleoside reverse transcriptase inhibitor Efavirenz (table 3) (Pereira et al., 2009). The enzymatic activity of PON-1 and the HDL serum levels become a potential marker that is worth exploring in order to evaluate the possible risk of cardiovascular diseases in HIV-infected patients and to assess the progression of the disease (Marsillach et al., 2008).

Table 2 - Clinical study comparing HDL levels in human serum between healthy subjects, infected HIV patients with or without ART and between the most used ART's.

	Controls	*HIV+ N ART*	*HIV+ PI VL+*	*HIV+ PI VL-*	*HIV+ NNRTI*
HDL (mg/dl)	56	45.1	54.4	56.1	54.1

ART – antiretroviral therapy; VL ± - viral load above or below detection limit (20 copies/ml); adapted from Siegel et al. (2015).

Table 3 - Comparison between biochemical variables between Caucasian HIV-infected patients without treatment and HIV-infected patients on antiretroviral therapy containing Efavirenz.

Parameters	*Untreated*	*Treated (Efavirenz)*
HDL (mg/dl)	48	46
PON-1 paraxaonase activity (U/L)	58,8	77,35
PON-1 activity /HDL ratio	1,3	1,88

Adapted from Pereira et al. (2009).

The evidence provided by this study is reinforced in a work presented by Marinho et al. (2016) in which is demonstrated once again that PON-1 activity increases when using cART containing a different NNRTI, Nevirapine (Marinho et al., 2016).

In a study published by Daminelli et al. (2008) (table 4), the HIV^+ group of patients showed that the LDL cholesterol and triglyceride levels in plasma are decreased than those of the control group, while HDL levels were identical. HIV+ patients frequently show reduced LDL, but triglycerides are generally described as increased (Treitinger et al., 2001), in contrast with the results of this study. However, increased triglyceride concentration leaded to a HDL cholesterol decrease, and the fact that triglycerides were not increased in the HIV^+ group ultimately facilitated the analysis of HDL. According to Daminelli et al. (2008), the three subgroups of HIV-infected patients did not differ among them in respect to LDL. Regarding HDL, however, its levels were higher in the subgroup treated with NRTI plus NNRTI combined therapy, when compared with the subgroup treated with NRTI plus PI therapy, with non-treated HIV^+ patients or with the values of the control group. The diminution of PON-1 activity in HIV-infected patients confirms the findings of Parra et al. (2007), in both treated and non-treated patients.

Table 4 - Comparison between biochemical variables in healthy subjects and treated and non-treated HIV$^+$ patients.

Parameters	*Controls*	*Untreated*	*NRTI + NNRTI*	*NRTI + PI*
HDL (mg/dl)	41	39	53	38
PON-1 (paraoxonase) (nmol.min^{-1}.ml^{-1})	72	46	55	34
LDL (mg/dl)	127	100	106	97
Triglycerides (mg/dl)	116	98	103	98

Adapted from Daminelli et al. (2008)

In fact, Parra et al. (2007) found a reduction in PON-1 activity with an increase in its concentration in HIV infected patients, which could be explained by the addition of a higher quantity of free radicals to PON-1, by lower serum concentrations of HDL and Apo A-1 and by changes in the liver that would influence the serum concentration of PON-1 (table 5).

Table 5 - Variations of PON-1 activity and concentration, HDL cholesterol ApoA-I and oxidized LDL with gender, presence of HCV co-infection, lipodystrophy, CD4+ cell count and viral load.

Characteristic	PON1 activity (U/L)	*P*	PON1 concentration (mg/L)	*P*	HDL-cholesterol (mmol/L)	*P*	Apo A-I (g/L)	*P*	ox-LDL (U/L)	*P*
Gender		0.67		0.17		0.10		0.04		0.29
Male	334.5 (166.3)		130.6 (149.8)		1.22 (0.44)		1.34 (0.30)		84.82 (31.90)	
Female	321.2 (140.9)		156.4 (157.9)		1.17 (0.51)		1.43 (0.33)		86.58 (36.36)	
HCV co-infection		<0.001		0.69		0.57		0.28		<0.001
Yes	312.5 (157.2)		149.5 (178.2)		1.18 (0.39)		1.35 (0.30)		77.06 (30.14)	
No	375.2 (157.1)		123.4 (103.3)		1.20 (0.62)		1.39 (0.34)		100.65 (37.9)	
Lipodystrophy		0.22		0.57		0.02		0.47		0.001
Yes	374.6 (181.4)		134.4 (169.4)		1.02 (0.37)		1.33 (0.24)		101.00 (40.00)	
No	333.0 (152.3)		135.4 (146.6)		1.25 (0.53)		1.37 (0.34)		81.63 (31.84)	
Viral load (copies/mL)		0.49		0.004		0.001		0.06		0.52
<200	329.7 (153.5)		108.9 (88.3)		1.23 (0.56)		1.40 (0.32)		83.78 (37.32)	
≥200	345.0 (164.4)		178.5 (209.2)		1.12 (0.36)		1.32 (0.27)		86.79 (30.16)	
CD4+ T lymphocytes (cells/mm^3)		0.22		0.94		0.06		0.30		0.79
<200	335.1 (157.8)		159.6 (129.9)		1.14 (0.39)		1.36 (0.31)		83.80 (35.50)	
200–500	357.6 (147.3)		128.2 (177.7)		1.15 (0.42)		1.33 (0.28)		86.08 (27.88)	
>500	317.2 (152.89		131.4 (156.7)		1.26 (0.63)		1.41 (0.35)		88.30 (38.10)	

Reprinted from Parra et al. (2007)

The results from this study indicated that the co-infection with HCV was associated with a significantly lower PON-1 activity and decreased amount of oxidized LDL. There were no significant differences regarding PON-1, HDL or Apo A-1 concentrations. Patients with an active viral replication had higher PON-1 concentrations and lower HDL cholesterol levels. It was not found any significant difference in PON-1 activity between these two groups. The three groups, stratified according to the CD4+ T

lymphocyte count, did not present any significant differences in PON-1 status, HDL-cholesterol, Apo A-1 or oxidized LDL levels. The presence of lipodystrophy was associated with lower HDL-cholesterol concentrations and higher oxidized LDL levels. CD4+ T and CD8+ T lymphocyte cell counts showed weak negative correlations with PON-1 concentration (Parra et al., 2007).

In regards to ethnicity, specifically between Caucasian and Black patients, Pereira et al. (2009) demonstrated that it is also important to take into account the genetic variability specifically those of individuals of different ethnicities, when evaluating PON-1 activity. Other studies do not take the genetic variability into account (Alonso-Villaverde et al., 2003; Maselli et al., 2014; Parra et al., 2007).

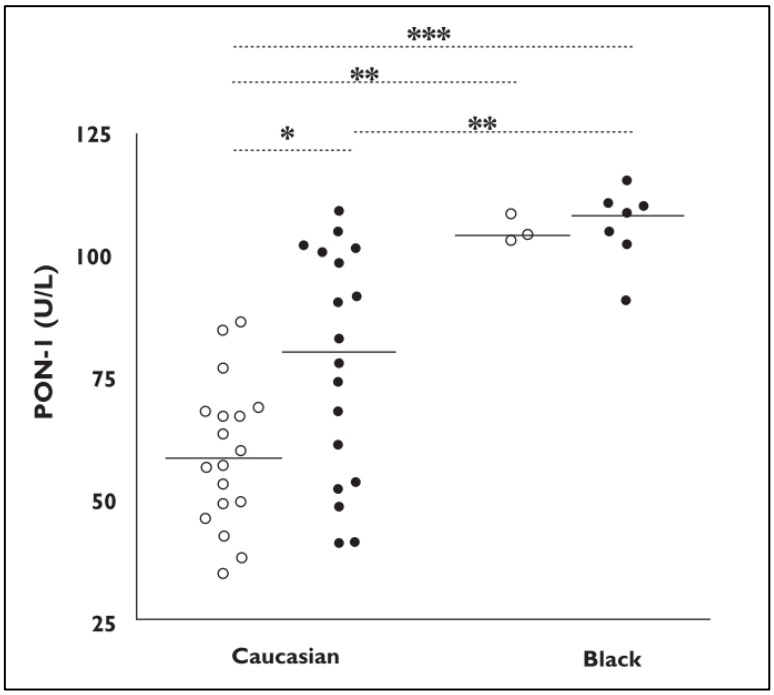

Fig. 12 - PON-1 activity HIV in infected individuals. PON-1 activity was lower in untreated [58.80 $U.L^{-1}$ (51.36, 66.23); n = 18] than in treated [77.35 $U.L^{-1}$ (65.66, 89.04); n = 18]; Caucasian patients and both were lower than Black patients despite treat- ment (*P < 0.05, **P < 0.01 and ***P < 0.001, one-way ANOVA plus Bonfer-roni's multiple comparison test); No treatment ◯ ; treated with Efavirenz ● . Adapted from Pereira et al. (2009).

Only a few studies have been published to date regarding the association between HIV infection and PON levels, and these have produced diverse results (Parra et al., 2007; Daminelli et al., 2008; Pereira et al., 2009). Daminelli et al. (2008) verified a significant decreased activity of PON-1 in HIV^{+} patients compared to healthy controls; ART

treatment did not reverse this deficiency. Parra et al. (2007) also found decreased PON-1 activity but also identified an increased PON-1 concentration in HIV^+ patients compared to heathy controls.

Siegel et al. (2015) measured both the activity and concentration of PON-1 in HDL particles, and found decreased levels of both. In light of the findings by Parra et al. (2007) it was proposed that a defect in incorporation of PON-1 into HDL, rather than low PON-1 abundance in these patients, was responsible for the low PON-1 concentrations. PON-1 activity measured in isolated HDL inversely correlates with age (Çakatay, Kayali, & Uzun, 2008) on which, given that the average age control group in Siegel et al. (2015) was younger than the one in the HIV^+ patients, could contribute to lower the PON-1 activity. On the other hand, Maselli et al. (2014) stated that there was no correlation between PON-1 activity and serum levels of HDL, possibly because the clinical data of the study does not provide significant statistical differences between groups.

2.1.3 – PON-1 ACTIVITY, $CD4^+$ AND $CD8^+$ T-CELLS IN HIV INFECTED PATIENTS

The Maselli et al. (2014) work showed lower serum PON-1 activity in HIV^+ patients with no ART (table 6). PON-1 activity showed a significant positive correlation with the number of CD4+ T-cells in HIV^+ patients with no ART, suggesting that the immune status may directly affect the activity of PON-1. Other studies have shown that enzyme levels are reduced in HIV^+ patients with no ART who have a reduced number of CD4+ T-cells (Le Moing et al., 2007). Thus, it might be proposed the existence of a relationship between the number of CD4+ T-cells and PON-1 activity, which would be directly related to a good outcome in a HIV infection. It remains uncertain whether the replication of the HIV virus itself would be related to PON-1. According to Maselli et al. (2014) the study established the factor that likely determines the reduction of PON-1 activity is the suppression of the immune system by HIV (Maselli et al. 2014).

Table 6 - Comparison between PON-1 activity and HIV markers of infection.

Parameters	***Controls***	***Untreated***	***Efavirenz/Nevirapine***	***Lopinavir/Ritonavir***
$CD4^+$ T-cells (cells/mm^3)	ND	404	483	486
$CD8^+$ T-cells (cells/mm^3)	ND	955	859	952
CD4:CD8 ratio	ND	0.4	0.5	0.5
HIV-1 RNA (copies/ml)	-	89.266	14.121	16.301
PON-1 paraoxonase activity (U/L)	117	81	109	106

Adapted from Maselli et al. (2014)

According to Pereira et al., (2009) there is also a possible relation between PON-1 activity and CD4 cell count, corroborated by other studies (Parra et al., 2007; Parra et al., 2010). It should be taken in consideration that in early studies there was no differentiation between HIV patients with and without co-infection by Hepatitis C virus, that can largely affect the outcomes on PON-1 activity, since this enzyme is mainly synthetized in the liver and a marker of hepatic function (Ferré et al., 2006).

2.1.4 THE ANTIOXIDANT AND ANTI-INFLAMATORY ROLE OF PON-1 IN HIV INFECTED PATIENTS

In the work publish by Dias et al. (2014), the lactonase activity was examined in a cohort of HIV infected patients, regardless of combined ART in use. Untreated patients and patients who had received continuous ART for more than one month were included. The study suggests that lower lactonase activity is associated with uncontrolled HIV infection, particularly with non-suppressed viremia, despite of ART. The data points for lactonase activity to have a role in HIV-infection, probably reflecting an increased formation of HCTL species. The study also proposes that a better understanding of the lactonase activity and its role in HCTL pathophysiology might identify new therapeutic targets in HIV infected patients (Dias et al., 2014).

2.1.5 PON-1 HAS A BIOMARKER FOR HIV INFECTION

According to Parra et al. (2007), serum PON-1 concentrations showed a strong correlation with β-2-microglobulin concentration [B=75.19 (50.32–100.06); $p<0.001$]. Given that β-2-microglobulin is a known effective marker of infection by HIV (Savès et al., 2001), this association might be worth exploring and could lead into new ways to better monitor the infection at multiple levels.

Maselli et al. (2014) demonstrated that the enzymatic activity of PON-1 is correlated with CD4+ T-cell count in patients not receiving ART. The demonstration that PON-1 activity was reduced in untreated patients, but not in individuals receiving ART, suggested that the activity of PON-1 is associated with the immune status of HIV-1 infected individuals, making the enzymatic activity a good candidate for an alternative biomarker (Maselli et al., 2014).

By quantifying the activity of PON-1 in HIV infected patients and being able to gradually establish correlations with treatment outcomes and the progression of the disease, it make it possible to progressively develop a new, efficient monitorization biomarker that is easy to quantify and evaluate (Parra et al., 2007; Maselli et al., 2014; Kulka, 2016).

2.2 – PON-1 AND VIRAL HEPATITIS (HBV AND HCV)

2.2.1 – PON-1 ACTIVITY AS A BIOMARKER FOR LIVER DISEASE

Chronic hepatitis is included in a group of liver disorders where the hepatic inflammation and necrosis lasts for more than six months. The severity of the disease might vary from minor forms, in which the progression is very slow and might go undetected for an extended period of time, to more serious forms which are linked with liver parenchymal scarring and structural disorders that progress to cirrhosis (Fauci, Kasper, Longo, Hauser & Jameson, 2015).

In chronic liver diseases of slow progression the traditional liver function tests often remain unaltered, until more severe forms of hepatic disorder becomes apparent.

In order to diagnose such slow progressive liver diseases before reaching advanced states such as hepatocellular necrosis and fibrosis, aside the outdated biochemical tests, alternative parameters become necessary to evaluate liver damage (Fauci, Kasper, Longo, Hauser & Jameson, 2015).

Currently histopathological analysis of liver biopsy samples are considered the best method to accurately diagnose liver disease. However, liver biopsy is an invasive technique with inevitable complications (Sanai & Keeffe, 2010). Once more, the need for a trustworthy and accurate diagnostic tool that does not require invasive and painful procedures emerges. In this regard, it would be convenient to find a hepatic biomarker, preferably one that can be easily and quickly assessed (Gangadharan, Antrobus, Dwek & Zitzmann, 2007; Marsillach et al., 2008).

The pathogenesis of numerous liver diseases are often associated with oxidative stress (Tanikawa & Torimura, 2006). The oxidative stress is strongly linked to the progression of alcoholic liver disease, non-alcoholic steatohepatitis and hepatitis B and C virus infection, which can lead to the development of acute hepatitis, chronic hepatitis, hepatocellular carcinomas and liver cirrhosis (Gil, Pla, Gonzalvo, Hernández & Villanueva, 1993).

The PON-1 enzyme is able to protect against oxidative stress by inhibiting lipid peroxidation (Aviram et al., 1998; Mackness, Durrington & Mackness, 2004). This raises the hypothesis that an association between oxidative stress and PON-1 activity in liver disease might exist (Mackness & Durrington, 1995).

Some of the studies performed in this context have detected a substantial reduction of

serum PON-1 activity in patients with chronic hepatitis (Ferré et al., 2002; Ferré et al., 2005; Kilic, Aydin, Kilic, Erman, Aydin & Celik, 2005).

Recently, Pyati (2015) showed that serum basal PON-1 activity is considerably reduced ($p < 0.001$) in patients with chronic hepatitis when compared to healthy controls. These findings are in agreement with the results from the studies reported by Başkol, Başkol, Deniz, Ozbakir, & Yücesoy, (2005); N. Ferré et al., (2002), (2005); Kilic, Aydin, Kilic, Erman, Aydin, Suleyman, et al., (2005) and Ali, Shehata, Ali-Labib, & Zahra, (2009).

According to Pyati (2015) (table 6), the activity of PON-1 is positively correlated with total serum protein levels and albumin levels, and are negatively correlated with serum bilirubin, alanine aminotransferase (ALT), and alkaline phosphate (ALP), with a great statistical significance ($p< 0.001$). No such correlation has been demonstrated between standard liver function tests and basal PON-1 activity in healthy controls. These results are consistent with previous works (Ferré et al., 2002; Ferré et al., 2005; Kilic, Aydin, Kilic, Erman, Aydin, Suleyman, et al., 2005; Başkol et al., 2005; Ali, Shehata, Ali-Labib, & Zahra, 2009).

Table 7 - Comparison of demographic variables, standard liver function tests and basal PON-1 activity in chronic hepatitis cases and healthy controls.

Parameters	***Controls***	***Chronic Hepatitis***	***p-value***
Age	45.6 ± 11.8	41.8 ± 9.8	0.080
Total Bilirubin (%)	0.74 ± 0.14	4.05 ± 1.74	< 0.001
Total Protein (%)	6.83 ± 0.72	6.2 ± 0.34	< 0.001
Albumin (g/dl)	3.72 ± 0.55	3.54 ± 0.34	0.35
ALT (U/L)	21.18 ± 6.87	135.92 ± 41.86	< 0.001
ALP (U/L)	88.78 ± 35.48	164.98 ± 47.42	< 0.001
Pon-1 activity (nmol/ml/min)	176.68 ± 25.88	101.88 ± 23.08	< 0.001

Adapted from Pyati (2015)

According to Pyati (2015), basal PON-1 activity seems to be a better marker for diagnosis of chronic hepatitis than total protein, albumin and ALP with a sensitivity of 68% and specificity of 100%. It also demonstrated a positive predictive value of 100%

and negative predictive value of 75%. However, although PON-1 activity seems to be a more reliable predictor of liver disease compared to total protein, albumin and ALP levels, when bilirubin and ALT are taken into account they still show better results in terms of predictability of disease than basal PON-1 activity. The analysis of the receiver operating characteristic (ROC) curve (figure 13) demonstrated that, among the study parameters, ALT demonstrated highest diagnostic accuracy (AUC=0.999) followed by total bilirubin (AUC=0.977), PON-1 (AUC=0.990), ALP (AUC=0.904), total protein (AUC=0.790) and albumin (AUC=0.595) (Pyati, 2015). These findings were similar to those observed in the study conducted by (Ferré et al. 2002).

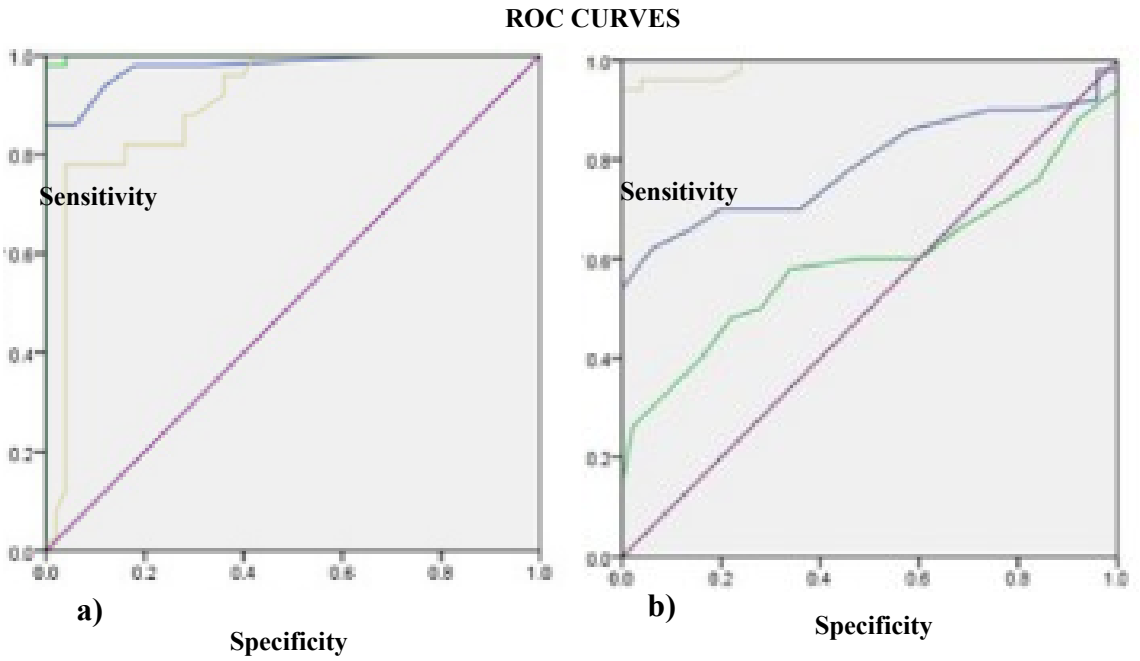

Fig. 13 – Diagnostic accuracy of serum biomarkers in the diagnosis of chronic hepatitis. a) bilirubin (**BLUE**), ALT (**GREEN**), ALP (**YELLOW**); b) total protein (**BLUE**), Albumin (**GREEN**) and PON-1 (**YELLOW**) ; reference line (**PURPLE**). Adapted from Pyati, (2015)

The measurement of PON-1 activity may represent a significant improvement in liver disease detection. The analysis of the data provided by the ROC curves (Ferré et al., 2002; Pyati, 2015) demonstrated that serum PON-1 activity is, in fact, worth exploring. The ROC analysis also supports that the measurement of baseline serum PON-1 activity is an excellent candidate to become a biomarker for liver disease. Although bilirubin and ALT seem to be more accurate biomarkers than PON-1, they are only indicatives of some types of liver injuries (Pyati, 2015).

The quantification of serum PON-1 activity is a simple, reliable, inexpensive and

easily automated method that can be performed in most automated analyzers used for standard biochemical liver function tests. A disadvantage of the assay is the elevated toxicity of paraoxon (in the cases were paraoxonase activity is in analisys) (Ferré, Camps, Cabré, Paul & Joven, 2001; Ferré et al. 2002; Pan, Ma, Bo & Guo, 2011; Pyati, 2015).

2.2.2 – PON-1 ACTIVITY AND OXIDATIVE STRESS IN THE LIVER

Hepatocytes are constantly exposed to reactive oxygen species (ROS), but protected from oxidative injury by a range of antioxidant pathways. The defenses against free radical-mediated injury include enzymatic deactivation and direct reaction with free radicals (Osman, Gabr, Lotfy, & Gabr 2007).

Although free radicals are normally produced by many reactions essential for cell metabolism and energy production, they are implicated also in the pathogenesis of several different human diseases, including viral infections such as HBV or HBC (Schwarz, 1996).

In patients with viral or alcoholic liver disease, the consequent alternation of cellular redox state is potentiated by a decrease in the antioxidant enzymes and an increase of free radical-mediated damage and apoptosis of liver cells (Loguercio & Federico, 2003).

Several studies suggested that free radical generation and oxidative stress play an important role in the mechanisms developed by HCV to survive and progress in the infected host (Cardin et al., 2001; Thorén, Romero, Lindh, Dahlgren & Hellstrand, 2004; Waris, Turkson, Hassanein & Siddiqui, 2005).

Lipid peroxidation is caused by free radicals leading to oxidative destruction of polyunsaturated fatty acids constitutive of cellular membranes. The destruction of polyunsaturated fatty acids leads to the production of toxic and reactive aldehyde metabolites such as malonaldehyde (MDA) (Levent et al., 2006). MDA content usually reflects the level of lipid peroxidation and indirectly reflects the extent of hepatocellular injury in vivo (Osman et al., 2007).

In the study by Ali et al. (2009), it was established that serum MDA levels were higher in patients with HCV than the healthy ones. This result considers the consequence of lipid peroxidation in the pathophysiology of patients with HCV. Serum MDA concentration in patients with chronic active hepatitis C as well as transaminase and bilirubin levels were found to be increased in many other studies (Mansurova, Mutikhova, & Olimova, 2005; Levent et al., 2006; Osman et al., 2007). MDA was showed to be elevated in the liver and the blood of patients with HCV as reported by De Maria et al. (1996). Moreover, Boya et al. (1999) reported that, the peripheral blood mononuclear cells separated from chronic hepatitis C patient had increased MDA concentrations.

According to Boya et al. (1999), serum arylesterase (AE) and PON-1 activities have decreased in the chronic and cirrhotic hepatitis C patients compared to the control group

Some recent studies are in agreement with the present investigation such as Gangadharan, Antrobus, Dwek, & Zitzmann, (2007) who found a decreasing in serum AE and PON-1 activities in chronic and cirrhotic HCV patients compared with healthy controls. The study proposed that serum AE and PON-1 carried in circulation bounded to HDL particles protect LDL from peroxidation, supported by Mackness, Durrington, & Mackness, (2004). Thus, there are two possible explanations for decreased PON-1 and AE activities in chronic hepatitis. Firstly, the decrease in PON-1 and AE enzymatic activities or gene expression could be the consequence of the hepatic dysfunction. The second is that serum PON and AE activities could be decreased as a consequence of an altered synthesis and/or secretion of HDL (Ali et al., 2009).

PON-1 and AE act as antioxidants that protect LDL from oxidative modifications and can reduce oxidized lipid in oxidized lipoproteins (Mackness et al., 2004; Kilic, Aydin, Kilic, Erman, Aydin, & Celik, 2005). Other important observation in the study by Ali et al., (2009) was that the activities of both enzymes have significantly decreased in cirrhotic more than in chronic HCV patients, indicating that the level of decrease in AE and PON-1 activity in the serum of patients with chronic liver diseases is probably a consequence of liver dysfunction. That could emphasize the prognostic value of AE and PON-1 in HCV infected patients and give clinicians a signal for deterioration of the liver function with the progression of the disease. The results also demonstrated that serum AE was negatively correlated with serum ALT and aspartate aminotransferase (AST) activities; whereas serum PON-1 was negatively correlated with serum ALT, AST and ALP activities and serum total bilirubin and direct bilirubin levels in HCV patients group. Kilic, Aydin, Kilic, Erman, Aydin, & Celik, (2005) are in agreement with Ali et al., (2009) that serum AE and PON-1 activities were also negative correlated with serum ALT and AST activities and albumin and bilirubin concentrations as well as between serum PON-1 and ALP activity.

Ali et al. (2009) states that a negative significant correlation was found between PON-1 and MDA in HCV patients. Ferré et al. (2006) reported that, increased serum PON-1 concentrations and raised hepatic PON-1 expression were associated with higher peroxidation in serum and liver biopsies. On the other hand, Başkol et al., (2005) found no statistically significant correlations between hepatic PON-1 activity and serum lipid levels or serum MDA levels in patients with non-alcoholic steatohepatitis. Moreover, PON-1 has been found to influence the anti-apoptotic ability of the HDL molecule, because it has the capacity to protect the lipoprotein against oxidation.

PON-1 has an active role in the regulation of oxidative stress, fibrosis and hepatic cell apoptosis in chronic liver diseases (Ferré et al., 2006). Marsillach et al. (2007) reported that, PON-1 activity measurement would be very useful in discriminating between alcoholic patients and healthy subjects.

When studying the ROC curves of both AE and PON-1, it was found that the areas under the curves were 0.968 and 0.966 respectively, and best cutoff values of serum AE and PON-1 were 764 and 482 nmol/min/ml respectively. Applying these cutoff values, the maximum sensitivity, specificity, positive predictive and negative predictive values of (AE and PON-1) were (86% and 79.5%), (90% and 100%), (95% and 100%) and (76% and 70%) respectively (Ali et al., 2009).

The study by Marsillach et al., (2007) reported that, PON-1 activity measurement would be very useful in discriminating between alcoholic patients and healthy subjects; on studying the ROC curve of PON-1 activity, the area under the curve of PON-1 activity was ≥0.90, the increased sensitivity up to 83.5–96.6% and specificity up to 95.8–99.6%. AE was the hepatitis C biomarker marker which showed the highest sensitivity, when measured as a single marker without any combination with others, while MDA or PON-1 was the marker which gave the highest specificity, when each of them was measured alone without combination. But in combination, the best combined ones for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were MDA plus albumin, PON-1 plus albumin and PON-1 plus AST (Marsillach et al., 2007).

The over release of the oxidative free radicals [MDA, nitric oxide (NO) and myeloperoxidase (MPO)] and the decrement of the antioxidants (PON-1 and AE) might play a role in the pathogenesis of viral hepatitis. Moreover, these markers are beneficial in testing liver dysfunction and detecting high-risk patients of cirrhotic from chronic HCV liver disease patients (Ali et al., 2009; Pyati, 2015).

Both PON-1 and AE could be considered as prognostic biomarkers of oxidative stress in HCV infection. If any abnormality of their levels is detected in chronic patients that could be a warning sign for occurrence of cirrhosis. Even more, changes in the levels of these markers in a normal person can give a risk sign to detect any early liver dysfunction. Therefore, these markers could produce a diagnostic and prognostic index which may improve sensitivity and specificity when combined with the other conventional liver function markers. Moreover, inclusion of antioxidants as adjunct in the management of chronic and cirrhotic HCV patients should be planned to prevent progressive deterioration and target liver damage (Ali et al., 2009).

2.2.3 – PON-1 ACTIVITY AND HBV INFECTIONS

Karsen et al. (2012) proposed that, serum PON-1 concentration was considerably lower in patients with chronic hepatitis B than in the control group. The results presented that patients with chronic hepatitis B are exposed to oxidative stress and showed decreased PON-1 activity. The study proposes that predisposal factors may also, in part, play a role in the pathogenesis of atherosclerosis in patients with chronic hepatitis B.

There is a well-established relation between the enzyme PON-1 in cardiovascular diseases and atherosclerosis (Michael I Mackness et al., 2004; Yilmaz, 2012; Kim, Marsillach, Furlong, & Jarvik, 2013; Kulka, 2016). (Draganov & La Du, 2004). HDL PON-1 activity is shown to be associated with modulation of endothelial functions and regulation of coronary vasomotor tone. (Malin et al., 2001; Pasqualini et al., 2005) Increased incidence of subclinical atherosclerosis in carotid arteries has been reported in patients with chronic hepatitis B (Alkhouri et al., 2010). On the other hand, other studies have been unable to demonstrate any difference between chronic hepatitis B and controls and indicated that HBs Ag seropositivity was not related with increased mortality risks of atherosclerosis related cardiovascular diseases (Wang, Chen, Lee, Yang & Hsiao, 2010).

The reduction of PON-1 activity under oxidative stress is an independent risk factor for coronary arterial disease and mostly attributed to changes in the redox status of the free sulfhydryl groups of proteins since sulfhydryl prevent the inhibition of PON-1 activity (Mackness et al., 2001). Free sulfhydryl groups of proteins constitute the main antioxidant component of serum and have been shown to be associated with the coronary heart disease (Rozenberg & Aviram, 2006).

Free radicals are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms. Organisms are protected against oxidative stress via enzymatic and non-enzymatic antioxidative mechanisms. Under normal conditions, a balance exists between free radical formation and their removal by antioxidant enzymes and other molecules (Mackness et al., 2001).

Karsen et al. (2012) also develops the claim that to measure impact of oxidative stress, the total antioxidant and oxidant parameters should be taken into consideration instead of individual compounds such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. This approach is supported by the fact of antioxidants act in combination with each other affecting total capacity and producing synergistic or antagonistic effects. Also, according to Karsen et al. (2012), free sulfhydryl

groups in plasma, and total antioxidant capacity levels were significantly lower in chronic hepatitis B subjects than observed in the control group, while LOOH, total oxidant status levels and oxidative stress index were significantly higher. These results represent the severe oxidative stress in patients with chronic hepatitis B infection. On one hand, chronic hepatitis B is the most common cause of hepatocellular carcinoma but on the other hand it could contribute to the development of atherosclerosis (Karsen et al., 2012).

In light of the results of the study by Karsen et al. (2012) it is possible to determine that oxidative stress is increased, while serum PON-1 is decreased, in chronic hepatitis B patients. The decrease in paraoxonase activity could contribute to the accelerated development of atherosclerosis in patients with chronic hepatitis B. This same conclusions are also stated by other authors (Farid & Horii, 2012)

CONCLUSIONS

Several studies conclude that significant alterations occur on the enzymatic activity of PON-1 in HIV, HBV and HCB infected patients. This modifications seem to occur due to the increased levels caused by the infections on the organism, leading to multiple disruption on antioxidant and anti-inflammatory pathways that seem were demonstrated to involve PON-1.

Although PON-1 activity alterations seem to be caused by malignant causes, positive and useful clinical information might be extracted by measuring such alterations. PON-1 possibly will be used with more research in future as a biomarker.

In HIV infections, there are well established markers for evaluating the infection status, however the insight provided by the additional monitoring of PON-1 activity could, in fact, be extremely useful. This additional assessment might lead to the prevention of the most common complications due to the chronic use of ART, such as cardiovascular disease and dyslipidemias. This prevention can be achieved by obtaining a more suitable diagnostic tool, for the disease progression and all its complications and by doing so, applying preventive measures that could avoid such complications (Coll et al., 2006; Parra et al., 2007; Pereira et al., 2009; Dias et al., 2014).

In regards to clarifying whether PON-1 could be used as a biomarker for HBV or HCV infections, several works including those of Ali et al., (2009), Pyati, (2015) Marsillach et al., (2007) demonstrated that the precision of PON-1 as a marker for disease is similar to those of ALT or bilirubin, already well accepted and used parameters of analysis (Pyati, 2015).

The enzyme PON-1, in addition to its possible biomarker applications in both HIV, HBV and HCV infections, revealed an extremely important role in the oxidative stress caused by those infections. As an enzyme with anti-oxidant proprieties and closely involved in regulating LDL peroxidation, in both cases the activity of the enzyme was closely associated with the progression of the disease which can definitely provide useful analytic tools for the diagnose and evaluation of those patients (Ferré et al., 2006; Mackness, Arrol, Abbott, & Durrington, 1993; Valyi-Nagy & Dermody, 2005).

More studies in this area are needed in order to better access whether PON-1 is worth to be taken into account or should be disregarded. However, the ground work done so far presents very positive outcomes for the future.

3. REFERENCES

ALDRIDGE, W. N. (1953). Serum esterases. II. An enzyme hydrolysing diethyl p-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *The Biochemical Journal*, *53*(1), 117–24. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13032042

Ali, E. M. M., Shehata, H. H., Ali-Labib, R., Esmail Zahra, L. M. & Zahra, L. M. E. (2009). Oxidant and antioxidant of arylesterase and paraoxonase as biomarkers in patients with hepatitis C virus. *Clinical Biochemistry*, *42*(13–14), 1394–1400. http://doi.org/10.1016/j.clinbiochem.2009.06.007

Alkhouri, N., Tamimi, T. A.-R., Yerian, L., Lopez, R., Zein, N. N. & Feldstein, A. E. (2010). The inflamed liver and atherosclerosis: a link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk., *55*(9). http://doi.org/10.1007/s10620-009-1075-y

Alonso-Villaverde, C., Coll, B., Gómez, F., Parra, S., Camps, J., Joven, J. & Masana, L. (2005). The efavirenz-induced increase in HDL-cholesterol is influenced by the multidrug resistance gene 1 C3435T polymorphism. *AIDS (London, England)*, *19*(3), 341–2. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15718846

Alonso-Villaverde, C., Segues, T., Coll-Crespo, B., Perez-Bernalte, R., Rabassa, A., Gomila, M., … Masana, L. (2003). High-density lipoprotein concentrations relate to the clinical course of HIV viral load in patients undergoing antiretroviral therapy. *AIDS (London, England)*, *17*(8), 1173–1178. http://doi.org/10.1097/01.aids.0000060360.78202.05

Assmann, G., Schulte, H., Von Eckardstein, A., & Huang, Y. (1996). High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis*, *124*(SUPPL.). http://doi.org/10.1016/0021-9150(96)05852-2

Aviram, M., Billecke, S., Sorenson, R., Bisgaier, C., Newton, R., Rosenblat, M., … La Du, B. (1998). Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *18*(10), 1617–1624. http://doi.org/10.1161/01.ATV.18.10.1617

Aviram, M., Rosenblat, M., Billecke, S., Erogul, J., Sorenson, R., Bisgaier, C. L., … La Du, B. (1999). Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radical Biology & Medicine*, *26*(7–8), 892–904. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10232833

Aviram, M., Rosenblat, M., Bisgaier, C. L., Newton, R. S., Primo-Parmo, S. L. & La Du, B. N. (1998). Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions: A possible peroxidative role for paraoxonase. *Journal of Clinical Investigation*, *101*(8), 1581–1590. http://doi.org/10.1172/JCI1649

B, J. J. C. P. R. S., & Aldridge, B. Y. W. N. (1953). Serum Esterases 1., (1949), 0–1.

Başkol, M., Başkol, G., Deniz, K., Ozbakir, O., & Yücesoy, M. (2005). A new marker for lipid peroxidation: serum paraoxonase activity in non-alcoholic steatohepatitis. *The Turkish Journal of Gastroenterology : The Official Journal of Turkish Society of Gastroenterology*, *16*(3), 119–23. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16245219

Bobin-Dubigeon, C., Biron, C., Volteau, C., Piroth, L., Biron, A., Perre, P., … Bard, J. M. (2013). Short communication: Paraoxonase 1 (PON1) in French HIV-infected patients under antiretroviral therapy: relationship with the metabolic syndrome and inflammation. *AIDS Research and Human Retroviruses*, *29*(12), 1571–1574. http://doi.org/10.1089/AID.2013.0010

Boya, P., de la Peña, A., Beloqui, O., Larrea, E., Conchillo, M., Castelruiz, Y., … Prieto, J. (1999). Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis C. *Journal of Hepatology*, *31*(5), 808–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10580577

Çakatay, U., Kayali, R. & Uzun, H. (2008). Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. *Clinical and Experimental Medicine*, *8*(1), 51–57. http://doi.org/10.1007/s10238-008-0156-0

Camps, J., Marsillach, J., & Joven, J. (2009). The paraoxonases: role in human diseases and methodological difficulties in measurement. *Critical Reviews in Clinical Laboratory Sciences*, *46*(2), 83–106. http://doi.org/Pii 909183322\rDoi 10.1080/10408360802610878

Cardin, R., Saccoccio, G., Masutti, F., Bellentani, S., Farinati, F., & Tiribelli, C. (2001). DNA oxidative damage in leukocytes correlates with the severity of HCV-related liver disease: validation in an open population study. *Journal of Hepatology*, *34*(4), 587–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11394660

Carr, A., & Cooper, D. A. (2000). Adverse effects of antiretroviral therapy. *Lancet (London, England)*, *356*(9239), 1423–30. http://doi.org/10.1016/S0140-6736(00)02854-3

Castaldo, E. T., & Chari, R. S. (2006). Liver transplantation for acute hepatic failure. *HPB : The Official Journal of the International Hepato Pancreato Biliary Association*, *8*(1), 29–34. http://doi.org/10.1080/13651820500465741

Cervellati, C., Romani, A., Bergamini, C. M., Bosi, C., Sanz, J. M., Passaro, A., & Zuliani, G. (2015). PON-1 and ferroxidase activities in older patients with mild cognitive impairment, late onset Alzheimer's disease or vascular dementia. *Clinical Chemistry and Laboratory Medicine*, *53*(7), 1049–56. http://doi.org/10.1515/cclm-2014-0803

Chambers, J. E. (2008). PON1 multitasks to protect health. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(35), 12639–40. http://doi.org/10.1073/pnas.0807062105

Chevaliez, S., & Pawlotsky, J.-M. (2006). *HCV Genome and Life Cycle*. *Hepatitis C Viruses: Genomes and Molecular Biology*. Horizon Bioscience. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21250393

Coffin, J. M., Hughes, S. H., & Varmus, H. E. (1997). *Retroviruses*. *Retroviruses*. Cold Spring Harbor Laboratory Press. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21433340

Coll, B., Alonso-Villaverde, C., Parra, S., Montero, M., Tous, M., Joven, J., & Masana, L. (2005). The stromal derived factor-1 mutated allele (SDF1-3'A) is associated with a lower incidence of atherosclerosis in HIV-infected patients. *AIDS (London, England)*, *19*(16), 1877–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16227796

Coll, B., Parra, S., Alonso-Villaverde, C., de Groot, E., Aragonés, G., Montero, M., … Masana, L. (2006). HIV-infected patients with lipodystrophy have higher rates of carotid atherosclerosis: the role of monocyte chemoattractant protein-1. *Cytokine*, *34*(1–2), 51–5. http://doi.org/10.1016/j.cyto.2006.03.013

Cost Considerations and Antiretroviral Therapy | Adult and Adolescent ARV Guidelines | AIDSinfo. (n.d.). Retrieved from https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/459/cost-considerations-and-antiretroviral-therapy

Daminelli, E. N., Spada, C., Treitinger, A., Oliveira, T. V., Latrilha, M. D. C., & Maranhão, R. C. (2008). Alterations in lipid transfer to high-density lipoprotein (HDL) and activity of paraoxonase-1 in HIV+ patients. *Revista Do Instituto de Medicina Tropical de Sao Paulo*, *50*(4), 223–227. http://doi.org/10.1590/S0036-46652008000400007

De Maria, N., Colantoni, A., Fagiuoli, S., Liu, G. J., Rogers, B. K., Farinati, F., … Floyd, R. A. (1996). Association between reactive oxygen species and disease activity in chronic hepatitis C. *Free Radical Biology & Medicine*, *21*(3), 291–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8855439

Depairon, M., Chessex, S., Sudre, P., Rodondi, N., Doser, N., Chave, J. P., … Mooser, V. (2001). Premature atherosclerosis in HIV-infected individuals--focus on protease inhibitor therapy. *Aids*, *15*(3), 329–334.

Dias, C., Marinho, A., Morello, J., Almeida, G., Caixas, U., Soto, K., … Pereira, S. (2014). Monitoring of the lactonase activity of paraoxonase-1 enzyme in HIV-1-infection. *Journal of the International AIDS Society*, *17*(4 Suppl 3), 19682. http://doi.org/10.7448/ias.17.4.19682

Downes, K. J., & Shah, S. S. (2012). Biomarkers in Infectious Diseases. *Journal of the Pediatric Infectious Diseases Society*, *1*(4), 343–6. http://doi.org/10.1093/jpids/pis099

Draganov, D. I., & La Du, B. N. (2004). Pharmacogenetics of paraoxonases: a brief review. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *369*(1), 78–88. http://doi.org/10.1007/s00210-003-0833-1

EASL. (2012). EASL Clinical Practice Guidelines : Management of chronic hepatitis B virus infection. *Journal of Hepatology*, *57*(1), 167–185. http://doi.org/10.1016/j.jhep.2012.02.010

Eckerson, H. W., Wyte, C. M., & La Du, B. N. (1983). The human serum paraoxonase/arylesterase polymorphism. *American Journal of Human Genetics*, *35*(6), 1126–1138. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6316781

Farid, A., & Horii, Y. (2012). Modulation of paraoxonases during infectious diseases and its potential impact on atherosclerosis. *Lipids in Health and Disease*, *11*, 92. http://doi.org/10.1186/1476-511X-11-92

Fauci AS, Kasper DL, Longo DL, B. E., & Hauser SL, Jameson JL, et al. editors. (2015). Harrison's principles of internal medicine. *McGraw Hill Medical*, *2*(17), 1955–69.

FDA. (2016). FDA-Approved HIV Medicines | HIV/AIDS Fact Sheets | Education Materials | AIDSinfo. Retrieved from https://aidsinfo.nih.gov/education-materials/fact-sheets/21/58/fda-approved-hiv-medicines

Feingold, K. R., & Grunfeld, C. (1992). Role of cytokines in inducing hyperlipidemia. *Diabetes*, *41*(SUPPL. 2), 97–101.

Feingold, K. R., Memon, R. A., Moser, A. H., & Grunfeld, C. (1998). Paraoxonase activity in the serum and hepatic mRNA levels decrease during the acute phase response. *Atherosclerosis*, *139*(2), 307–315. http://doi.org/10.1016/S0021-9150(98)00084-7

Fernández-Hidalgo, N., Almirante, B., & Pahissa, A. (2009). Reply to Lomas et al. *Clinical Infectious Diseases*, *48*(8), 1166–1166. http://doi.org/10.1086/597501

Ferré, N., Camps, J., Cabré, M., Paul, A., & Joven, J. (2001). Hepatic paraoxonase activity alterations and free radical production in rats with experimental cirrhosis. *Metabolism: Clinical and Experimental*, *50*(9), 997–1000. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11555827

Ferré, N. L., Camps, J., Prats, E., Vilella, E., Paul, A., Figuera, L., & Joven, J. (2002). Serum Paraoxonase Activity: A New Additional Test for the Improved Evaluation of Chronic Liver Damage, *48*(2), 261–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11805006

Ferré, N., Marsillach, J., Camps, J., Mackness, B., Mackness, M., Riu, F., … Joven, J. (2006). Paraoxonase-1 is associated with oxidative stress, fibrosis and FAS expression in chronic liver diseases. *Journal of Hepatology*, *45*(1), 51–59. http://doi.org/10.1016/j.jhep.2005.12.018

Ferré, N., Marsillach, J., Camps, J., Rull, A., Coll, B., Tous, M., & Joven, J. (2005). Genetic association of paraoxonase-1 polymorphisms and chronic hepatitis C virus infection. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *361*(1–2), 206–10. http://doi.org/10.1016/j.cccn.2005.05.024

Gangadharan, B., Antrobus, R., Dwek, R. A., & Zitzmann, N. (2007). Novel serum biomarker candidates for liver fibrosis in hepatitis C patients. *Clinical Chemistry*, *53*(10), 1792–9. http://doi.org/10.1373/clinchem.2007.089144

Garg, H., Sarin, S. K., Kumar, M., Garg, V., Sharma, B. C., & Kumar, A. (2011). Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. *Hepatology (Baltimore, Md.)*, *53*(3), 774–80. http://doi.org/10.1002/hep.24109

Gil, F., Pla, A., Gonzalvo, M. C., Hernández, A. F., & Villanueva, E. (1993). Rat liver paraoxonase: subcellular distribution and characterization. *Chemico-Biological Interactions*, *87*(1–3), 149–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8393736

Grimm, D., Thimme, R., & Blum, H. E. (2011). HBV life cycle and novel drug targets. *Hepatology International*, *5*(2), 644–653. http://doi.org/10.1007/s12072-011-9261-3

Harel, M., Aharoni, A., Gaidukov, L., Brumshtein, B., Khersonsky, O., Meged, R., … Tawfik, D. S. (2004). Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. *Nature Structural & Molecular Biology*, *11*(5), 412–9. http://doi.org/10.1038/nsmb767

Hassett, C., Richter, R. J., Humbert, R., Chapline, C., Crabb, J. W., Omiecinski, C. J., & Furlong, C. E. (1991). Characterization of cDNA clones encoding rabbit and human serum paraoxonase: the mature protein retains its signal sequence. *Biochemistry*, *30*(42), 10141–10149. http://doi.org/10.1021/bi00106a010

Hepatitis B and C - Hepatitis B and C Treatments. (2016). Retrieved October 15, 2016, from http://www.fda.gov/forpatients/illness/hepatitisbc/ucm408658.htm

Jain, S., Gautam, V., & Naseem, S. (2011). Acute-phase proteins: As diagnostic tool. *Journal of Pharmacy & Bioallied Sciences*, *3*(1), 118–127. http://doi.org/10.4103/0975-7406.76489

Jakubowski, H. (1997). Metabolism of homocysteine thiolactone in human cell cultures: Possible mechanism for pathological consequences of elevated homocysteine levels. *Journal of Biological Chemistry*, *272*(3), 1935–1942. http://doi.org/8999883

Jakubowski, H. (2000). Calcium-dependent human serum homocysteine thiolactone hydrolase. *The Journal of Biological Chemistry*, *275*(6), 3957–3962. http://doi.org/10.1074/jbc.275.6.3957

Jakubowski, H., & G??owacki, R. (2011). Chemical Biology of Homocysteine Thiolactone and Related Metabolites. *Advances in Clinical Chemistry*, *55*, 81–103. http://doi.org/10.1016/B978-0-12-387042-1.00005-8

Karabina, S. A. P., Lehner, A. N., Frank, E., Parthasarathy, S., & Santanam, N. (2005). Oxidative inactivation of paraoxonase - Implications in diabetes mellitus and atherosclerosis. *Biochimica et Biophysica Acta - General Subjects*, *1725*(2), 213–221. http://doi.org/10.1016/j.bbagen.2005.07.005

Karsen, H., Binici, I., Sunnetcioglu, M., Baran, A. I. I., Ceylan, M. R. R., Selek, S., & Celik, H. (2012). Association of paraoxonase activity and atherosclerosis in patients with chronic hepatitis B. *African Health Sciences*, *12*(2), 114–118. http://doi.org/10.4314/ahs.v12i2.6

Kilic, S. S., Aydin, S., Kilic, N., Erman, F., Aydin, S., Suleyman, İ. C., … Celik, İ. (2005). Serum arylesterase and paraoxonase activity in patients with chronic hepatitis. *World J Gastroenterol World Journal of Gastroenterology ISSN J Gastroenterol*, *11*(1146), 7351–7354. Retrieved from www.wjgnet.com

Kilic, S. S., Aydin, S. S., Kilic, N., Erman, F., Aydin, S. S., & Celik, I. (2005). Serum arylesterase and paraoxonase activity in patients with chronic hepatitis. *World Journal of Gastroenterology*, *11*(46), 7351–7354. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16437641

Kim, D. S., Marsillach, J., Furlong, C. E., & Jarvik, G. P. (2013). Pharmacogenetics of paraoxonase activity: elucidating the role of high-density lipoprotein in disease. *Pharmacogenomics*, *14*(12), 1495–515. http://doi.org/10.2217/pgs.13.147

Kulka, M. (2016). A review of paraoxonase 1 properties and diagnostic applications, *19*(1), 225–232. http://doi.org/10.1515/pjvs-2016-0028

Laborde, C. M., Mourino-Alvarez, L., Akerstrom, F., Padial, L. R., Vivanco, F., Gil-Dones, F., & Barderas, M. G. (2012). Potential blood biomarkers for stroke. *Expert Review of Proteomics*, *9*(4), 437–49. http://doi.org/10.1586/epr.12.33

Lang, S., Mary-Krause, M., Cotte, L., Gilquin, J., Partisani, M., Simon, A., … Costagliola, D. (2010). Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS*, *24*(8), 1228–1230. http://doi.org/10.1097/QAD.0b013e328339192f

Le Moing, V., Thiébaut, R., Chêne, G., Sobel, A., Massip, P., Collin, F., … Choutet, P. (2007). Long-term evolution of CD4 count in patients with a plasma HIV RNA persistently <500 copies/mL during treatment with antiretroviral drugs. *HIV Medicine*, *8*(3), 156–163. http://doi.org/10.1111/j.1468-1293.2007.00446.x

Lenten, B. J. Van, Hama, S. Y., Beer, F. C., Stafforini, D. M., Mcintyre, T. M., Prescott, S. M., … Navab, M. (1995). Anti-inflammatory HDL Becomes Pro-inflammatory during the Acute Phase Response. *Journal of Clinical Investigation*, *96*, 2758–2767.

Lenten, B. J. Van, Wagner, A. C., Nayak, D. P., Hama, S., Navab, M., & Fogelman, M. (2001). High-Density Lipoprotein Loses Its Anti-Inflammatory, 2283–2289.

Lessire, F., Gustin, P., Delaunois, A., Bloden, S., Nemmar, A., Vargas, M., & Ansay, M. (1996). Relationship between parathion and paraoxon toxicokinetics, lung metabolic activity, and cholinesterase inhibition in guinea pig and rabbit lungs. *Toxicology and Applied Pharmacology*, *138*(2), 201–10. http://doi.org/10.1006/taap.1996.0118

Levent, G., Ali, A., Ahmet, A., Polat, E. C., Aytaç, C., Ayşe, E., & Ahmet, S. (2006). Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. *Journal of Translational Medicine*, *4*, 25. http://doi.org/10.1186/1479-5876-4-25

Locarnini, S. (2004). Molecular virology of hepatitis B virus. *Seminars in Liver Disease*, *24 Suppl 1*(4), 3–10. http://doi.org/10.1055/s-2004-828672

Loguercio, C., & Federico, A. (2003). Oxidative stress in viral and alcoholic hepatitis. *Free Radical Biology & Medicine*, *34*(1), 1–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12498974

Mackness, B., Davies, G. K., Turkie, W., Lee, E., Roberts, D. H., Hill, E., … Mackness, M. I. (2001). Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *21*(9), 1451–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11557671

Mackness, M. I., Arrol, S., Abbott, C., & Durrington, P. N. (1993). Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*, *104*(1–2), 129–135. http://doi.org/10.1016/0021-9150(93)90183-U

Mackness, M. I., Arrol, S., & Durrington, P. N. (1991). Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Letters*, *286*(1–2), 152–154. http://doi.org/10.1016/0014-5793(91)80962-3

Mackness, M. I., & Durrington, P. N. (1995). HDL, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis*, *115*(2), 243–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7661883

Mackness, M. I., Durrington, P. N., & Mackness, B. (2004). The role of paraoxonase 1 activity in cardiovascular disease: potential for therapeutic intervention. *American Journal of Cardiovascular Drugs : Drugs, Devices, and Other Interventions*, *4*(4), 211–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15285696

Malin, R., Knuuti, J., Janatuinen, T., Laaksonen, R., Vesalainen, R., Nuutila, P., … Lehtimäki, T. (2001). Paraoxonase gene polymorphisms and coronary reactivity in young healthy men. *Journal of Molecular Medicine (Berlin, Germany)*, *79*(8), 449–58. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11511975

Mansurova, F. K., Mutikhova, K. S., & Olimova, S. O. (2005). [Lipid peroxidation and anti-oxidative protection in patients with chronic type C hepatitis]. *Klinicheskaia Meditsina*, *83*(5), 39–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15984581

Marinho, A. T., Dias, C. G., Pinheiro, P. F., Lemos, A. R., Antunes, A. M. M., Marques, M. M., … Pereira, S. A. (2016). Nevirapine modulation of paraoxonase-1 in the liver: An in vitro three-model approach. *European Journal of Pharmaceutical Sciences*, *82*, 147–153. http://doi.org/10.1016/j.ejps.2015.11.019

Marsillach, J., Ferré, N., Vila, M. C., Lligoña, A., Mackness, B., Mackness, M., … Camps, J. (2007). Serum paraoxonase-1 in chronic alcoholics: Relationship with liver disease. *Clinical Biochemistry*, *40*(9–10), 645–650. http://doi.org/10.1016/j.clinbiochem.2007.01.020

Marsillach, J., Parra, S., Coll, B., Joven, J., & Camps, J. (2008). Chapter 11 Paraoxonase-1 in Chronic Liver Diseases , Neurological Diseases and Hiv Infection, 187–198.

Maselli, L. M. F., Cunha, J. Da, Gutierrez, E. B., Maranh??o, R. C., Spada, C., & Bydlowski, S. P. (2014). Human paraoxonase-1 activity is related to the number of CD4+ T-cells and is restored by antiretroviral therapy in HIV-1-infected individuals. *Disease Markers*, *2014*. http://doi.org/10.1155/2014/480201

MAZUR, A. (1946). An enzyme in animal tissues capable of hydrolysing the phosphorus-fluorine bond of alkyl fluorophosphates. *The Journal of Biological Chemistry*, *164*, 271–89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20989488

Miyamoto, T., Takahashi, Y., Oohashi, T., Sato, K., & Oikawa, S. (2005). Bovine paraoxonase 1 activities in serum and distribution in lipoproteins. *J Vet Med Sci.*, *67*(3), 243–8. http://doi.org/10.1292/jvms.67.243

Ng, C. J., Shih, D. M., Hama, S. Y., Villa, N., Navab, M., & Reddy, S. T. (2005). The paraoxonase gene family and atherosclerosis. *Free Radical Biology & Medicine*, *38*(2), 153–63. http://doi.org/10.1016/j.freeradbiomed.2004.09.035

Novak, F., Vavrova, L., Kodydkova, J., Hynkova, M., Zak, A., & Novakova, O. (2010). Decreased paraoxonase activity in critically ill patients with sepsis. *Clinical and Experimental Medicine*, *10*(1), 21–25. http://doi.org/10.1007/s10238-009-0059-8

Olszewski, A. J., & McCully, K. S. (1993). Homocysteine metabolism and the oxidative modification of proteins and lipids. *Free Radical Biology and Medicine*, *14*(6), 683–693. http://doi.org/10.1016/0891-5849(93)90151-J

Ono, a, & Freed, E. O. (2001). Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(24), 13925–30. http://doi.org/10.1073/pnas.241320298

Osman, H. G., Gabr, O. M., Lotfy, S., & Gabr, S. (2007). Serum levels of bcl-2 and cellular oxidative stress in patients with viral hepatitis. *Indian Journal of Medical Microbiology*, *25*(4), 323–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18087079

Otvos, J. D., Jeyarajah, E. J., & Cromwell, W. C. (2002). Measurement issues related to lipoprotein heterogeneity. *The American Journal of Cardiology*, *90*(2), 22i–29i. http://doi.org/10.1016/S0002-9149(02)02632-2

Pan, D., Ma, S., Bo, X., & Guo, L. (2011). Electrochemical behavior of methyl parathion and its sensitive determination at a glassy carbon electrode modified with ordered mesoporous carbon. *Microchimica Acta*, *173*(1–2), 215–221. http://doi.org/10.1007/s00604-011-0551-1

Parra, S., Alonso-Villaverde, C., Coll, B., Ferr??, N., Marsillach, J., Aragon??s, G., … Camps, J. (2007). Serum paraoxonase-1 activity and concentration are influenced by human immunodeficiency virus infection. *Atherosclerosis*, *194*(1), 175–181. http://doi.org/10.1016/j.atherosclerosis.2006.07.024

Pasqualini, L., Cortese, C., Marchesi, S., Siepi, D., Pirro, M., VAUDO, G., … Mannarino, E. (2005). Paraoxonase-1 activity modulates endothelial function in patients with peripheral arterial disease. *Atherosclerosis*, *183*(2). http://doi.org/10.1016/j.atherosclerosis.2005.03.030

Pereira, S. A., Batuca, J. R., Caixas, U., Branco, T., Delgado-Alves, J., Germano, I., … Monteiro, E. C. (2009). Effect of efavirenz on high-density lipoprotein antioxidant properties in HIV-infected patients. *British Journal of Clinical Pharmacology*, *68*(6), 891–897. http://doi.org/10.1111/j.1365-2125.2009.03535.x

Périard, D., Telenti, A., Sudre, P., Cheseaux, J.-J., Halfon, P., Reymond, M. J., … Mooser, V. (1999). Atherogenic Dyslipidemia in HIV-Infected Individuals Treated With Protease Inhibitors. *Circulation*, *100*(7).

Primo-Parmo, S. L., Sorenson, R. C., Teiber, J., & La Du, B. N. (1996). The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics*, *33*(3), 498–507. http://doi.org/S0888754396902256 [pii]

Pyati, A. K. (2015). Serum Basal Paraoxonase 1 Activity as an Additional Liver Function Test for the Evaluation of Patients with Chronic Hepatitis. *Journal of Clinical and Diagnostic Research*, *9*(11), 12–15. http://doi.org/10.7860/JCDR/2015/15917.6850

Riddler, S., Li, X., Chu, H., Kingsley, L., Dobs, A., Evans, R., … Sharrett, A. (2007). Longitudinal changes in serum lipids among HIV-infected men on highly active antiretroviral therapy. *HIV Medicine*, *8*(5), 280–287. http://doi.org/10.1111/j.1468-1293.2007.00470.x

Rose, H., Hoy, J., Woolley, I., Tchoua, U., Bukrinsky, M., Dart, A., & Sviridov, D. (2008). HIV infection and high density lipoprotein metabolism. *Atherosclerosis*, *199*(1), 79–86. http://doi.org/10.1016/j.atherosclerosis.2007.10.018

Rose, H., Woolley, I., Hoy, J., Dart, A., Bryant, B., Mijch, A., & Sviridov, D. (2006). HIV infection and high-density lipoprotein: the effect of the disease vs the effect of treatment. *Metabolism*, *55*(1), 90–95. http://doi.org/10.1016/j.metabol.2005.07.012

Rosenson, R. S., Brewer, H. B., Chapman, M. J., Fazio, S., Hussain, M. M., Kontush, A., … Schaefer, E. J. (2011). HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clinical Chemistry*, *57*(3), 392–410. http://doi.org/10.1373/clinchem.2010.155333

Rozenberg, O., & Aviram, M. (2006). S-Glutathionylation regulates HDL-associated paraoxonase 1 (PON1) activity., *351*(2). http://doi.org/10.1016/j.bbrc.2006.10.059

Sanai, F., & Keeffe, E. (2010). Liver biopsy for histological assessment - the case against. *Saudi Journal of Gastroenterology*, *16*(2), 124. http://doi.org/10.4103/1319-3767.61244

Savès, M., Morlat, P., Chêne, G., Peuchant, E., Pellegrin, I., Bonnet, F., … Beylot, J. (2001). Prognostic Value of Plasma Markers of Immune Activation in Patients with Advanced HIV Disease Treated by Combination Antiretroviral Therapy. *Clinical Immunology*, *99*(3), 347–352. http://doi.org/10.1006/clim.2001.5033

Schwarz, K. B. (1996). Oxidative stress during viral infection: a review. *Free Radic Biol Med*, *21*(5), 641–649. http://doi.org/0891584996001311 [pii]

Shih, D. M., Gu, L., Xia, Y. R., Navab, M., Li, W. F., Hama, S., … Lusis, A. J. (1998). Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature*, *394*(6690), 284–7. http://doi.org/10.1038/28406

Siegel, M. O., Borkowska, A. G., Dubrovsky, L., Roth, M., Welti, R., Roberts, A. D., … Fitzgerald, M. L. (2015). HIV infection induces structural and functional changes in high density lipoproteins. *Atherosclerosis*, *243*(1), 19–29. http://doi.org/10.1016/j.atherosclerosis.2015.08.036

Sierra, S., Kupfer, B., & Kaiser, R. (2005). Basics of the virology of HIV-1 and its replication. *Journal of Clinical Virology*, *34*(4), 233–244. http://doi.org/10.1016/j.jcv.2005.09.004

Simon, V., Ho, D., & Karim, Q. (2010). HIV/AIDS epidemiology, pathogenesis, prevention and treatment. *The Lancet*, *368*(9534), 489–504. http://doi.org/10.1016/S0140-6736(06)69157-5.HIV/AIDS

Stein, J. H., Komarow, L., Cotter, B. R., Currier, J. S., Dubé, M. P., Fichtenbaum, C. J., … Torriani, F. J. (2008). Lipoprotein changes in HIV-infected antiretroviral-naïve individuals after starting antiretroviral therapy: ACTG Study A5152s. *Journal of Clinical Lipidology*, *2*(6), 464–471. http://doi.org/10.1016/j.jacl.2008.08.442

Stein, L. L., & Loomba, R. (2009). Drug targets in hepatitis B virus infection. *Infectious Disorders Drug Targets*, *9*(2), 105–16. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19275699

Strimbu, K., & Tavel, J. A. (2010). What are biomarkers? *Current Opinion in HIV and AIDS*, *5*(6), 463–6. http://doi.org/10.1097/COH.0b013e32833ed177

Tanikawa, K., & Torimura, T. (2006). Studies on oxidative stress in liver diseases: important future trends in liver research. *Medical Molecular Morphology*, *39*(1), 22–7. http://doi.org/10.1007/s00795-006-0313-z

Tassopoulos, N. C., Papaevangelou, G. J., Sjogren, M. H., Roumeliotou-Karayannis, A., Gerin, J. L., & Purcell, R. H. (1987). Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. *Gastroenterology*, *92*(6), 1844–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3569758

Thorén, F., Romero, A., Lindh, M., Dahlgren, C., & Hellstrand, K. (2004). A hepatitis C virus-encoded, nonstructural protein (NS3) triggers dysfunction and apoptosis in lymphocytes: role of NADPH oxidase-derived oxygen radicals. *Journal of Leukocyte Biology*, *76*(6), 1180–6. http://doi.org/10.1189/jlb.0704387

Tillmann, H. L., Hadem, J., Leifeld, L., Zachou, K., Canbay, A., Eisenbach, C., … Manns, M. P. (2006). Safety and efficacy of lamivudine in patients with severe acute or fulminant hepatitis B, a multicenter experience. *Journal of Viral Hepatitis*, *13*(4), 256–63. http://doi.org/10.1111/j.1365-2893.2005.00695.x

Treitinger, a, Spada, C., da Silva, L. M., Hermes, E. M., Amaral, J. a, & Abdalla, D. S. (2001). Lipid and acute-phase protein alterations in HIV-1 infected patients in the early stages of infection: correlation with CD4+ lymphocytes. *The Brazilian Journal of Infectious Diseases : An Official Publication of the Brazilian Society of Infectious Diseases*, *5*(4), 192–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11712964

UNAIDS. (2016). *GLOBAL AIDS UPDATE*.

Usuki, S. (2012). Prospective Role of β-Cell-Specific IGF-1 for Oxidative Stress in the Pathogenesis of Diabetic Neuropathy. *Journal of Diabetes & Metabolism*, *1*(S5). http://doi.org/10.4172/2155-6156.S5-009

Valyi-Nagy, T., & Dermody, T. S. (2005). Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histology and Histopathology*, *20*(3), 957–967.

van Himbergen, T. M., van Tits, L. J. H., Roest, M., & Stalenhoef, A. F. H. (2006). The story of PON1: how an organophosphate-hydrolysing enzyme is becoming a player in cardiovascular medicine. *The Netherlands Journal of Medicine*, *64*(2), 34–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16517986

Vasan, R. S. (2006). Biomarkers of cardiovascular disease: Molecular basis and practical considerations. *Circulation*, *113*(19), 2335–2362. http://doi.org/10.1161/CIRCULATIONAHA.104.482570

Wang, C. C. (2012). Goals of Treatment in the Patient With Chronic HBV Infection. Retrieved from http://www.medscape.org/viewarticle/751570

Wang, C.-H., Chen, C.-J., Lee, M.-H., Yang, H.-I., & Hsiao, C. K. (2010). Chronic hepatitis B infection and risk of atherosclerosis-related mortality: A 17-year follow-up study based on 22,472 residents in Taiwan. *Atherosclerosis*, *211*(2). http://doi.org/10.1016/j.atherosclerosis.2010.03.008

Waris, G., Turkson, J., Hassanein, T., & Siddiqui, A. (2005). Hepatitis C Virus (HCV) Constitutively Activates STAT-3 via Oxidative Stress: Role of STAT-3 in HCV Replication. *Journal of Virology*, *79*(3), 1569–1580. http://doi.org/10.1128/JVI.79.3.1569-1580.2005

WHO. (2016a). WHO | Hepatitis B. Retrieved November 15, 2016, from http://www.who.int/mediacentre/factsheets/fs204/en/

WHO. (2016b). WHO | Hepatitis C.

Yilmaz, N. (2012). Relationship between paraoxonase and homocysteine: Crossroads of oxidative diseases. *Archives of Medical Science*, *8*(1), 138–153. http://doi.org/10.5114/aoms.2012.27294

Zeisel, M., Crouchet, E., Baumert, T., & Schuster, C. (2015). Host-Targeting Agents to Prevent and Cure Hepatitis C Virus Infection. *Viruses*, *7*(11), 5659–5685. http://doi.org/10.3390/v7112898

Zekri, A. N., Youssef, A. S., El-Desouky, E. D., Ahmed, O. S., Lotfy, M. M., Nassar, A. A., & Bahnassey, A. A. (2016). Serum microRNA panels as potential biomarkers for early detection of hepatocellular carcinoma on top of HCV infection. *Tumour Biol*. http://doi.org/10.1007/s13277-016-5097-8