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**Biological markers of the depressive
disorder**

PhD THESIS

- Abstract -

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Key words: depression, depressive disorder, biological markers, inflammations, CRP-hs

GENERAL PART

CHAPTER I: Introductions and the problem scale

The term "biological marker" can be comprehensively defined as a modification of a biological constant associated with Major Depressive Disorder (MDD) that could be used to indicate its presence and severity, but also to provide predictable values as treatment response indicators [1]. Data from the literature reveal the changes at the neurobiochemical level in Major Depressive Episode (MDE) at the noradrenergic [2], serotonergic [3,4], acetyl cholinergic system [8] and the possible involvement of gamma-aminobutyric acid-GABA- or of neurokinins, especially substance P, through its connections with receptor subfamilies (NK1, NK2, NK3) plays an important role in modulating the adaptive response to psychostress, with its increased levels in patients with depressive disorder [18] and low following antidepressant therapy [19]. Other theories refer to the role of melatonin in MDD, evidenced by the involvement of circadian rhythm abnormalities in MDE [20], as well as the involvement of neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF) and neuroplasticity theory [9]. The latest approaches in EDM also integrate the inflammatory theory that we will use in this thesis because it covers and integrates all other theories [21].

In this context, the research project Biological markers in depressive disorder starts from a broader theme, the predictive value of biological indicators in the screening, diagnosis and evaluation of depression, and aims to establish a series of clinical, biological, and statistical correlations between clinical data and a series of paraclinical data, with potential as an indicator of MDD presence for screening, diagnosis and evolution purpose.

CHAPTER II: DEPRESSIVE DISORDER: diagnosis and clinical aspects

The notion of MDD is widely used but more specifically we should refer to it as an unique or as a series of MDE (more easily defined from nosological point of view in terms of categories), that includes a varied phenomenology, from changes in disposition compatible with a relatively normal life because the impairment of functionality can be minimal, to psychotic manifestations, which evolve both with the disturbance of the affective state and with the diminution of cognitive, psychomotor and cognitive possibilities, so the patient is almost non-functional in any sphere (if we refer to an evolutionary dimensional definition) [23].

MDD is considered to be composed single / first or recurrent MDE and the diagnosis is made according to ICD-10 (Classification of Mental and Behavioral Disorders, World Health

Organization) and DSM- IV-TR (Diagnostic and Statistical Manual of Mental Disorders, edited by the American Psychiatric Association) [28,29]. The differential diagnosis of depression disorder is based on the correct use of DSM IV TR or ICD 10 diagnostic criteria, and the intensity of the disorder is established by confirming the clinical impression rating scales [30]. The main differential diagnoses are made in relation to the following disorders [28, 29]: sadness and pathological grief; dysthymia; depression disorder adaptation disorder; mixed anxiety-depressive disorder; bipolar affective disorder with current depressive episode; borderline personality disorder; affective disorder due to a general medical condition, which in turn must be differentiated by the somatic comorbidities following the major depressive disorder, in which the depressive episode can be considered as an etiopathogenic element; masked depression; alcohol and drug use accompanied by depressive manifestations.

MDD Therapy

MDE requires prompt, correct diagnosis and effective treatment for several reasons: depressive patients have a 2x higher risk of mortality than the general population, resulting from direct causes, such as suicide, 30x more commonly in the population of people with MDD [31]. The need to treat an MDE as soon as possible comes because we want to avoid chronic depression and incomplete remissions. The major goal of the effective therapeutic treatment of depressive patients is to reach full and complete remission [31] and the best results in achieving and maintaining long-term quality remission have been on the combined therapy (pharmacotherapy and psychotherapy) [32].

In this model, the three stages of disease progression correspond to the three treatment periods as follows: (1) the acute phase corresponds to the period from the initiation of the treatment to the remission, (2) the phase of continuation of the treatment corresponds to the period in which the remission is stabilized. , and (3) the maintenance phase is a prophylactic one, regarding the emergence of new episodes of depression or suicide. The acute period lasts 6-12 weeks, the retention period, at least 6 months after obtaining the remission and aims to prevent relapses or recurrence, rehabilitation and long-term monitoring of the remission [32]. We talk about recovery after episodes when the patient has been asymptomatic for about 6 months. The term "recovery" is used only for individual episodes of the disease and does not imply that the patient is prevented from recurrence [34]. The therapeutic options follow the three standard approaches to the treatment of depression: pharmacotherapy, psychotherapy, light therapy and neurostimulation therapies. In the WHO classification, entitled Anatomical Therapeutic Chemical Classification System (ATC), for the classification of drugs and other

products, code N06 is reserved for psychoanaleptics. The N does not show the antidepressants belonging to the nervous system medication.

Other therapeutic means would be Psychotherapies and Neurostimulation Therapies: Electroshock therapy (ECT), Transcranial Magnetic Stimulation (TMS), Deep Brain Stimulation (DBS) and Vagus Nerve Stimulation (VNS). The current psychotherapeutic approaches have been developed and refined according to the culture and particularities of the target population, being forced to face other types of intrapsychic conflicts conditioned by the alienating potential of modern life [33]. Several types of psychotherapy are used, each with response rates and method limitations [30].

CHAPTER III - Epidemiology and etiopathogenesis MDD

Epidemiological data

Currently, at the global level, MDD is the leading cause of disability, both by presentism and absenteeism [25]. Moreover, MDD is considered in the top 10 causes of mortality worldwide, accounting for about 4% of all medical-related deaths [24]. The incidence of MDD is between 80 and 200 / 100,000 / year in the male population and between 250 and 7800 / 100,000 / year in women, and the prevalence in Western countries is between 1.8 and 3.2% in men and between 2 and 9, 3% in women [39].

Etiopathogenesis elements of the MDD

Theorized etiopathogenic models for the MDD support a multifactorial etiopathogenesis, in which numerous links are distinguished. The genetic model is based on studies that have objectified the risk for depression by presynaptic synthesis deficiency (free tryptophan values, serotonin transporters) and post-synaptic signaling (5HT receptors) for serotonin [2], being considered as genetic risk indicators of serotonin dysfunction. from depression

The multi-systemic approach to depression allows the correct evaluation of the multiple enteropathogenic factors involved in depressive pathology, of the neuralgic points that are not resolved so far from the therapeutic point of view (neurodegenerative risk, blood-brain dysfunction, vascular perfusion, etc.) and the search for valid markers. biological type that can be easily used and relatively low cost, providing indications on the evolutionary stage of the disease (markers of endothelial dysfunction, markers of proinflammatory hyperactivity, disturbance of immune function, hyperactivity of the HHCS axis) [9]. The neurobiochemical model, in close correlation with the psychopharmacology of depressive disorder,

antidepressant medication having the corrective role for one, two or three lines of neurotransmitters and rebalancing synaptic functioning [9]. Neuroprotection, neuroplasticity and neurogenesis could be correlated with circadian rhythms, the suprachiasmatic nucleus and melatonin being true sensors of this cellular activity. The seasonal depressive disorder model was the basis of the neurobiological studies regarding the correlation of the mechanisms of the depressive disorder and the influence of the circadian rhythms on the imbalance or functional balance of the cerebral circuits involved in the etiopathogenesis of depression. In this perspective, light therapy has made "history", but the excess of the light excitation caused secondary glutamatergic activations and diminished the mechanisms of neuroplasticity and neurogenesis. From these observations, it can be argued that the balance of cortico-subcortical circuits involving the cerebral amygdala, hippocampus, cingulate cortex and frontal cortex can only be rebalanced by maintaining the functional relationship between dysplastic (excitotoxic) and pro-neurogenetic factors [9, 31].

Conservation of the neuroplasticity becomes an important target of therapeutic strategies for preserving connectivity between the main brain structures involved in depression (prefrontal cortex, anterior cingulate cortex, cerebral amygdala and hippocampus). Decreased cerebral amygdala volume could be observed in both neuroimaging and post-mortem studies in cases diagnosed as depression with therapeutic resistance [9].

CHAPTER IV: SECONDARY MDE AND ITS MOST FREQUENT COMORBIDITIES

Depression and diabetes: MDD frequently associate type II diabetes, a comorbid association that significantly influences the rehabilitation level of patients with this diagnosis. The prevalence of functional disabilities caused by the association between depressive disorder and diabetes is assessed at the following values [72]: functional disabilities caused by major depressive disorder: 51.3%; functional disabilities caused by diabetes: 58.1%; functional disabilities caused by the association of diabetes with major depressive disorder: 77.8%. The comorbid association between depression and diabetes significantly increases the mortality rate for all medical or neurological conditions [72].

The mechanisms involved in the evaluation of micro- and macrovascular risk in depression comorbidity with type II diabetes may be correlated with biological indicators: chronic vulnerability of the hypothalamus-pituitary-adrenal axis with high cortisol levels and reduction of insulin sensitivity; involvement of immune systems with possible peripheral

biological markers (increase of lipid level, free cholesterol and conjugate); proinflammatory factors (increased fibrinogen and C-reactive protein) and cytokines (IL-6, TNF- α) [74].

Depression and cardiovascular disease: depression can be considered as an adverse prognostic factor in patients with risk factors for coronary heart disease or those who already have a confirmed coronary heart disease [75]. The prevalence of depressive disorder in patients with cardiovascular disease is estimated at about 20% [76], considering that depression is three times more common in patients who have had a recent myocardial infarction, than in the general population. MDE is an independent risk factor for the evolution of myocardial infarction, the biological and behavioral factors being superimposed to a large extent for both conditions, interconnecting. These data are also valid for the association of MDD with strokes. Moreover, the medication used in cardiology can cause depressive symptoms. However, it should be noted that beta-blockers cause increased depression, chronic fatigue syndrome and sexual dysfunction, significantly decreasing medication compliance and adherence, yet cardiological benefits should not be forgotten [77, 78].

Depression in neurodegenerative diseases: Neurodegenerative diseases frequently exhibit depressive manifestations during the prodromal period, depression being considered as a precipitating factor of neurodegenerative invasion. For example, Parkinson's Disease has obvious depressive symptoms in the prodromal phase, and after confirmation of the diagnosis, depression is encountered in 20-40% of patients and is resistant to treatment or has residual symptoms (85).

SPECIAL PART

Chapter V: Hypothesis, Objectives, Method and Materials

It becomes necessary to identify a biological marker of MDD, which should be highly sensitive, specific and which can be performed at a reasonable cost together with the routine analyzes and thus we can identify people with depressive symptomatology (who do not have insight of the disorder or who have it but does not show up in the specialized services for diagnosis and treatment because of the stigma or other factors) or with undiagnosed MDE in order to refer them to specialized care.

As the immune system and central nervous system (CNS) have two-way communication and the activation of inflammatory markers is demonstrated in MDE, we set out to investigate this area of biological markers [50]. This would be possible because this protein is secreted by the vascular endothelium and the relation of the endothelium to the

blood-brain barrier, perivascular space and CNS is much closer to CRP which is secreted by the liver or by the rest of the interleukins or cytokines secreted in the periphery [50]. This bidirectional relationship between the CNS and the immune system via the endothelium also appears to be confirmed by the high rate of depression in cardiovascular diseases, which are also the most common comorbidities [80].

So far, biological markers whose changes were significantly correlated with depressive disorder were [90]: Cortisol (variable data), TNF α (limited studies), IL 2, IL 6, IL 10 (contradictory data), C-reactive protein (contradictory data) and CRP-hs (limited data).

C-reactive protein (CRP) is a protein that is not secreted under normal conditions, it is secreted only during the inflammatory process. CRP occurs in the bloodstream in response to the action of cytokines such as interleukin-6 (IL6). CRP is theoretically non-existent in the blood flow of healthy persons. CRP is one of the most sensitive biological markers of acute inflammation. However, CRP does not have a good specificity although it is very sensitive [91].

There are two ways to evaluate the CRP. The first assesses CRP in the general way to monitor inflammatory processes, to detect infections or to follow the transplant or transplant rejection. The reported values are between 0.3 and 20mg / dl or 3 up to 200mg / l. The second method is a high-sensitivity one (high-sensitivity C-reactive protein or CRP-hs) that can detect low levels of the protein and are a marker of the risk of cardiovascular events. The sensitivity is 0.01mg / dl (0.10mg / l). CRP-hs is therefore useful for assessing the risk of developing myocardial infarction in patients with acute coronary syndrome [91]. The American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC) have made the following recommendations for testing hs-CRP for cardiac risk assessment: - In patients with systemic inflammation, with signs of infection or trauma, testing should not be performed due to false positive results; - To evaluate the cardiovascular risk optimally, it is recommended to use the average value of the hs-CRP test. This average value is obtained from a minimum of 2 values measured at an interval of 2 weeks. If one of the determinations is detected a level > 10 mg / L an inflammatory cause should be suspected and the test will be repeated over another 2 weeks; - People in whom the calculation of standard markers has estimated a moderate risk (For example: a 10-20% risk of developing cardiovascular disease in the next 10 years) and the doctor wants additional information to recommend the optimal preventive treatment, will benefit the most by this test; - In secondary prevention, the role of hs-CRP (For example: in patients already diagnosed with

cardiovascular disease) is limited. The main reason in this case is generated by the fact that the interventions must be aggressive, regardless of the hs-CRP values, because the evaluation of this biological marker does not influence the therapeutic decisions; -The evaluation of hs-CRP should not be substituted for the already standardized markers of cardiovascular risk but it is an additional element with the net benefits mentioned above. This is why screening of the entire adult population using hs-CRP is not recommended.

Risk groups are defined as follows by CRP-hs [91]:

- low risk: below 1.0 mg / L
- average risk: from 1.0 to 3.0 mg / L
- increased risk: over 3.0 mg / L

The main objective of the research is to establish the level of validity that highly specific / sensitive C reactive Protein (PCR-hs / hs-CRP) can have as a screening biological marker for depressive disorder and to evaluate its reliability and accuracy.

The secondary objective of the research is to identify, if possible, other possible biological markers of the depressive disorder and to correlate the possible values with the severity of the depressive symptomatology.

CRP-hs determination method [91]:

The present research is represented by a prospective and naturalistic study on a batch of 100 random persons that came at the INGG Ana Aslan ambulatory for routine bloodtests within the time interval (1 Feb-30 June 2016). The constitution of the study group was made on the basis of informed consent, in accordance with the ethical norms of respecting the confidentiality of information and of anonymizing the identity of patients in the database and after obtaining the necessary approvals from the Ethics Commissions of the I.O.S.U.D. U.M.F. from Craiova, as well as a protocol for collaboration with INGG Ana Aslan and Innomedica.

First thing was the patient information and training with informed consent signing. Afterwards, he completed the scales and the blood sample was collected from the venous blood according to the standard procedure. The vacutainer was without anticoagulant and without separator gel. The minimum volume of the sample was 0.5 mL serum. After that the sample was put to centrifugation to separate the serum. Possible causes for sample rejection were: intensely hemolysed or intensely lipemic specimens. Sample stability: serum is stable

for 3 days at room temperature; 8 days at 2-8°C; prolonged time (12-24 months) at -20°C. In the case of the present study the serum were stored at -20°C and worked at the end of the harvest in two batches. The method used in the present study was latex-immunoturbidimetry and the limit of detection was 0.15 mg / L. For waves > 10 mg / L causes of non-cardiovascular origin were considered.

We also considered the main limits and possible interferences because the increases in hs-CRP values may be nonspecific and therefore should be interpreted only in the clinical context of the patients. Moreover, in the case of large values, the determination was repeated. [91, 94, 95].

HADS scale (self-administration scale - which can be completed automatically on an HP tablet thanks to innovative software developed together with Innomedica and which automatically communicates with the database). There were also printed versions of this scale for patients who wanted to participate in the research but did not want to complete the data on a tablet. Patients who achieved high scores on the HADS scale on depression or anxiety had the opportunity to schedule a free psychiatric consultation to be diagnosed according to ICD-10 and / or DSM IV TR criteria or to deny the diagnosis. The study was conducted between February 1, 2016 - June 30, 2016 at the INGG polyclinic Ana Aslan in Bucharest.

The following biological variables were also measured: hemolymphogram, GGT, blood glucose, total cholesterol, triglycerides, TSH, fibrinogen, VSH, CRP and CRP-hs.

Psychometric instruments and analyzers

1. Major Anxiety and Depression Scale (HADS) Self-Assessment Scale. The evaluation of depression in clinical trials requires objective calibration and the HADS scale allows a good quality quantification of the clinical impression according to Zigmond AS and Snaith RP from 1983 being recognized for the fidelity and validity of the Bjelland I bag from 2002 [43, 44].

2. Paraclinical tests: C-reactive protein and C-reactive protein with high sensitivity - CRP-HS plus all the usual analyzes performed by patients.

3. Konelab 30- with method validation to work CRP, CRP-HS and the rest of the immunological and Biochemical analyzes.

4. Celltac F microsed- for VSH work.

5. Quadadata 2001 - for fibrinogen work.

6. Aia 360 - for thyroid hormone work.

7. Single channel automatic vats and pipettes with variable volume (5-50 µl / 50-200 µl).

8. CRP and CRP-HS reagents plus 2ml control for CRP-HS validation method.

For data analysis we used the Microsoft Excel program (Microsoft Corp., Redmond, WA, USA), along with the XLSTAT 2014 add-on for MS Excel (Addinsoft SARL, Paris, France) and the IBM SPSS Statistics 20.0 program (IBM Corporation, Armonk, NY, USA). We initially recorded the data in the Microsoft Excel files, then processed them statistically, in order to analyze the relationships between the clinical and paraclinical data of the patients.

CHAPTER VI: RESULTS ON THE STUDY LOT

The average age of the group was 67 years, high compared to the general population but explainable because the study took place in the ambulatory of the National Institute of Geriatrics and Gerontology and also because of this it is normal that most of them came to the analysis to be retirees. The hemolymphogram and gamma-glutamyltranspeptidase (GGT) were in 76% of patients within normal range. Cholesterol and triglycerides are part of the evaluation of lipid profile and this is a group of tests used to determine the risk of coronary heart disease had pathological values in 46% of cases, respectively 32% [97]. Thyroid stimulation hormone (TSH) was greater than 4.3ng / dl in 11% of cases. The inflammatory markers taken into account, except of course of CRP-HS, were - CRP, fibrinogen and VSH. Most patients, being older, had at least one diagnosis, only 9% of the total group did not suffer from any pathology. Of the 91% patients who had a main diagnosis 53% had no secondary diagnosis. 38% of patients had at least two somatic comorbidities. 47% of the patients in the group suffered from a cardiovascular disease, which is above the average found in the specialized literature, but it is explainable because the average age of the group was one advanced. The most common secondary diagnosis in the study group was that of metabolic diseases (diabetes and dyslipidemia) because 15% of them had this secondary diagnosis. The association of the two pathologies is an aggravating element from the point of view of the evolutionary complications, as well as of the direct consequences on the management of the diseases, by therapeutic non-adherence and risk behaviors. cardiovascular associated with the main diagnosis, being considered with an increased risk of morbidity-mortality.

CHAPTER VII: STATISTICAL ANALYSYS AND DISCUSSIONS

Statistical analysis of variables for defining potential markers

The results obtained in the Shapiro-Wilk and Anderson-Darling tests for all parameters measured or recorded in this research show that most of the variables do not have Gaussian distribution, and for this reason **we will use nonparametric tests**. The standard deviation for

general parameters analyzed in relation to the HADS-A and HADS-D scores varies within the group between a minimum of 12.46 for age and a maximum of 60.45 for triglyceride level. For inflammatory markers the smallest standard deviation is within the TSH values of 1.48, followed by the CRP value with 3.16 and CRP-HS with 4.48. It can be seen from Table 23 that the highest standard deviation value is for fibrinogen.

The HADS scale is composed of 14 items of which 7 are used for the evaluation of depressive symptoms and 7 are for the evaluation of anxiety symptoms. A score higher than 8 for each domain indicates an increased risk for depression or anxiety. The overall variation of the scores was between 2 and 34 points with a median of 17 points. Only the CRP and CRP-HS values are statistically significantly correlated with the risk of anxiety and for the estimation of the risk of depression, the only biological indicators that are statistically significantly correlated are CRP, CRP-hs, VSH, fibrinogen and interestingly the age of the patients (Mann-analysis). Whitney). The correlation matrix (rho Spearman type) already shows us which are the first markers that can be correlated with the depressive symptomatology objectified by the HADS scale.

VARIABLE	AGE	GGT	GLY	CHOL	TRYGL	Fibrinogen	VSH	TSH	CRP	CRP HS	HADS- global	HADS-A	HADS-D
AGE		0.000 (0.997)	0.109 (0.281)	-0.131 (0.195)	-0.174 (0.083)	0.323 (0.001)	0.058 (0.568)	-0.016 (0.871)	0.185 (0.065)	0.296 (0.003)	0.315 (0.001)	0.168 (0.095)	0.461 (0.000)
GGT	0.000 (0.997)		0.420 (< 0.0001)	0.119 (0.240)	0.464 (< 0.0001)	0.110 (0.274)	0.151 (0.133)	0.125 (0.215)	0.079 (0.432)	0.154 (0.125)	0.120 (0.233)	0.061 (0.543)	0.144 (0.153)
GLY	0.109 (0.281)	0.420 (< 0.0001)		0.075 (0.455)	0.457 (< 0.0001)	0.116 (0.250)	-0.105 (0.298)	0.010 (0.918)	-0.082 (0.414)	0.006 (0.949)	0.018 (0.860)	0.026 (0.795)	0.017 (0.868)
CHOL	-0.131 (0.195)	0.119 (0.240)	0.075 (0.455)		0.394 (< 0.0001)	0.082 (0.414)	0.246 (0.014)	0.043 (0.673)	0.044 (0.660)	0.002 (0.987)	-0.014 (0.888)	-0.025 (0.804)	-0.012 (0.904)
TRYGL	-0.174 (0.083)	0.464 (< 0.0001)	0.457 (< 0.0001)	0.394 (< 0.0001)		0.050 (0.620)	0.165 (0.100)	0.108 (0.283)	-0.049 (0.628)	0.080 (0.426)	-0.100 (0.321)	-0.062 (0.540)	-0.147 (0.143)
Fibrinogen	0.323 (0.001)	0.110 (0.274)	0.116 (0.250)	0.082 (0.414)	0.050 (0.620)		0.514 (< 0.0001)	0.110 (0.276)	0.391 (< 0.0001)	0.381 (0.000)	0.303 (0.002)	0.183 (0.069)	0.360 (0.000)
VSH	0.058 (0.568)	0.151 (0.133)	-0.105 (0.298)	0.246 (0.014)	0.165 (0.100)	0.514 (< 0.0001)		0.139 (0.166)	0.383 (< 0.0001)	0.385 (< 0.0001)	0.251 (0.012)	0.139 (0.167)	0.320 (0.001)
TSH	-0.016 (0.871)	0.125 (0.215)	0.010 (0.918)	0.043 (0.673)	0.108 (0.283)	0.110 (0.276)	0.139 (0.166)		0.036 (0.720)	0.097 (0.337)	0.138 (0.170)	0.172 (0.087)	0.052 (0.608)
CRP	0.185 (0.065)	0.079 (0.432)	-0.082 (0.414)	0.044 (0.660)	-0.049 (0.628)	0.391 (< 0.0001)	0.383 (< 0.0001)	0.036 (0.720)		0.415 (< 0.0001)	0.358 (0.000)	0.228 (0.023)	0.445 (< 0.0001)
CRP HS	0.296 (0.003)	0.154 (0.125)	0.006 (0.949)	0.002 (0.987)	0.080 (0.426)	0.381 (0.000)	0.385 (< 0.0001)	0.097 (0.337)	0.415 (< 0.0001)		0.347 (0.000)	0.228 (0.023)	0.406 (< 0.0001)
HADS- global	0.315 (0.001)	0.120 (0.233)	0.018 (0.860)	-0.014 (0.888)	-0.100 (0.321)	0.303 (0.002)	0.251 (0.012)	0.138 (0.170)	0.358 (0.000)	0.347 (0.000)		0.913 (< 0.0001)	0.802 (< 0.0001)
HADS-A	0.168 (0.095)	0.061 (0.543)	0.026 (0.795)	-0.025 (0.804)	-0.062 (0.540)	0.183 (0.069)	0.139 (0.167)	0.172 (0.087)	0.228 (0.023)	0.228 (0.023)	0.913 (< 0.0001)		0.513 (< 0.0001)
HADS-D	0.461 (< 0.0001)	0.144 (0.153)	0.017 (0.868)	-0.012 (0.904)	-0.147 (0.143)	0.360 (0.000)	0.320 (0.001)	0.052 (0.608)	0.445 (< 0.0001)	0.406 (< 0.0001)	0.802 (< 0.0001)	0.513 (< 0.0001)	

rho Spearman correlation matrix

Biological markers

The correlation matrix shows us which are the markers that can be statistically significant associated with the depressive symptoms objectified by the HADS scale. For CRP, the rho Spearman correlation coefficient value was 0.2278, which corresponds to a significance level $p = 0.0227 < 0.05$, and for CRP-HS the rho coefficient was 0.2281, which corresponds to a p value. = 0.0228 < 0.05.

The HADS-D score was also correlated with other markers of inflammation, fibrinogen and VSH value, the rho Spearman correlation coefficient values being 0.3600 for fibrinogen ($p = 0.0023 < 0.05$) and 0.3202 for VSH ($p = 0.0121 < 0.05$), the correlations being, and in these cases, directly proportional, that is, the HADS-D score is higher in the patients in whom we identified high values of fibrinogen or VSH (obviously, there is also a correlation directly between the two inflammatory markers, $\rho = 0.514$, corresponding to a p value < 0.001, highly statistically significant, as can be seen from table 36).

CRP proved to be a much more reliable inflammatory marker for anxiety assessment, the difference between patients with HADS-A ≤ 8 score and those with HADS-A > 8 score being statistically significant, $p = 0.026 < 0.05$, with CRP values being higher for those with high scores on the HADS-A subscale.

CRP-HS identifies a clearer difference between patients with HADS-A ≤ 8 score and those with HADS-A score > 8 , the Mann-Whitney test result being statistically significant, $p = 0.011 < 0.05$, CRP-HS values being more high for those with high scores on the HADS-A subscale.

Patients who had higher values of HADS-D are older than those with lower values, on average 73.52 years old, compared to 63.93, the difference being highly statistically significant ($p < 0.001$). This result is in agreement with the correlation observed between HADS-D and age, by the coefficient rho Spearman = 0.4612, which corresponds to a p value < 0.001, highly statistically significant.

As with the HADS-A subscale, CRP and CRP-HS also proved to be more sensitive markers for both HADS-D, for both variables registering highly significant differences between patients with HADS-D ≤ 8 score and those with score HADS-D > 8 , the Mann-Whitney test result being lower than the 0.001 threshold. However, in the Chi square test only the hypothesis that CRP-HS can assess the risk of depression was valid (p Chi square was 0.004).

Statistical variables and discussions

We analyzed the relationships between the levels of the scores recorded on the psychometric subscales used and the threshold levels proposed in the literature for the demographic variables and the biological constants recorded, to see if they are relevant in the pathology studied in the present work.

We performed the **Chi square test** for the incidence tables generated by the cross-sectional analysis of HADS-A, respectively HADS-D, with each parameter separately. Unfortunately, this type of analysis proved less sensitive than the one performed by the Mann test. -Whitney, of the biological variables *only the risk evaluated by the CRP-HS values for the patients with HADS-D \leq 8 score and those with HADS-D score $>$ 8 having a statistical significance (p Chi square = 0.004 <0.05).*

If we are comparing study results with the data from other studies on inflammatory markers and depression in elderly population, we should mention also the study of Dr. Heiki Luukinen, which aimed to study the relationship between CRP-hs value and the incidence of depression in 1113 elderly people in Scandinavian countries had some similar results even if the methodology was different[116] . In this study, CRP-hs value is significantly correlated with depressive symptomatology evaluated using the Zung Rating Scale regardless of comorbidities or sex [116]. And the study of Dr. Marian L. Valter performed on 390 European patients using CRP-hs measurement and the PH-Q8 questionnaire, confirms CRP-hs as a biomarker for depression, regardless of gender [117].

Chapter VIII: CONCLUSIONS

1. The primary objective of the study was reached because we were able to demonstrate that CRP-HS can be used as a biomarker to determine the risk of depression. In the study group we determined the following: 26% of patients had normal CRP-HS values with minor cardiovascular risk, 28% had average risk and 46% major risk. The variation of CRP-HS values in the batch was large, between 0.01mg / L and 18.57 mg / L. The p-value according to the Mann-Whitney analysis for anxiety was 0.106 and for depression 0.0001. The rho Spearman value for anxiety risk was 0.028 and for depression 0.0001. However, in chi square analysis only the value of the statistical significance of the validity of the theory for depression was valid (0.004).

2. Secondary goals were partially achieved because the other parameters monitored from a global point of view do not correlate with depression, but the value of CRP, CRP-HS,

VSH and fibrinogen correlates statistically with the severity of depression measured by the HADS scale.

Proposal for the study data future usage

The data obtained may initially be used to replicate the study on a larger and more diverse group in order to validate the biological markers of depression identified in the present work for other population. After this step, these markers may be used as a screening method for major depressive episode during routine analyzes. The CRP- HS biological marker for the risk of depression, may also be used in another scientific research or even in the clinic.

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