**"OVIDIUS" UNIVERSITY CONSTANTA**
**FACULTY OF NATURE SCIENCES AND AGRICULTURAL SCIENCES**
**DOCTORAL SCHOOL OF APPLIED SCIENCES**
**FUNDAMENTAL FIELD: BIOLOGY-BIOCHEMISTRY**

# PhD. THESIS

# STUDY OF BIOCHEMICAL MARKERS IN CHRONIC LIVER DISEASE ASSOCIATED WITH RHEUMATOID ARTHRITIS

## SUMMARY

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**CONSTANȚA**
**2014**

**STUDY OF BIOCHEMICAL MARKERS IN CHRONIC LIVER DISEASE ASSOCIATED WITH RHEUMATOID ARTHRITIS**

**CONTENT**



Key words: chronic hepatitis C, rheumatoid arthritis, hepatic steatosis, oxidative stress

## OBJECTIVES AND AIM OF THE STUDY

Liver pathology is varied and can be included in several acute or chronic diseases. The studies in general populations show a raised incidence of viral liver diseases which affect a significantly percent from world population. Into classification of hepatic chronic liver diseases, viral hepatitis C infection is considered o real health issue. Another liver disease considered really frequent in general population is liver steatosis which is caused by deposit of lipids in liver cells. Both are considered chronic liver diseases, but with different etiologies, but also conditions that can be associated with other diseases.

Rheumatic pathology is characterized by a chronic inflammation, immunological implications of which include a series of rheumatic diseases affecting both sexes of all ages. This category includes rheumatoid arthritis, chronic inflammatory autoimmune pathology that can be associated with various chronic liver diseases. The monitoring of the liver associated to a rheumatic pathology it is important because the damage of the liver in rheumatoid arthritis may be due to several factors such as toxic drug therapy for liver, the presence of viral infections with liver tropism, autoimmune hepatitis and disturbances in lipid metabolism with development of dyslipidemia and non-alcoholic liver steatosis. Moreover, one must consider the implications of the presence of immunosuppressive therapy for viral infections associated with rheumatic disease. These factors can coexist at some point and can act synergistically on the liver.

A number of studies on liver pathology in the context of rheumatic pathology believe that liver disease may progress to more severe forms of disease, such as cirrhosis and cancer of the liver, serious conditions that lead to complications of the underlying disease, of rheumatic treatment and also altering the functionality of the entire body. Treatment guidelines recommend monitoring liver function in patients with rheumatic diseases to prevent the occurrence of toxic hepatitis secondary to therapy administered.

Various studies on the effects of the disturbances in lipid metabolism and the presence of hepatitis C virus suggests that they could induce a chronic inflammatory process in the body with the occurrence of rheumatic manifestations. Also, research in recent years are emerging new hypotheses about the possible implication of chronic inflammation in rheumatoid arthritis by induction of changes in liver function that result in the emergence of lipid metabolism disorders. Also, researches in recent years have emerging new hypotheses about the possible implication of chronic inflammation involved in rheumatoid arthritis by inducing changes in liver function that result in the emergence of lipid metabolism disorders.

Liver diseases present in patients diagnosed with rheumatoid arthritis have complicated disease and essential treatment. It is important to monitor patients with multiple comorbidities, particularly those related to liver functioning and to identify biochemical markers that may provide a better assessment of the functional status of the whole organism.

Currently, the focus is on new concepts of treatment by applying personalized therapies. For appropriate treatment, it is necessary to study and to understand the pathological mechanisms involved in associated diseases, particularly the mechanisms involved in the development of liver diseases. It is important to identify and the most predictive biomarkers for early diagnosis of the disease and how to apply optimal therapeutic methods to reduce the risk and development of severe liver disease with increased quality of life.

The main objective of this study is the evaluation of biochemical markers and to establish the presence of correlations and patterns (models) between these biochemical markers in the context of chronic liver disease associated with rheumatoid arthritis, which contributes to:

- Understanding the mechanisms underlying these diseases and how that influence and mutually reinforcing each other.
- The improvement of evolution and prognosis of rheumatoid pathology
- Establish appropriate treatment and finding new therapeutic guidelines for rheumatoid arthritis and related diseases.
- Improving patient quality of life

These are the objectives underlying the goals international medical organizations involved in improving the patient quality of life and life expectancy, with fewer deaths due to associated complications.

To achieve the main objective were studied and compared several groups of patients:

- Group of patients diagnosed with rheumatoid arthritis (PR)
- Group of patients diagnosed with hepatic steatosis associated with rheumatoid arthritis (PR + SH)
- Group of patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis (HCVC + PR)
- Group of patients diagnosed with chronic hepatitis C (HCV)
- Group of healthy control patients (M)

In this thesis were conducted two studies whose main objectives were:

**1. Oxidative stress and the variation of biochemical parameters in patients diagnosed with hepatic steatosis associated with rheumatic arthritis**

**2. Oxidative stress and changes in biochemical markers in patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis**

The blood biochemical parameters were determined in laboratory Hospital CF Constanta Port, the following parameters:

- Enzymatic antioxidant: superoxide dismutase (SOD), glutathione peroxidase(GPx), total antioxidant status (TAS)
- Serum glucose (GL)
- Total cholesterol (CT), high-density lipoprotein (HDL), low-density lipoprotein (LDL)
- Triglyceride (TG)
- Serum total protein (TP)
- Iron serum level (Fe)
- Fibrinogen (FIB
- Erythrocyte sedimentation rate( ESR or VSH)
- Transaminases (AST, ALT), γ-glutamyl-transferase (GGT)
- Total bilirubin (BT), direct bilirubin(BD) and indirect bilirubin (BI)
- Alkaline phosphatase (ALP)
- Urea (U)
- Creatinine (CR)
- Uric Acid (AU)

## PART II
## PERSONAL CONTRIBUTIONS

### INTRODUCTION

**The main objective** is the evaluation of liver functional status in rheumatoid arthritis in the context of two comorbidities presence of liver steatosis and chronic hepatitis C.

**Secondary objectives**:

- Evaluation of various biochemical markers and oxidative stress markers in each group of patients.
- Comparison of different biochemical markers and oxidative stress markers analyzed in different groups of patients.
- Creation and establishment of correlations between different biochemical markers.
- Ultrasound evaluation for the presence of liver steatosis in patients with rheumatoid arthritis.

In this thesis were conducted **two studies whose main objectives** were:

1. **Oxidative stress and the variation of biochemical parameters in patients diagnosed with hepatic steatosis associated with rheumatic arthritis**
2. **Oxidative stress and changes in biochemical markers in patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis**

The study was conducted on a total of 93 patients, by performing three experiments. Patients were distributed in several groups according to pathology:

- Patients diagnosed with rheumatoid arthritis (PR)
- Patients diagnosed with chronic hepatitis C virus (HCVC)
- Patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis (HCVC + PR)
- Patients diagnosed with hepatic steatosis associated with rheumatoid arthritis (PR + SH)
- Healthy control group patients (M)

The study was conducted in 2009-2012. For this study we worked with Port CF II Hospital, Internal Medicine Department II and Laboratory Department, based on a contract with Hospital Research Department. The study was conducted with the approval of the Board of Directors of the Hospital. For this study we also have collaborated with a family medicine cabinet.

Enrolled patients were informed about the use of data for research purposes and they have been asked the consent. Statistical evaluations used to validate hypotheses obtained were achieved by Student methods (t-student), analysis of variance one-factor ANOVA and Pearson linear correlation.

### MATERIALS AND METHODS

- Biochemical determinations were performed on serum, plasma and red blood cell lysate.
- Biochemical determinations were carried out on biochemical analyzer DOCHEM 300 and oxidative stress markers have been carried out on biochemical analyzer Randox.
- To determine haematological tests were used the automatic hematology analyzer SYSMEX-K1000.

- To determine fibrinogen was used the automatically machine named Coagulometers ACL-1000.
- Reagent kits used for determination of oxidative stress markers were purchased from Randox Laboratories Company, UK.
- Reagent kits used for determining different biochemical marker are complying with international standards and standardized methods.

## METHODS OF ASSESSMENT

The kits of reagents used were produced by Randox Laboratories, United Kingdom. Reagent kits are intended to be used for manual or semi-automatic method, for the automatic analyzer used were preserved the concentrations of reagents, the ratios between the reactants (reagents and the sample) and the reaction times.Calibration was performed with the calibrator or standard solution, slope factor was built on the ratio between the factor's concentration and the extinction read.The reaction was controlled with control sera. The results were very close to the optimum value (average of the minimum and maximum limits), allowing to be continued the determinations for the enzyme's activities.

To this end, the following parameters were determined in laboratory of Hospital CF Constanta Port:

- Enzymatic antioxidants: superoxide dismutase, glutathione peroxidase, total antioxidant status
- Glucose
- Cholesterol, HDL, LDL, triacylglycerols
- Total protein serum
- Serum iron
- Fibrinogen and erythrocyte sedimentation rate (ESR)
- Aminotransferases (AST, ALT), γ-glutamyltransferase(GGT);
- Total bilirubin, direct bilirubin and indirect bilirubin
- Alkaline phosphatase
- Urea
- Creatinine
- Uric acid

## RESULTS AND DISCUSSIONS

### 6.1 Oxidative stress and the variation of biochemical parameters in patients diagnosed with hepatic steatosis associated with rheumatic arthritis

**Introduction**

The liver is the main organ implied in the lipid metabolism. The expression of the lipid metabolism disturbances in PR is represented by the change of the serum lipids concentration and accumulation of lipids in the hepatic cells that determines the appearance of the hepatic steatosis (SH) and non-alcoholic steatosis (ROSOIU and VERMAN, 2008). The pathogenesis of SH is not known but it is considered that an altered redox balance and oxidative stress are implied (GAMBINO et al., 2011).

It is considered that hepatic steatosis may be implied in the activity of rheumatoid arthritis and can be a negative predictor for attending and maintaining a minimal activity of PR (MINNO et al., 2012). A study on the prevalence, risk factors and hepatic disease features in rheumatic arthritis and psoriasis arthritis shows that the prevalence of the liver disease is increased in this pathology and that the most frequent liver disease is SH (fatty liver) that is a main risk factor in PR (SANTIAGO et al., 2012). Modifications of the lipid metabolism induce disturbances of different systems of the body generating cardio-vascular and hepatic diseases.

Research studies emerged hypothesis that patients with chronic inflammation, as in PR, present an increased risk to develop cardio-vascular diseases. The suppression of chronic inflammation will reduce the cardiovascular risk. The markers of chronic inflammation, even at low levels of plasma concentration in patients diagnosed with PR are predictive factors for coronary events (SATTAR et al., 2003).

In PR, citokines set free from the synovial tissue to the blood circulation (McINNES, 2001) act on distal tissues altering their activity. Citokines may act on the liver, modify the endothelial function or determine the appearance of pro-atherogenic conditions or oxidative stress, modulate the immune response and mediate different metabolic effects. The result is the intense metabolism in different organs and change of lipid metabolism (HILL and et al., 1998).

Prospective studies evaluated the lipid profile in PR patients and demonstrated the presence of a pattern of lipid metabolism disturbance (SATTAR et al., 2003; COJOCARU et al., 2012): reduced total cholesterol, LDL and HDL, increased TG (HELDENBERG et al., 1983). This is not the only pattern described. Studies on the plasma concentrations of CT and TG in rheumatic pathology indicate the presence of increased concentration of both parameters; others show decreased concentration of them; the lipid profile is with an unfavorable atherogenic index CT/HDL more than 4 (TRACY, 2003; KAKAFIKA et al., 2006; NISAR et al., 2012; GEORGIADIS et al., 2006). The mechanisms of PR dyslipidemia are considered to be determined by the action of citokines at tissue level and the dyslipidemia is secondary to chronic inflammation in PR (KHOVIDHUNKIT et al., 2000; LEE et al., 2000). The pro-oxidant model of dyslipidemia in PR associated with citokines activity determines the oxidation of LDL The oxidative activity is revealed by the positive corelations between the reactants of acute phase and negative correlations between the plasma concentration of antioxidant vitamins and acute phase reactants (SATTAR et al., 2003).

Aims of the study:

1. Evaluating the oxidative stress and other biochemical markers in patients with PR versus patients diagnosed wth PR and SH.
2. Quantitative analysis of the results obtained in the two groups for revealing significant differences between them.
3. Comparing the obtained values with reference values provided by the literature.
4. The analysis of statistic correlations between TAS and iron, cholesterol, fibrinogen and between fibrinogen and markers of lipid profile (CT, LDL, HDL, TG, RA)

**Materials and methods**

In this research we evaluated 22 patients diagnosed with PR hospitalized in the Hospital CF Port Constanta, Department of Internal Medicine II.

Exclusion criteria were chronic cardiovascular, respiratory, renal diseases, chronic users of alcohol, other chronic hepatic diseases.

The biological samples were analysed:

- The cytolytic syndrome: ALT, AST,
- Serum iron
- Inflammatory syndrome : fibrinogen, ESR (or VSH)
- Lipid profile: total cholesterol, triacylglycerides (TG), lipoproteins (LDL, HDL, index of atherogenic risk= RA=CT/HDL)
- Evaluation of oxidative stress: total antioxidant status (TAS).
- All the patients were investigated with abdominal ultrasound for revealing steatosis.

Related to the ultrasound investigation there are 2 groups: 12 patients with PR and 10 patients with hepatic steatosis and PR.

For all the patients the average values of the biochemical parameters were determined and compared. The analysis of correlations between markers was performed:

- TAS and Fe (TAS-Fe)
- TAS and total cholesterol (TAS-CT)
- TAS and fibrinogen ( TAS-FIB)
- Fibrinogen and lipid profile (CT, LDL-C, HDL-C, TG, RA)

  The statistic analysis was performed using Student-t Test

**Results and discussion**

The demographic analysis of patients age and gender reveal that females represent the majority (20 females, 90.91%) and only 2 patients are males. The average age is 64.68 16 and there is no statistic significance between the two groups.The abdominal ultrasound investigation of the 22 patients with PR, diagnosed 10 with hepatic steatosis (45.45%).

**The hepatic cytolysis** is revealed by aminotransferases activity with increased values of ALT and AST in the patients that associate the SH, as shown in figures 5 and 6. The statistically significant difference between the two groups show that there is an intracellular stress determined by the presence of hepatocellular lesions resulting in the increased permeability of the cell membrane for these enzymes.

**The serum iron** concentration does not show statistic significant differences between the two groups (fig.7). A tendency of decrease closer to the minimal limits of the normal values is noticed. The liver has an important role in the iron homeostasis. The iron is implied in pro-oxidant mechanisms. The excessive deposits of iron have cytotoxic effects inducing hepatocellular.

**The oxidative stress**. Between the PR and PR+SH groups there is a statistically significant difference for the average values of TAS (fig.4), showing a trend of decrease of the average values in the PR+SH group, revealing an increase of the oxidative stress as a result of the decrease of the antioxidant mechanisms. These are secondary to the existence of a hepatocellular injury due to the hepatic steatosis and iron deposits. The decrease can be explained by their consumption during the process of neutralization of the reactive species of oxygen.

The possibility of the existence of a correlation between the serum concentration of TAS and total cholesterol, TAS and fibrinogen, TAS and iron between the two groups of patients but no correlation was identified.
The presence of the oxidative stress determined by the hepatic steatosis is a negative factor that should be taken into account when anti-rheumatic medication is prescribed, because some of them are toxic for the liver and the fatty liver is vulnerable to faster develop toxicity. There is an interconnection between steatosis and oxidative stress. Thus, the steatosis is progressing to steatohepatitis increasing the oxidative stress, that will determine the evolution to chronic liver disease.

**The inflammatory profile**. The inflammation markers (fibrinogen, ESR) are increased in both groups of patients, but with no significant differences between the average values of the two groups (fig.13, fig.14). The presence of SH presumes a degree of hepatocellular injury and the induction of a degree of inflammation. No significant differences between the two groups were generated.

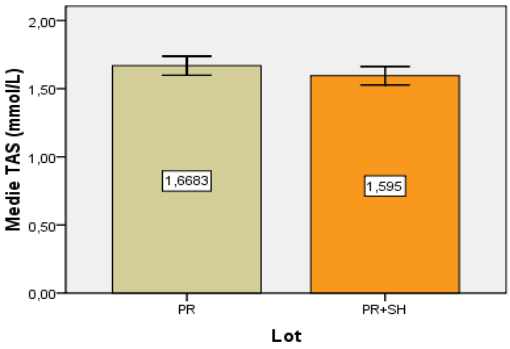
**Lipid profile**. In the speciality research the studies on PR patients describe a dyslipidemia with no specific pattern.There are no statistically significant differences between the lipid status parameters in the analyzed groups PR and PR+SH, but there is a difference for RA=CT/HDL with an increase of this ratio in PR group versus PR+SH (fig.12). Patients with steatosis show an average value of RA close to the upper normal limit, revealing the tendency to develop cardiovascular diseases. The risk is very high for patients with PR.

Evaluation of lipids reveal high concentration of total cholesterol higher than 200mg/dl in 13 patients (59.09%) with 9 (36.36%) higher than 240 mg/dl, with high risk of atherosclerosis (RA) and cardiovascular diseases. LDL pro-atherogenic, is increased in 13 patients (59.09%), HDL anti-atherogenic, is decreased close to the lower normal limit. High values of TG are present in 6 patients (27.27%).

To estimate the atherogenic risk (RA) and the risk to develop cardiovascular diseases the CT/HDL ratio. Values higher than 4, were calculated for 15 patients (68.18%) showing a high risk for cardiovascular disease and death due to cardiovascular cause. Half of the patients presented high values of CT, LDL, RA.

There is an important percentage of patients that associate hyperfibrinogenemia with dyslipidemia (table 14). The existence of correlation between fibrinogen a marker of inflammation and dyslipidemia. No correlation between fibrinogen and TG and HDL but there is a negative correlation between fibrinogen, fibrinogen şi CT ($r = -0,500$, $p = 0,018<0,05$), FIB-LDL ($r = -0,544$, $p = 0,009<0,01$). şi FIB- RA($r = -0,481$, $p = 0,023<0,05$), (table 9-11).

A recent study conducted by JOHNSSON et al., in 2013 in Scotland on a large group with chronic systemic inflammation followed up the variation of CT and HDL with a marker of inflammation (CRP) considering that there is a inverse association between CRP, and CT or HDL. Our study reveals a negative correlation between fibrinogen and CT, LDL and PR confirming this hypothesis.



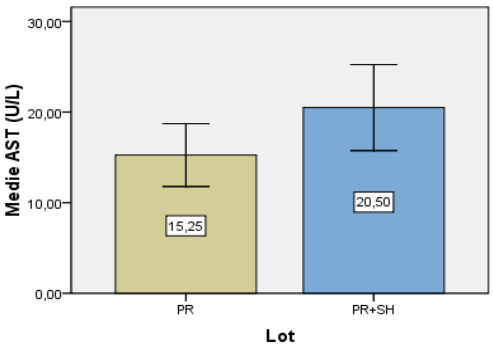
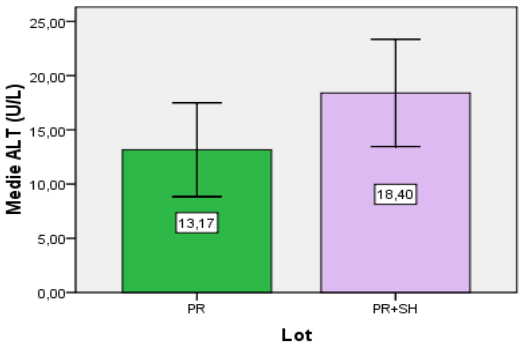




**Fig.4.** TAS- The mean value and standard deviation PR and PR+ SH

**Fig.5.** AST- The mean value and standard deviation PR and PR+ SH



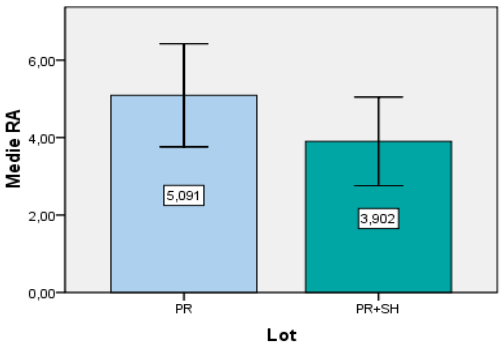
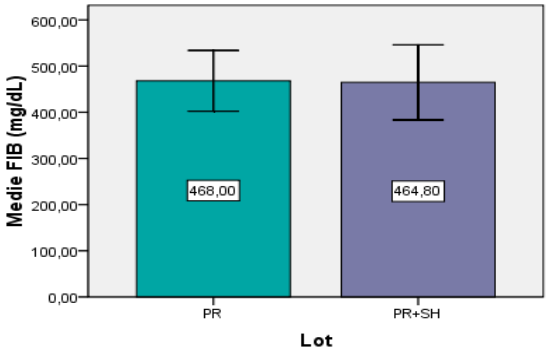




**Fig.6.** ALT- The mean and standard deviation PR and PR+SH

**Fig.12.** RA=CT/HDL - The mean and standard deviation PR and PR+SH



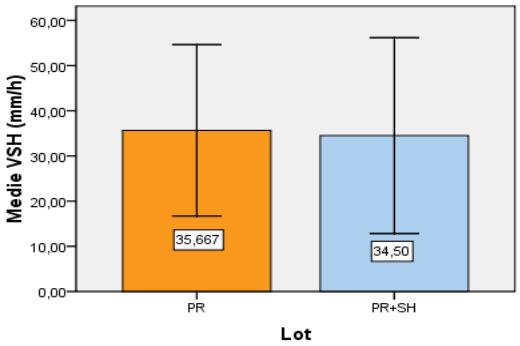




**Fig.13.** FIB- The mean value and standard deviation PR and PR+ SH

**Fig.14.** VSH- The mean value and standard deviation PR and PR+ SH

**Tabel 9.** Statistical correlations between fibrinogen and CT in the general group of patients.

| | | FIB (mg/dL) | CT (mg/dL) |
|---|---|---|---|
| FIB (mg/dL) | Corealația Pearson **(r)** | 1 | -0,500* |
| | Sig. (2-tailed) **(p)** | | 0,018 |
| | N | 22 | 22 |
| CT (mg/dL) | Corealația Pearson | -0,500* | 1 |
| | Sig. (2-tailed) | 0,018 | |
| | N | 22 | 22 |

*.Correlation is significant at the 0.05 level (2-tailed).

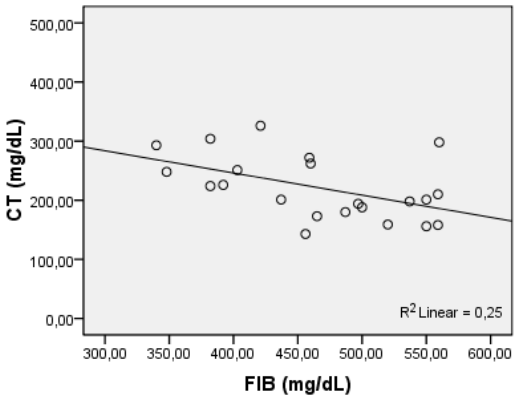



**Fig.15.** Correlations between fibrinogen and CT in the general group of patients

**Tabel 10.** Statistical Correlations between fibrinogen and LDL in the general group of patients

| | | FIB (mg/dL) | LDL (mg/dL) |
|---|---|---|---|
| FIB (mg/dL) | Corealația Pearson (r) | 1 | -0,544** |
| | Sig. (2-tailed) (p) | | 0,009 |
| | N | 22 | 22 |
| LDL (mg/dL) | Corealația Pearson(r) | -0,544** | 1 |
| | Sig. (2-tailed) (p) | 0,009 | |
| | N | 22 | 22 |

**. Correlation is significant at the 0.01 level (2-tailed).

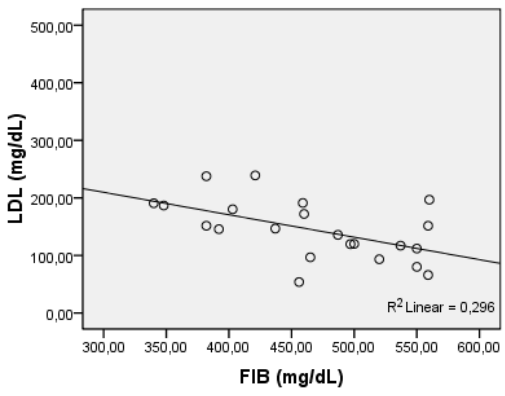



**Fig.16.** Correlations between fibrinogen and LDL in the general group of patients.

**Tabel 11.** Statistical correlations between fibrinogen and RA in the general group of patients.

| | | FIB (mg/dL) | RA |
|---|---|---|---|
| FIB (mg/dL) | Corealația Pearson (r) | 1 | -0,481* |
| | Sig. (2-tailed) (p) | | 0,023 |
| | N | 22 | 22 |
| RA | Pearson Correlation(r) | -0,481* | 1 |
| | Sig. (2-tailed) (p) | 0,023 | |
| | N | 22 | 22 |

*Correlation is significant at the 0.05 level (2-tailed).

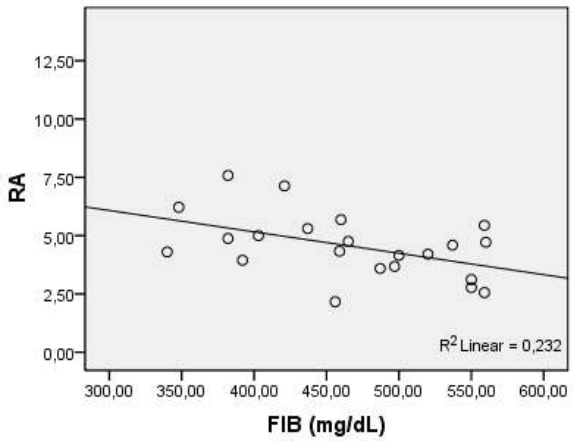



**Fig.17.** Correlations between fibrinogen and RA in the general group of patients.

**Tabel 14.** Hyperfibrinogenemia associated with various dyslipidemias

| Correlations | no. patients | % patients |
|---|---|---|
| Fibrinogen ↑+ cholesterol ↑ | 8 | 36,36% |
| Fibrinogen ↑+ triglycerides ↑ | 5 | 22,73% |
| Fibrinogen ↑+ LDL↑ | 8 | 36,36% |
| Fibrinogen ↑+ RA ↑(>4) | 11 | 50% |

**Conclusions**

1. Analysis by gender and age demographic of this experiment indicate that the majority of patients are women (90.91%). The average age of patients in the study were between 60-70 years old, that is specific to developing rheumatic diseases, but between the two pathological groups, rheumatoid arthritis (PR) and rheumatoid arthritis associated with hepatic steatosis (PR+SH), no statistically significant differences exist.

2. The presence of oxidative stress in the group of patients with PR+SH is reflected in statistically significant differences for TAS and serum transaminases. The increasing trend of these enzymes and decreasing trend of TAS in this group is a sign of the presence of liver injury.

3. There were no correlations between TAS-Fe, TAS-CT and TAS-fibrinogen in the two pathological groups.

4. Hepatic steatosis increases the sensitivity of the liver to extra stress, and it is possible that liver toxicity develop more rapidly after medication used for rheumatoid arthritis. The existence of a vicious circle between hepatic steatosis and oxidative stress results in the progression of hepatic steatosis and increased oxidative stress and will lead to the development of chronic liver disease.

5. The presence of steatosis, oxidative stress and rheumatic therapy can lead to earlier manifestation of toxic hepatitis and therefore the therapy should be supplemented with an antioxidant drug and appropriate diet.

6. Statistical analysis for lipid markers, CT, TG, HDL, LDL, between the two groups of patients shows that statistically significant differences are not present.

7. Statistical analysis for atherogenic risk (RA=CT/HDL) revealed a statistically significant difference between PR and PR+SH groups. Patients with PR+SH have a tendency to develop cardiovascular disease. Patients with PR have a higher atherogenic risk and thus, a higher risk to develop cardiovascular disease.

8. There is a dyslipidemia pattern: hypercholesterolemia, elevated LDL and an unfavorable atherogenic risk ratio in patients with rheumatoid arthritis with or without hepatic steatosis and with an increased risk of developing cardiovascular disease. Half of patients with rheumatoid arthritis are simultaneously presenting elevated levels of CT, LDL and RA.

9. Fibrinogen, a marker of inflammation, is associated in significant percentages with different levels of dyslipidemia; most notably that 50% of patients present an association between hyperfibrinogenemia and increased atherogenic risk.

10. Still, the fibrinogen shows negative correlations with CT, LDL and RA, which confirms the hypothesis that lipid markers decrease with increasing fibrinogen. These results are broadly similar to results from other studies, but the correlations were applied for the other inflammatory parameters.

11. There is no correlation between fibrinogen - TG and fibrinogen - HDL.

12. This study confirms the hypothesis of a modulation of the inflammatory process due to the variation of serum lipids (specific dyslipidemia pattern)

13. The inflammatory process identified by fibrinogen and ESR (VSH), has not generated statistically significant differences between the two groups; mean values of inflammatory markers are elevated in both groups and are indicating that there is no additional inflammatory process induced by the presence of hepatic steatosis.

14. Patients in this group are at risk of developing anemia through low serum iron level due to sequestration of iron in the liver. Thus, in patients with hepatic steatosis an extra oxidative stress is induced and thus, the level of hepatotoxicity in rheumatoid arthritis is reached earlier.

15. We believe that our results contribute to the development of new research of:
    - study and better understanding of the evolution of dyslipidemia pattern in the presence of inflammatory conditions;
    - the use of inflammatory and lipid-lowering medication;
    - better assessment of the risk for developing cardiovascular disease and death in patients with rheumatoid arthritis that may associate liver steatosis

### 6.2. Oxidative stress and changes in biochemical markers in patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis

Rheumatoid arthritis (PR) is a chronic inflammatory disease, autoimmune, with extra-articular manifestations, of unknown etiology. Mechanisms for the production of tissue damage in joints and systemic lesions are not fully understood. It is considered to be involved genetic modifications influenced by environmental factors and the presence of oxidative stress in the tissue. The PR is now considered a multifactorial disease (TOBÓN et al., 2009).

Epidemiological studies in the general population show a high prevalence of PR and the risk of developing multiple comorbidities that complicate underlying disease and increased risk of mortality (GABRIEL and MICHAUD, 2009).

Studies of opportunistic infections in PR indicate an increased risk of bacterial, fungal and viral infections (B, C), which are more common in active and severe forms of PR, as a result of immunosuppressive therapy, and complicate basic rheumatic disease (GABRIEL and MICHAUD, 2009; CROWSON et al., 2012; VASSILOPOULOS et al., 2012).

Studies on PR and hepatitis with HCV revealed a change in intracellular defense mechanisms with the emergence of oxidative stress, which is considered a key element in the development of these diseases (OZKAN et al., 2007, CLÉMENT et al., 2009).

In the context of association of several comorbidities in patients with PR, it is important to assess liver functions to allow a better evaluation of the functional status of the whole organism. Also, it is important to establish new biochemical markers and correlations that can be useful in setting prognosis of PR evolution when infected with hepatitis C virus.

## Study 1. Comparing patients with associated HCVC+PR and HCVC

### Objectives of the study

1. Assessment of biochemical markers and oxidative stress markers by determining enzymatic and non-enzymatic antioxidants in patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis (HCVC+PR) compared with patients diagnosed with chronic hepatitis C (HCVC) and a control group of healthy patients.
2. Quantitative analysis of the obtained results, in order to observe significant differences between the three groups.
3. The comparison of the obtained values with from the literature references.
4. Establishing the presence of statistically significant differences between the three groups and within each pathological group.
5. Establish the presence or absence of statistical correlations between SOD, GPx and other biochemical parameters, uric acid, BT, serum iron and fibrinogen, in HCVC and HCVC+PR.

### Materials and methods

This study was conducted in collaboration with the Clinical Hospital of Constanta Port, Medical Department II, and a private practice of family medicine, during 2009-2012.
The study included 38 patients divided into two groups and a group of healthy controls:

- 16 patients diagnosed with chronic hepatitis with C virus (HCVC)
- 19 patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis (HCVC + PR)
- 18 healthy controls (M).

Patients were diagnosed based on ACR diagnostic criteria for PR and immunological laboratory diagnosis of infection with HCV.
Biological samples were collected in the hospital. Processing and determination of biochemical markers was performed in the laboratory department of the hospital.

Dosage of biochemical tests was performed on serum, plasma and whole blood; the erythrocyte lysate was assesed for SOD and blood collected on anticoagulant (heparin) for GPx, using laboratory standards.

Biochemical markers dosed in this study are as follows :

- Superoxide dismutase (SOD) and glutathione peroxidase (GPx) - enzymatic markers of oxidative stress
- Serum aminotransferases: aspartataminotransferase (AST), alanine aminotransferase (ALT ) - hepatic cytolysis syndrome

- γ-glutamiltranspeptidaza (GGT), alkaline phosphatase (ALP), total, direct and indirect bilirubin (BT, BD, BI) - cholestasis syndrome
- Serum iron
- Fibrinogen (FIB) - inflammatory syndrome
- Total cholesterol (TC), triacylglycerols (TG) - lipid profile
- Urea, creatinine, uric acid
- Total protein
- Serum glucose

Statistical evaluation was performed by applying ANOVA , showing them in tables and graphs. Exclusion criteria from the study are patients with chronic respiratory, renal and malignant disease, chronic alcoholism and patients with other liver diseases .

**Results and discussion**

**The demographic analysis** revealed that in both groups of patients females predominate, with a percentage of 68.42% in HCVC+PR group and 62.50% in HCVC group. The age distribution of patients who associate the two diseases (HCVC+PR) is between 41-80 years and of patients with HCVC between 51-80 years. Most patients in HCVC+PR (36.84%) were in 61-70 years and most patients with HCVC (50%) were in 71-80 years decade**.**

**Superoxide dismutase (SOD)**. By evaluating the mean values of SOD activity is observed that there is a statistically significant difference between groups HCVC and M ($p < 0.001 < 0.05$) and HCVC+PR and M ($p = 0.006 < 0.05$); a decrease of averages in pathological groups is observed compared to control group. Statistical comparison of mean values of SOD between groups HCVC and HCVC+PR confirms that there are satistically significant differences between these groups ($p = 0.007 < 0.05$). It is noted that although the mean values of both groups tend to decrease, the SOD decrease is less pronounced in the patients of HCVC+PR group compared to the HCVC group (fig. 24) .

**Glutathione peroxidase (GPx).** Statistical evaluation of the mean values of GPx activity confirms that there are statistical differences between HCVC+PR group and M group, with increased values in the pathological group ($p = 0.001 < 0.05$). Although average values of GPx activity are higher in HCVC compared to M group, the evaluation of the mean values of GPx allowed the assumption that there are no statistical differences between these groups ($p > 0.05$). Between HCVC and HCVC+PR groups there is significant difference ($p > 0.05$), with a tendency of increase in GPx in HCVC+PR group (Fig. 25).

For pathological groups, statistical correlations have been achieved between SOD and GPx activity and other biochemical parameters: uric acid, total bilirubin, serum iron and fibrinogen. In the HCVC group, a strong positive correlation was observed between SOD and GPx activities ($r = 0.707$, $p = 0.002 < 0.05$) (Table 88), SOD and uric acid, GPx and uric acid, which indicates a high activity of intracellular antioxidant enzymes to neutralize ROS. Our data reveal a pronounced oxidative stress in patients with HCVC.

Following statistical correlations for HCVC+PR group, a weak to moderate negative correlation between SOD and GPx ($r = -0.491$, $p = 0.033 < 0.05$) was revealed; corelating this with statistical evaluation of the mean values of SOD and GPx activity, we found that when the primary defense mechanism (SOD) tends to decrease, the second line of antioxidant defense tends to increase (GPx), as there is an excess of peroxide that needs to be neutralized; the excess

is due to the increased presence of superoxide ion.

In the pathological groups, HCVC and HCVC+PR, SOD and GPx activity changes secondary to excess production of ROS, reflecting an imbalance between prooxidant and antioxidant systems, which reveal the presence of oxidative stress. The global stress is higher in HCVC+PR group, that present a decreased SOD and an increased GPx activity.

Between SOD activity and serum Fe a strong negative correlation was established in the HCVC group ($r=-0.312$, $p=0.001<0.05$), and no correlations in the HCVC+PR group. Between GPx and Fe no correlations are present in both pathologic groups. Between SOD activity and serum uric acid a positive moderate correlation is present ($r=0.519$, $p=0.039<0.05$) and between GPx activity and serum uric acid has also established a moderate positive correlation ($r=0.618$, $p=0.011< 0.05$). These positive correlations may indicate that uric acid is an antioxidant and the increase of its concentration indicates an increase of antioxidant systems, thus it is considered a marker of oxidative stress. Among the three groups studied, no statistically significant differences were found between the mean values of uric acid .

**Serum iron**. The mean values of serum iron are within limits, but statistical evaluation accepts the hypothesis that there is significant difference between the group of patients HCVC the other two groups studied ($p<0.05$). The mean values increase in HCVC group and decrease to the lower limit of normal in HCVC+PR group (Fig.40).

Studies on serum iron in C virus infection believes that there is an alteration in the mechanisms regulating iron homeostasis; hepcidin is involved in these mechanisms. Secretion of hepcidin is modulated by various factors. It was observed that iron deposits and inflammation activates transcription of hepcidin in hepatocytes and hypoxia, anemia and increased erythropoiesis leads to decreased expression of hepcidin ( SABĂU et al., 2009). A recent study (LIU et al., 2012) on the implications of hepcidin in HCV shows a suppression of hepcidin expression in hepatocytes infected with the virus. Thus, in the HCVC group, the tendency to hipersideremia may indicate a mechanism for the inhibition of the expression of hepcidin in the liver. In the HCVC+PR group, a decrease in serum iron to the lower limits of normal was observed.

**Serum glucose**. Mean chiro these lots are within normal limits and statistical analysis supports the hypothesis that there are statistically significant differences between the groups of patients studied ($p> 0.05$).

**Serum transaminases**. By statistic evaluation, the hypothesis is accepted that there is statistical significance between the mean values of AST activity in the control group and HCVC ($p=0.001<0.05$) and HCVC+PR groups ($p=0.007<0.05$) with an increase of AST value in pathological groups. Moreover, one hypothesis is accepted that there are statistically significant differences between the two pathological groups, with high activity of AST in HCVC group ($p=0.001<0.05$). The same assumptions are valid for ALT.

Statistical assessment of de Ritis ratio admits the hypothesis that there is present a significant difference between M and HCVC group. De Ritis ratio value decreases to the normal value of 1.3 in the HCVC group ($p=0.001<0.05$). No significant differences between M and HCVC+PR and between HCVC and HCVC+PR groups ($p>0.05$).

Following this evaluation there is an increase of the membrane permeability for these enzymes, more pronounced in the HCVC group. Cytolysis syndrome was not present in the HCVC+PR group.

**Gamma-glutamiltranspeptidase (GGT)**. By statistical evaluation of mean values of GGT activity the hypothesis is accepted that there are statistically significant differences between HCVC and M and between HCVC and HCVC+PR. High activity of GGT was found in HCVC group ($p<0.001<0.05$). Significant differences are not

present between M and HCVC+PR group ($p>0.05$).

**Alkaline phosphatase (ALP).** Statistical evaluation of the mean values of ALP activity confirmed that the only statistically significant difference exist between HCVC and HCVC+PR groups, with a tendency of increase in HCVC group ($p=0.020<0.05$). Among the other groups there are no significant differences ($p>0.05$).

**Serum bilirubin.** Bilirubin concentrations are modified, so statistically significant differences are found for the mean values of BD between M and pathological groups ($p<0.05$) but not between the two pathological groups ($p>0.05$). Significant differences exist for mean BT concentration between HCVC+PR and the other groups ($p<0.05$), with a trend of decreasing values in HCVC+PR group. Among other groups, M and HCVC, no significant differences are present ($p>0.05$). Serum bilirubin is considered a non-enzymatic antioxidant. By statistical evaluation of the two pathological groups, no correlation was established between BT and oxidative stress markers - GPx and the SOD activity ($p<0.05$). The assessment shows that there is a tendency of increase for ALT, AST, GGT, ALP activities and serum bilirubin in HCVC+PR group due to the presence of liver viral disease. These biochemical parameters are higher in the group of HCVC patients, revealing that there is a profound impairment of virus infected hepatocytes (GHIDUS et al., 2013).

**Total cholesterol (TC)** and **serum triglycerides (TG).** Statistical evaluation of the mean values of TC between the studied groups, indicates that there are statistically significant differences between HCVC and HCVC+PR groups, with an increasing trend of CT in HCVC+PR group. For the other groups, statistically significant differences are not present ($p>0.05$). There are no significant differences for the mean values of serum TG for the three groups ($p>0.05$). Serum CT and TG reflects the synthesis ability of the liver; hepatitis affects this function, leading to disturbances in the lipid metabolism with lipid accumulation in hepatocytes (hepatic steatosis) and altered serum lipid concentration. CT and TG are also important in the liver infectivity of HCV (YAMAMOTO et al., 2011).

**Urea** and **creatinine** are two biochemical markers involved in the evaluation of renal function. It appears that there are statistically significant differences between mean values of urea between M and HCVC+PR groups ($p<0.001<0.05$). No statistical significant differences exist between M and HCVC or HCVC and HCVC+PR ($p>0.05$). By statistical evaluation of the mean values of serum creatinine between pathologic groups no significant differences are found. The average values of these biochemical markers are within the normal range. Renal function assessed by urea and creatinine levels in these patients is not affected.

**Fibrinogen**. The statistical assessment of mean values of fibrinogen shows that there are significant differences between M and HCVC+PR or HCVC and HCVC+PR ($p<0.001<0.05$). Among other groups no significant differences are found ($p>0.05$) (Fig. 41). No statistical correlations are found between fibrinogen and antioxidant enzymes SOD and GPx .Mean fibrinogen concentration in HCVC+PR group is higher than the upper limit of the reference interval and due to statistical considerations above, it can be said that the presence of inflammation reflected by plasma levels of fibrinogen is only due to chronic inflammation in rheumatoid arthritis.

**Total protein**. Between mean values of PT in groups HCVC and HCVC+PR no statistically significant differences are shown ($p>0.05$). Between HCVC+PR and M groups, statistically significant differences exist between mean values of PT, but the values are in the normal range ($p<0.05$).

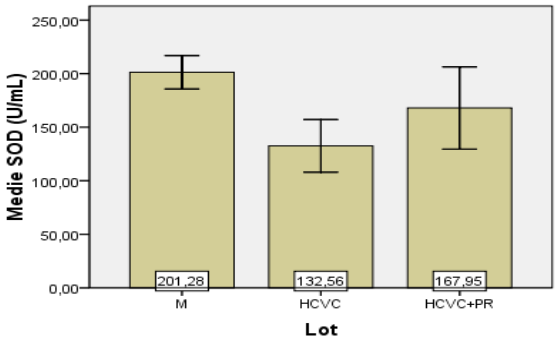



**Fig.24.** SOD variation

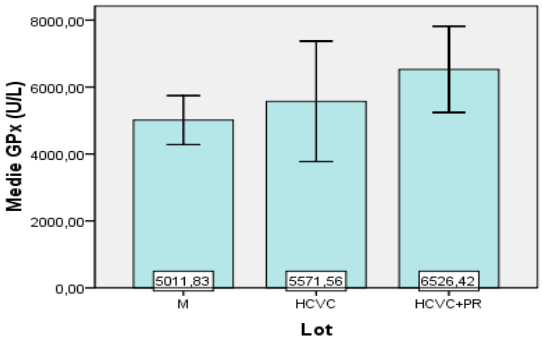



**Fig. 25.** GPx variation

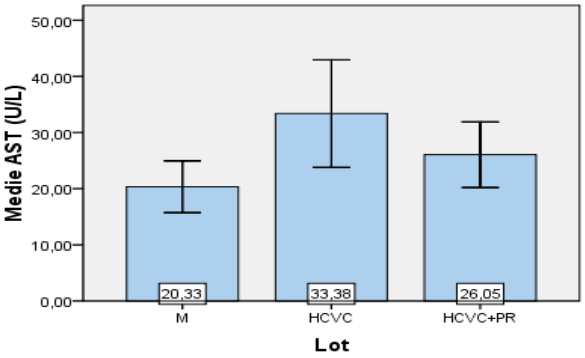



**Fig.27**.AST variationi

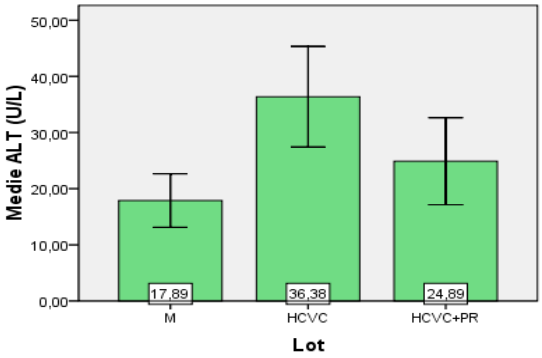



**Fig. 28.** ALT variation

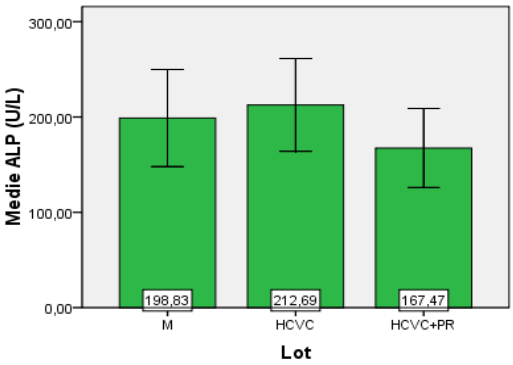



**Fig.30.** GGT variation

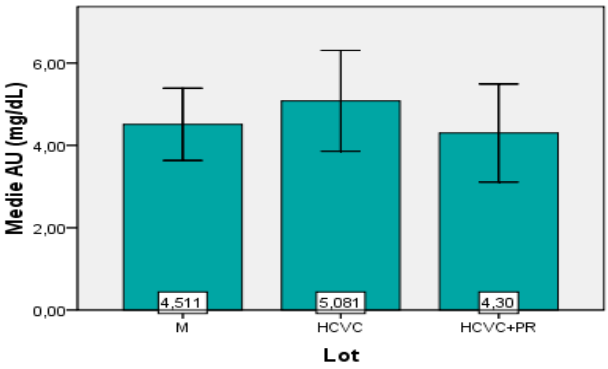



**Fig.37.** Uric acid variation

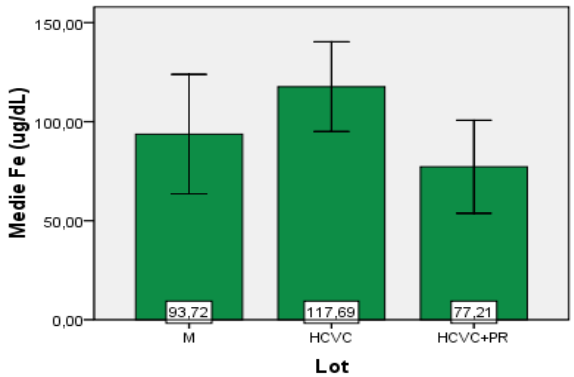



**Fig. 40.** Serum iron (Fe)variation

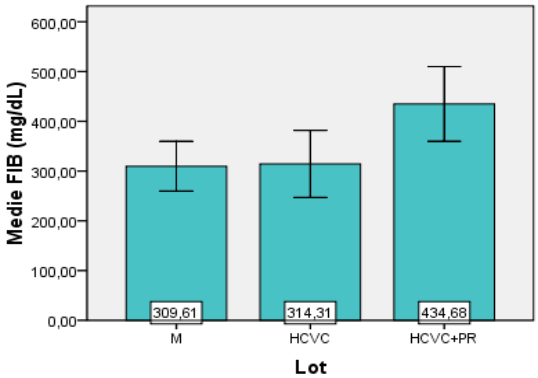



**Fig. 41. F**ibrinogen variation

**Tabel 88**.Statistical correlations between SOD and GPx in HCV + PR and HCVC

| Lot | | | SOD (U/mL) | GPx (U/L) |
|---|---|---|---|---|
| HCVC | SOD (U/mL) | Corelația Pearson **(r)** | 1 | 0,707** |
| | | Sig. (2-tailed) **(p)** | | 0,002 |
| | | N | 16 | 16 |
| | GPx (U/L) | Corelația Pearson | 0,707** | 1 |
| | | Sig. (2-tailed) | 0,002 | |
| | | N | 16 | 16 |
| HCVC+R | SOD (U/mL) | Corelația Pearson | 1 | -0,491* |
| | | Sig. (2-tailed) | | 0,033 |
| | | N | 19 | 19 |
| | GPx (U/L) | Corelația Pearson | -0,491* | 1 |
| | | Sig. (2-tailed) | 0,033 | |
| | | N | 19 | 19 |

**. Correlation is significant at the 0.01 level (2-tailed).

**Tabel 89.** Statistical correlations between SOD and uric acid (AU) in HCV + PR and HCVC

| Lot | | | SOD (U/mL) | AU (mg/dL) |
|---|---|---|---|---|
| HCVC | SOD (U/mL) | Corelația Pearson | 1 | 0,519* |
| | | Sig. (2-tailed) | | 0,039 |
| | | N | 16 | 16 |
| | AU (mg/dL) | Corelația Pearson | 0,519* | 1 |
| | | Sig. (2-tailed) | 0,039 | |
| | | N | 16 | 16 |
| HCVC+PR | SOD (U/mL) | Corelația Pearson | 1 | 0,154 |
| | | Sig. (2-tailed) | | 0,529 |
| | | N | 19 | 19 |
| | AU (mg/dL) | Corelația Pearson | 0,154 | 1 |
| | | Sig. (2-tailed) | 0,529 | |
| | | N | 19 | 19 |

*. Correlation is significant at the 0.05 level (2-tailed).

**Tabel 92.** Statistical correlations between SOD and serum iron (Fe) in HCV + PR and HCVC

| Lot | | | SOD (U/mL) | Fe (ug/dL) |
|---|---|---|---|---|
| HCVC | SOD (U/mL) | Corelația Pearson | 1 | -0,731** |
| | | Sig. (2-tailed) | | 0,001 |
| | | N | 16 | 16 |
| | Fe (ug/dL) | Corelația Pearson | -0,731** | 1 |
| | | Sig. (2-tailed) | 0,001 | |
| | | N | 16 | 16 |
| HCVC+PR | SOD (U/mL) | Corelația Pearson | 1 | 0,150 |
| | | Sig. (2-tailed) | | 0,540 |
| | | N | 19 | 19 |
| | Fe (ug/dL) | Corelația Pearson | 0,150 | 1 |
| | | Sig. (2-tailed) | 0,540 | |
| | | N | 19 | 19 |

**. Correlation is significant at the 0.01 level (2-tailed).

**Tabel 93.** Statistical correlations between GPx and GPx in HCV + PR and HCVC

| Lot | | | GPx (U/L) | AU (mg/dL) |
|---|---|---|---|---|
| HCVC | GPx (U/L) | Corelația Pearson | 1 | 0,618* |
| | | Sig. (2-tailed) | | 0,011 |
| | | N | 16 | 16 |
| | AU (mg/dL) | Corelația Pearson | 0,618* | 1 |
| | | Sig. (2-tailed) | 0,011 | |
| | | N | 16 | 16 |
| HCVC+PR | GPx (U/L) | Corelația Pearson | 1 | -0,085 |
| | | Sig. (2-tailed) | | 0,730 |
| | | N | 19 | 19 |
| | AU (mg/dL) | Corelația Pearson | -0,085 | 1 |
| | | Sig. (2-tailed) | 0,730 | |
| | | N | 19 | 19 |

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

**Conclusion**

1. In both groups, with chronic hepatitis with virus C (HCVC) and chronic hepatitis with virus C associated with rheumatoid arthritis (HCVC+PR), the majority of patients are women, but with a higher percentage in group HCVC+PR (68.42%) and because PR is specific to females.
2. The higher percentage of patients with HCVC+PR is represented by women of 61-70 years, as confirmed by literature data, whereas rheumatoid arthritis is specific for women of this decade of age. In the HCVC group the increased percentage of women is found in the decade of life 71-80 years.
3. Between the two groups there is a difference between age decades: HCVC+PR affects younger patients (aged 41-80 years) and HCVC affects patients aged 51-80 years.
4. **Oxidative stress** is present in both pathological groups as highlighted by modified activity of SOD and GPx in pathological groups compared to the control group, with statistically significant differences.
5. **SOD** activity tends to decrease in both groups, more intense in HCVC group.
6. **GPx** activity tends to increase in both pathological groups.
7. Strong positive correlation between SOD and GPx in group HCVC indicates increased consumption of these antioxidant enzymes to neutralize SRO, which reveals a pronounced oxidative stress.
8. Positive correlations between SOD and uric acid and GPx and uric acid in the HCVC group, and SOD and GPx negative correlation in the HCVC+PR group, revealed the presence of increased oxidative stress.
9. Decreased SOD activity may be due to its increased consumption for neutralizing the superoxide ion and production of an excess of peroxide ($H_2O_2$). Increased GPx activity may reflect increased production of peroxides, because there is a growing need of GPx to neutralize intracellular peroxides.
10. In HCVC and HCVC+PR is found that when the primary defense mechanisms (SOD) tend to decrease, and there is an amplification process of secondary antioxidant defense mechanisms (GPx).
11. Patients who associate the two comorbidities, HCVC+PR, present a more accentuated global stress due to decreased activity of SOD and the increase of GPx activity.
12. Serum **iron** shows statistically significant differences between pathological groups, with increased serum iron in HCVC and downward trend in serum iron in HCVC+PR, this is due to the mechanisms of activation and blocking of hepcidin in the liver, with the change of iron homeostasis, change of serum iron concentration and Fe retention in different tissue deposits.
13. A strong negative correlation between SOD activity and serum iron concentration in the group of patients with HCVC is present. Iron is involved in oxidative stress, being a pro-oxidant factor and has a negative correlation with SOD which may indicate the presence of increased oxidative stress, leading to reduced amount of SOD due to its increased consumption.
14. The study reveals that the liver is damaged; this is evidenced by the growing trend for enzymes as **ALT, AST, GGT, ALP, de Ritis ratio** and serum **bilirubin** in the group of patients with HCVC+PR, due to the presence of the viral hepatitis. These biochemical markers of liver function are more pronounced in the group of patients with HCVC, who have a deeper damage of hepatocytes in the presence of viral infection.
15. **Lipid** profile (total cholesterol and triglycerides) is partially modified by the growing trend of CT values, statistically significant for HCVC+PR group compared with control group, but not compared to HCVC group.

16. Renal function, as assessed by **urea** and **creatinine**, is not affected in the two pathological groups. There is only a trend of serum urea to increase, with statistical significance in patients in group HCVC+PR compared to control group.
17. Serum **glucose** did not show statistically significant differences between the three groups.
18. High concentration of **fibrinogen** is statistically significant in patients from HCVC+PR group compared to control group and only due to the presence of inflammatory rheumatic disease and not due to an extra hepatic inflammatory process.
19. The average values of **total protein** did not differ statistically significant between groups HCVC and HCVC+PR.
20. In patients with HCVC were not found correlations between SOD-FIB, SOD-BT, GPx-FIB, GPX-BT, GPx-Fe, and in the group of patients with HCVC+PR were not found correlations between SOD-uric acid, SOD-FIB, SOD-Fe, SOD-BT, GPx-uric acid , GPx-FIB, GPx-BT, GPx-Fe.
21. Patients who associate the two comorbidities (HCVC+PR) have the liver affected to a lesser extent than patients with HCVC, but have an increased overall oxidative stress probably due to the coexistence of the two diseases, which indicates an effort of the organism to combat excess ROS.
22. To reduce the overall oxidative stress antioxidant should be considered that antioxidant therapy to be part of the treatment.

## 6.2 Comparing the groups HCVC+PR and PR

**Objectives of the study**

1.Evaluation and biochemical markers of oxidative stress by determining enzymatic and non-enzymatic antioxidants of patients diagnosed with chronic hepatitis C associated with rheumatoid arthritis compared with patients diagnosed with rheumatoid arthritis and a control

2.Quantitative analysis compared the results obtained to observe significant differences between the three groups that share studied

3.The comparison of the values obtained from the literature rereferinţă with the reference trial

4.Establishing the presence of statistically significant differences between the three groups and within each group the pathological

5.Establish the presence or absence of statistical correlations between SOD, GPx and other biochemical parameters: uric acid, BT, and fibrinogen serum iron in HCV + PR and PR groups

**Materials and methods**

This study was performed in collaboration with Constanta Port Hospital, Medical Department and private offices of family medicine, during 2009-2012. The study includes 38 patients and a control group:

- 19 patients diagnosed with rheumatoid arthritis (PR),
- 19 patients diagnosed with CHCV associated with rheumatoid arthritis (HCVC+PR),
- 18 healthy control patients (M).

The two groups of patients were established according to diagnostic criteria for RA and on the basis of immunologic laboratory diagnosis of infection with hepatitis C virus.

The assay of the biochemical parameters was performed on serum, plasma and whole blood, according to the standards.

The biochemical markers analyzed in this study are the following:

- Assessment of oxidative stress: the activity of antioxidant enzymes: SOD and GPx,
- Hepatic cytolysis: Aminotransferases AST, ALT
- Cholestasis: γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), serum bilirubin - total (BT), direct (BD) and indirect (BI),
- Minerals: serum iron
- Inflammatory Syndrome: fibrinogen (FIB)
- Carbohydrate metabolism: Glycemia (G)
- Lipid metabolism: total cholesterol (CT), triacylglycerols (TG)
- Protein metabolism: total serum proteins (PT), creatinine (CR), urea (U), uric acid (AU)

Statistical evaluation was performed using ANOVA test.

Exclusion criteria of the study are patients with chronic kidney and respiratory disorders, malignant diseases, chronic alcohol consumers and patients with other liver disorders.

**Results and discussion**

**Demographic analysis** indicates the following: the group with PR included 19 patients aged between 31 and 81 years, of which 89.47% were women (17 patients) with a predominence in 50-70 years group and 10.53% men (2 patients). The group with HCVC+PR included 19 patients aged between 45 and 78 years, of which 68.42% women (13 patients) with a major number in 40-80 group of age, and 31.58% men (6 patients). There is an extension to the lower age decades of PR, the presence of viral infection associated with this disease may be involved early in its manifestation.

Epidemiological studies on the prevalence of PR in the age and sex of the patient indicates an increased frequency in women than men, the mechanism by which gender affects susceptibility to PR is not known, but is thought sex hormones are involved; the maximum occurrence of PR in the 5th decade of life, when there are changes in hormonal equilibrium of female.

**Superoxide dismutase (SOD)** (fig.45) and **Glutathione peroxidase** (GPx) (fig.46) In the group of patients that combines the two diseases (HCVC+ PR) oxidative stress is more clearly reflected in the downward trend in activity of the SOD and strong increase of GPx activity as a cellular compensatory mechanism. Combining both HCVC and PR pathologies causes an increased total oxidative stress in these patients compared with the PR group.

**Serum glucose.** Mean blood glucose levels in these groups are within normal limits, although there is a lower average in HCVC+PR group, but statistical analysis supports the hypothesis that there are statistically significant differences between the control groups, HCVC, HCVC+PR ($p<0.05$).

**Serum transaminases** AST and ALT are modified in the group of patients that associates the two comorbidities (HCVC+PR). The mean values of ALT and AST are in the limits of reference values, but there is a tendency to increase the activity of these enzymes and a statistically significant difference from the control group and the PR group ($p<0.05$). De Ritis ratio (AST/ALT) did not show statistically significant differences between the groups ($p>0.05$). The increase trend of transaminases activity reflects a certain degree of permeability of the hepatocellular membrane for the enzymes due to the damage of hepatocytes.

**Serum bilirubin** (direct, indirect and total) are slightly modified in the group of patients HCVC+PR, with slight increases in BD; there are statistical differences between the mean values of BD between the control group and the group HCVC+PR ($p<0.001<0.05$) that are explained by the presence of viral hepatitis that alters hepatocellular functions of conjugation and excretion of bilirubin. No statistical correlations were established between BT and SOD and GPx.

**Gamma-glutamiltranspeptidase (GGT)** and **alkaline phosphatase (ALP)**, in patients with HCV+PR are not changed, which does not indicate the presence of cholestasis syndrome in these patients.
For the group of patients with PR an increase of the GGT and ALP average values to the upper limits of normal was revealed. For serum GGT there is a significant difference between PR and control groups ($p< 0.05$), but not between other groups.

**Total cholesterol (CT)** and **serum triglycerides (TG)**. There is hypercholesterolemia in groups HCVC+PR and PR with statistically significant difference compared to control group ($p<0.001<0.05$). In HCVC+PR group, CT values are lower than PR group, but without statistical significance ($p>0.05$). Between the mean values of serum TG there are not present statistically significant differences for the three studied groups. Average values are in the reference interval. The association of the two diseases PR and infection with HVC is important. Since both diseases are associated with altered lipid metabolism disorders and may involve non-alcoholic hepatic steatosis.

**Urea, creatinine and uric acid.**Serum urea concentration did not differ significantly between the two groups of pathology ($p>0.05$); the mean values of urea are within the normal range. There are significant differences between HCVC+PR and PR groups compared to control group ($p<0.001$). This can be explained by a more intense protein catabolism, or a tendency of decrease in the glomerular filtration rate in these patients. Serum uric acid and creatinine did not differ significantly between pathological groups and control group ($p>0.05$). Serum creatinine in the normal range shows that the renal function in these patients is normal. There are no statistical correlations between uric acid and SOD in HCVC+PR group and between uric acid and GPx in the PR and HCVC+PR patients
.

**Serum iron**. Mean serum iron level in these groups is normal, but with a tendency to fall toward the lower limit of normal in the pathology groups. There is no statistically significant difference between the control group and pathological groups but there is a significant difference between the group of patients HCVC+PR and PR group ($p=0.039<0.05$). The combination of the two co-morbidities may induce alteration of mechanisms of hepcidin expression, increased in PR and decreased in HVCV. The assay revealed decreased serum iron in HCVC+PR group compared to the PR group. There were no correlations between iron and SOD and GPx in pathological groups.

**Total protein**. Between the mean plasma there are no statistically significant differences in all the studied groups ($p = 0.807 > 0.05$).

**Fibrinogen.** The average values in the two groups of patients are exceeding the upper limit of normal (400 mg/dl) showing that the inflammatory syndrome is present in both studied groups of patients. By comparing the mean values of fibrinogen in HCVC+PR and PR groups, there are statistical differences compared to control group ($p<0.05$). Although, in the patients of PR+HCVC group the fibrinogen is higher compared to the patients with PR, but there is no statistical significance ($p=0.477 >0.05$). No statistical correlations were established between plasma fibrinogen and antioxidant enzymes SOD and GPx.

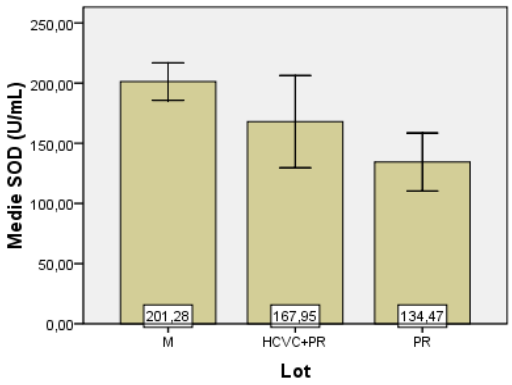



**Fig. 45.** Superoxide dismutase variaton

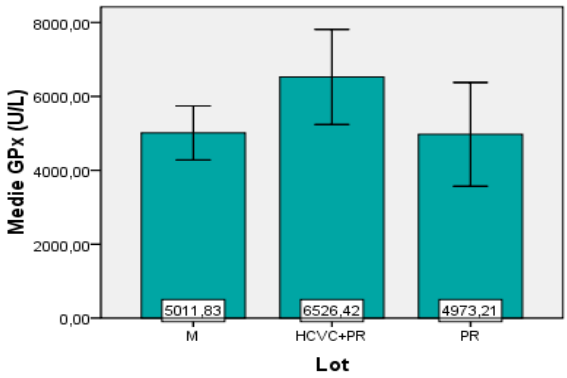



**Fig.46.** Glutathione peroxidase variation

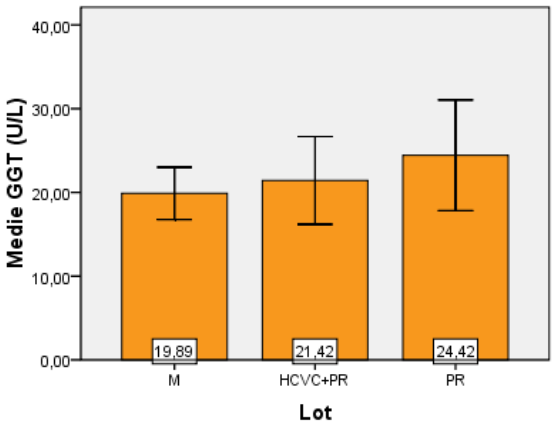



**Fig.51.** Gamma-glutamyltransferase variation

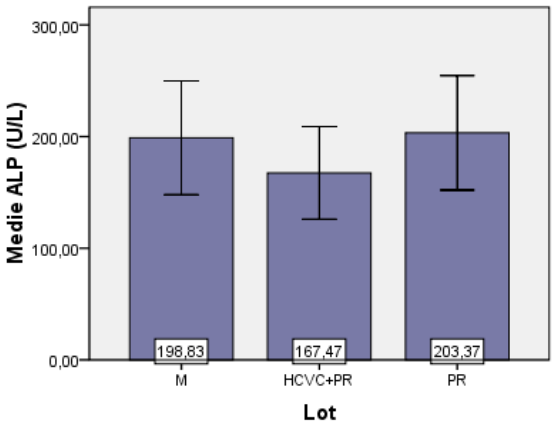



**Fig.52.** Alkaline phosphatase variation

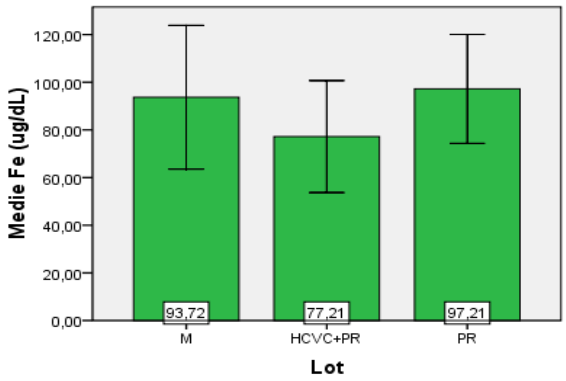



**Fig.61.** Serum iron variation

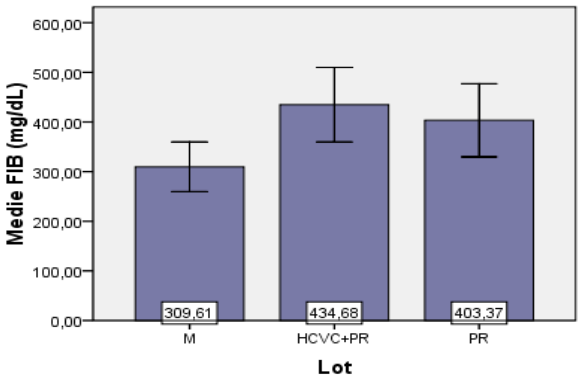



**Fig.62.** Ffibrinogen variation

**Tabel 165.** Statistical correlations between SOD and GPx in HCV + PR and PR

| Lot | | | SOD (U/mL) | GPx (U/L) |
|---|---|---|---|---|
| HCVC+PR | SOD (U/mL) | Corelația Pearson **(r)** | 1 | -0,491* |
| | | Sig. (2-tailed) **(p)** | | 0,033 |
| | | N | 19 | 19 |
| | GPx (U/L) | Corelația Pearson | -0,491* | 1 |
| | | Sig. (2-tailed) | 0,033 | |
| | | N | 19 | 19 |
| PR | SOD (U/mL) | Corelația Pearson | 1 | -0,166 |
| | | Sig. (2-tailed) | | 0,497 |
| | | N | 19 | 19 |
| | GPx (U/L) | Corelația Pearson | -0,166 | 1 |
| | | Sig. (2-tailed) | 0,497 | |
| | | N | 19 | 19 |

*. Correlation is significant at the 0.05 level (2-tailed).

**Tabel 166.** Statistical correlations between SOD and uric acid (AU)in HCV + PR and PR

| Lot | | | SOD (U/mL) | AU (mg/dL) |
|---|---|---|---|---|
| HCVC+PR | SOD (U/mL) | Corelația Pearson | 1 | 0,154 |
| | | Sig. (2-tailed) | | 0,529 |
| | | N | 19 | 19 |
| | AU (mg/dL) | Corelația Pearson | 0,154 | 1 |
| | | Sig. (2-tailed) | 0,529 | |
| | | N | 19 | 19 |
| PR | SOD (U/mL) | Corelația Pearson | 1 | 0,459* |
| | | Sig. (2-tailed) | | 0,048 |
| | | N | 19 | 19 |
| | AU (mg/dL) | Corelația Pearson | 0,459* | 1 |
| | | Sig. (2-tailed) | 0,048 | |
| | | N | 19 | 19 |

*. Correlation is significant at the 0.05 level (2-tailed).

.

**Conclusions**

1. Rheumatoid arthritis (PR) affects mostly women with disease manifestation between 50-70 years due to changed hormonal status corresponding to the period of menopause.
2. The majority of patients diagnosed with chronic hepatitis with virus C associated with rheumatoid arthritis (HCVC+PR) are women aged 40 to 80 years, and an earlier manifestation of rheumatoid arthritis symptoms can be due to the presence of the viral infection.
3. Oxidative stress is present in both pathological groups HCVC+PR and PR, and is evidenced by changes in **SOD** and **GPx** activity by excess SRO production and exceeding the defense capacity of the antioxidant system. This modulation of the activity of antioxidant systems, secondary to the excess of SRO, is reflected by the positive correlation between SOD and uric acid present in the PR group.
4. The combination of both comorbidities, hepatitis C and rheumatoid arthritis (HCVC + PR), causes a more pronounced oxidative stress, as reflected by the downward trend in SOD activity and the sharp rise in GPx activity as a cellular compensatory mechanism revealed by the presence of a negative correlation between SOD and GPx. So is a growth of secondary antioxidant defense mechanisms (GPx) when the first line of defense (SOD) decreases.
5. The association of two pathologies as HCVC and PR determines the presence of an additional oxidative stress, an increased overall oxidative stress compared to the oxidative stress noted in the group of patients with PR only.
6. Inflammatory syndrome is present in both pathological groups with increased mean values for fibrinogen, but no statistically significant differences between HCVC+PR and PR group, which can be said that the presence of viral hepatitis pathology does not induce an extra inflammatory process.
7. In the group of patients with HCVC+PR, a cholestasis syndrome is not observed. But an impaired indirect bilirubin serum level can be explained by hepatocyte dysfunction, associated with the increasing trend of serum transaminases due to increased permeability of the cell membrane.
8. The group of patients diagnosed with PR shows a tendency of increase for GGT and ALP activity. There is a statistically significant increase in GGT activity compared to control group. That may reflect decreased intracellular reduced glutathione (G-SH) and the presence of oxidative stress in patients diagnosed with PR.
9. Dyslipidemia due to high **cholesterol** level is present in both pathological groups. Total cholesterol tends to increase in the two groups and is more pronounced in the group with PR compared to the HCVC+PR group. Mean serum **triglyceride** levels are not changed. Dyslipidemia is important in PR pathology, as it supports the cardiovascular manifestations in PR; lipids are also involved in the mechanisms of hepatitis C virus infectivity.
10. Both pathologies, chronic hepatitis C and rheumatoid arthritis, are associated with disturbances in lipid metabolism, alterations of serum lipid profile and also can associate nonalcoholic steato-hepatitis.
11. The study of dyslipidemia in the context of associated pathologies can be a starting point for future studies to observe the implications of the association of rheumatoid arthritis, viral hepatitis C and non-alcoholic hepatic steatosis on the viral load and drug therapy of these diseases.

12. Low serum iron level is more pronounced in the group of patients HCVC+ PR compared to the PR group, and was noted through a statistically significant difference. The combination of the two co-morbidities may induce additional hyposideremia.
13. By evaluating the mean values of serum glucose, serum total protein, serum urea, uric acid and creatinine were observed no statistically significant differences.
14. In the group of patients HCVC+PR were not found correlations between SOD-uric acid, SOD-FIB, SOD-Fe, SOD-BT, GPx-uric acid, GPx-FIB, GPX-BT, GPx-Fe.
15. In the group of patients with PR were not found correlations between SOD-GPx, SOD-FIB, SOD-Fe, SOD-BT, GPx-uric acid, GPx-FIB, GPx-Fe, GPx-BT.

## GENERAL CONCLUSIONS

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The results of studies conducted in this thesis and the conclusions drawn at the end of the study, may issue the following comments and conclusions.

1. Patients diagnosed with rheumatoid arthritis associated with various chronic liver pathologies shows some variations versus patients diagnosed with only viral hepatitis or rheumatism.

**2.Demographic data.** Gender and age demographic analysis confirms that rheumatic pathology is specific to females that dominate the groups of patients with rheumatoid arthritis with or without chronic liver disease.

In the group of patients with rheumatoid arthritis (PR) is found a maximum number in the decades between 50-70 years. Patients diagnosed with rheumatoid arthritis associated with hepatic steatosis (PR+SH) have the average value between 61-70 years. It is observed that there is a wider age range in patients with chronic hepatitis C associated with rheumatoid arthritis (HCVC+PR), but lower than in rheumatoid arthritis (PR) patients maintaining a high percentage of patients in these intervals, with a maximum in the decade 61-70 years.

These ranges refer to the period of age of women hormonal changes. Increased percentages of patients diagnosed with HCVC+PR in different decades may be due to the presence of viral infection.

**3. Oxidative stress**. In the group of patients diagnosed with PR+SH, the oxidative stress is evidenced by the decrease in Total Antioxidant Status (TAS); the presence of fatty liver determines an increase of hepatic sensitivity to additional stress.

Patients diagnosed with HCVC+PR, have a more pronounced oxidative stress compared with the oxidative stress in HCVC, PR and compared to control group. Decreased activity of SOD and increased activity of GPx suggest that when primary antioxidant mechanisms (SOD) decreases, an overexpression of secondary antioxidant mechanisms occurs, as a cellular compensatory mechanism. These processes are highlighted by the presence of negative correlation between antioxidant enzymes SOD and GPx.

In the group of patients diagnosed with HCVC and in the PR group, the modified enzymes SOD and GPx compared to control group, and the presence of correlations between these enzymes, confirm the involvement of oxidative stress in both rheumatic pathology and viral pathology.

In study 2 (HCVC+PR versus PR) in the group of patients with PR, the oxidative stress is evidenced by the decreased activity of SOD, increased activity of GPx and the presence of positive correlation between SOD and uric acid.

The presence of oxidative stress in PR, HCVC and associated pathologies highlight a modulation of primary and secondary antioxidant systems that occurs due to excess ROS.

The association of the two diseases cause an increased overall oxidative stress causing additional stress for the organism.

4. **Hepatic impairment**. In group with PR+SH there is an increase of AST and ALT activities compared to the PR group, with statistically significant difference, demonstrating a certain degree of liver injury, with increased hepatocellular membranes permeability to these enzymes.In the groups with HCVC+PR and HCVC there is a trend of increase for AST, ALT, GGT, ALP activities, de Ritis ratio, serum bilirubins, but this is higher in HCVC group. This trend of increased hepatic markers show that in the group HCVC+PR there is a higher degree of liver damage due to the presence of viral infection.

In group with PR there is an increase of ALP and GGT activity. This may be determined by an elevated ALP bone isoenzyme, patients being at risk for osteoporosis. The increased GGT is a sign of oxidative stress in this pathology.

**5.** Concentration of serum iron varies depending on the underlying disease. There is a tendency of decreased values in PR+SH and PR+HCVC group. Hyposideremia accompanies rheumatic diseases and studies show that hepatic steatosis may cause an increase of liver deposit of iron. Sequestration of iron in various tissues including the liver, is cytotoxic, thus iron is involved in pro-oxidant mechanisms with increased oxidative stress.

Iron involvement in oxidative stress can be demonstrated the in strong negative correlation between the activity of the antioxidant enzyme SOD and Fe, present in the group of patients with HCVC.

There is a more intense decrease of iron in patients with HCVC+PR than the PR or HCVC groups. These changes show that there is an alteration of the mechanisms regulating the iron homeostasis, in which the hepcidin polypeptide, synthesized by the liver, plays an important role. Impaired expression of hepcidin mechanisms may result in decreased serum iron in groups of patients with PR and PR associated with chronic liver disease. Monitoring of these patients is important to prevent the occurrence of severe iron deficiency anemia that may lead to various hematologic and metabolic complications.

**6.** Evaluation of lipid profile in pathological groups revealed some changes in the concentration of lipid markers such as total cholesterol (CT), serum triglycerides (TG), HDL, LDL. It was found that rheumatoid arthritis is associated with various dyslipidemias. Studies on lipid metabolism in patients with PR suggest different dyslipidemic patterns.

By assessing lipid markers in the group with hepatic steatosis associated with rheumatoid arthritis (PR+SH) no statistically significant differences were obtained compared to the group with rheumatoid arthritis.

Atherogenic ratio (AR=CT/HDL) present significant statistical difference, with increased values, more in the group with PR than in group PR+SH.

By evaluating the group of patients PR+SH and PR, a dyslipidemia pattern was found, characterized by hypercholesterolemia, high LDL and atherogenic ratio unfavorable in patients diagnosed with PR with or without

hepatic steatosis. These patients present an increased risk of developing atherosclerosis and cardiovascular disease; 50% of these patients present elevated values for CT, LDL and AR.

Total cholesterol in the group of patients with HCVC+PR and PR group differ significantly from the control group, with increased CT in these groups, but without the presence of a significant difference between HCVC and HCVC+PR groups.

We say that a dyslipidemia pattern is emerging in patients with HCVC+PR and PR, but more pronounced in the group with rheumatoid arthritis. Not so marked increase of cholesterol in HCVC+PR group may be due to viral infection, which may affect to some degree the liver anabolism.

**7. The presence of inflammatory process**. The mean values of fibrinogen and ESR are elevated in PR and PR+SH groups but with no statistical differences, which indicates that there is not an inflammatory process induced by the presence of extra hepatic steatosis but elevated values are due to rheumatic pathology .

By evaluating all groups of patients was found that increased fibrinogen is associated with various dyslipidemias; half of the patients associated hyperfibrinogenemia. Still, negative correlations are present between fibrinogen and CT, LDL and AR, which confirms that there is an inverse association between inflammatory markers and markers of lipid.

High concentration of fibrinogen is present in patients with HCVC+PR and PR. No statistical significant differences between the two groups, suggesting that increased inflammatory process in HCVC+PR is due to rheumatic disease and no additional inflammatory process is induced by the presence of hepatitis C.

**8.** We consider that the results of this study involving the presence of chronic liver disease in rheumatoid arthritis, is a database for deepening and expanding research in this area. Thus emerges the need for more extensive research on groups of patients to identify and elucidate the changes that occur at the molecular level in biochemical processes in rheumatoid arthritis associated with hepatic steatosis and chronic hepatitis C.

**9.** We consider that we need to extend these studies to lead to a combination of antioxidant therapy with conventional therapy, in order to apply the most appropriate targeted and personalized treatment, to aim the increase of the quality of life.

**10**. Elements of originality of this thesis are:

- Research on chronic liver disease pathology associated with rheumatoid arthritis is into current trends in medical studies on the presence and influence of comorbidities in rheumatoid arthritis.
- Studies in this thesis attempt to identify and explain some pathological mechanisms of chronic liver disease associated with other pathology, correlated with chronic inflammation, through confirmation or refutation of hypotheses that are currently issued in actuality.
- The global assessment of oxidative stress in liver pathology associated with rheumatoid arthritis, this paper brings new priority data to completing the state of knowledge in the field of biomedical importance.
- The available specialty literature, I did not find studies that carry out an assessment of the antioxidant system and oxidative stress in the pathology of chronic liver disease associated with rheumatoid arthritis, particularly liver pathology of viral etiology. In this respect, we believe that this thesis contribute to the deepening and broadening of knowledge worthy of high current biomedical field, providing a particularly useful source of information for professionals interested in the approached issues.

Design, diversification and correlative application of specific investigative methods and techniques have major implications for the development of analytical biochemistry in medical clinic, obviously contributing to the foundation and evaluation of medical knowledge at the molecular and system level.

In conclusion, the data obtained in this thesis and thorough processing of a rich specialty literature is a worthy contribution to the foundation of current knowledge and broadening approached issue, highlighting the importance of clinical laboratory data elucidating the biochemical mechanisms in rheumatoid arthritis associated with chronic liver disease.

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### B. SCIENTIFIC PAPERS PRESENTED ON INTERNATIONAL SCIENTIFIC CONFERENCES AND PUBLISHED IN SUMMARY

**1. Ghiduş D.**, Mihailov C., Roşoiu N., Verman I.G., Ion M.N. Bichemical Profile of Rheumatic Disease Associated with Non-Alcholic Fatty Liver Disease. The Annual International Conference of the Romanian Society of Biochemistry and Molecular Biology & The Conference on „Cellular and Molecular Biology Biotechnologies on Medical Application". Rom. J. Biochem, vol.48, supplementa. 28- 30 September, Craiova, Ed. Academiei Române, (2011). **Revistă indexată B+, indexată şi cu abstracte în ISI Thomson Scientific Master Journal List,**

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## C. PARTICIPATION - NATIONAL AND INTERNATIONAL SCIENTIFIC CONFERENCES

**1**. **Sesiunea de referate și comunicări științifice** ale cadrelor didactice și studenților di facultatea de Medicină a univeritătii OVIDIUS Constanța (2010)-prezentare poster.

**2. RSBMB Internațional Meeting** (Craiova , România, 2011)- prezentare poster.

**3. 32nd Balkan Medical Week and 80 Anniversary of Balkan Medical Union** (Nis, Serbia, 2012)- prezentare poster.

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**5. Sesiunea Științifică de Toamnă 2013 a Academiei Oamenilor de Știință din România. Eco – economia și Dezvoltarea Durabilă** (Brașov, Români, 2013)- prezentare orală.