

**UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL**

PHD THESIS

HEMATOLOGICAL CHANGES IN HEART FAILURE

-ABSTRACT-

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Key words: heart failure, haematological parameters, anemia

STATE OF KNOWLEDGE

CHAPTER 1 - MODIFICATIONS OF THE ERITROCITARY SERIES IN HEART FAILURE

1.1 ANEMIA AND HEART FAILURE

Anemia is a hematological comorbidity that has been increasingly studied in recent years. Until now, the causes of anemia in patients with heart failure (HF) and the mechanisms by which anemia aggravate HF are not exactly known. Although there is no clear causal relationship between HF and anemia, there are multiple mechanisms whose complex interactions can contribute to anemia: chronic renal dysfunction, erythropoietin deficiency, nutritional deficits, chronic inflammation, malnutrition due to chronic HF, dysfunction haematogenous marrow, neurohormonal activation, hemodilution and secondary drug treatment ^[1]. The hemodynamic compensatory response induced by the presence of anemia can contribute to worsening of heart failure on the one hand and, on the other hand, to a further decrease in hemoglobin (Hb), thereby closing down a vicious circle.

1.2 ERYTHROCYTOSIS IN HEART FAILURE

Increasing the number of erythrocytes increases the blood's ability to carry oxygen but high hematocrit may increase the risk of thrombotic events by increasing blood viscosity and may interfere with remodeling and cardiac apoptosis in HF by activating leukocytes and production of iron-induced proinflammatory cytokines (Fe²⁺) from the hemoglobin structure ^[2].

CHAPTER 2

INTERRELATION LEUKOCYTE – HEART FAILURE

The role of chronic inflammation in the onset of HF has been demonstrated in several clinical trials, the inflammatory process having an important role in HF, especially in men ^[3,4]. Increased production of proinflammatory cytokines from circulating monocytes and activation of the immune system can directly reduce the number of lymphocytes ^[5] and increase oxidative stress in neutrophils, lymphocytes and especially in circulating monocytes in decompensated HF ^[6, 7]. In addition, the intrinsic dysfunction in the HF are characterized by reduction in bone marrow hematopoietic stem cell differentiation in the myeloid and erythroid progenitor cells in similar proportions ^[8].

CHAPTER 3

MODIFICATIONS OF THE THROMBOCYTE SERIES IN HEART FAILURE

Several morphological and functional changes of platelets were observed in heart failure: reduction of platelet life, increase in mean platelet volume and increased platelets activation and reactivity ^[9, 10]. Also, inflammatory conditions are associated with reactive thrombocytosis and the presence of leukocyte-thrombocyte complexes may be a pathophysiological link between thrombosis and inflammation ^[11, 12]. Iron deficiency anemia can cause reactive thrombocytosis in 13-50% of cases ^[13, 14] and increases oxidative stress, thus increasing the risk of thromboembolic complications associated with hypercoagulant status in HF.

PERSONAL RESEARCH

CHAPTER 4 - MATERIALS AND METHODS

I've conducted a descriptive-analytic study, observational, prospective study involving 397 consecutive patients hospitalized for decompensated HF, from 10.10.2010 to 30.09.2013 in the Clinic of Cardiology of the Filantropia Municipal Clinical Hospital of Craiova. All patients included in the study were over 18 years old, with chronic HF and NYHA functional class III or IV, diagnosed at least 1 year before inclusion in the study and the left ventricular ejection fraction (LVEF) $\leq 45\%$ at echocardiography. Patients with acute or chronic conditions accompanied by hematological changes or undergoing therapies that may influence the haematological profile, vegetarian diet, pregnancy, uncooperative patients and those who refused to participate in the study were excluded. All patients confirmed their participation by signing an informed consent form.

Patients were followed for 3 years after inclusion in our study. During the study, patients who were identified with a condition mentioned in the exclusion criteria were excluded from the study group.

For the statistical analysis of the data we used the EXCEL 2016 and SPSS 20 (Statistical Package for Social Sciences). We considered a statistically significant value of $p < 0.05$.

CHAPTER 5 – STUDY I - PREVALENCE OF HEMATOLOGICAL CHANGES IN PATIENTS WITH HEART FAILURE

5.1 PURPOSE AND SPECIFIC OBJECTIVES OF STUDY I

The purpose of the research was to identify haematological changes that may be risk factors for heart failure decompensation with FEVS \leq 45%.

The objectives of the study are: to describe the haematological parameters of patients with heart failure enrolled in the study and to compare them with the values considered normal among the general healthy population; analysis of aspects of the relationship between chronic decompensated cardiac disease, associated pathologies, drug therapy and the presence of haematological changes; identifying haematological variables with predictive role in worsening heart failure.

5.2 RESULTS

5.2.1 GENERAL DATA OF THE STUDIED LOT

The study analyzes a group of elderly patients (mean age 73.3 ± 7.8 years) with ischemic etiology of HF (45%) followed by valvular etiology (34.8%), multiple comorbidities [primary hypertension (HTN) - 63.7%, diabetes mellitus - 39.3%, chronic obstructive pulmonary disease (COPD) - 19.1%, renal dysfunction - 49.6%, atrial fibrillation - 58.9%), a severe clinical picture presentation [functional class New York Heart Association (NYHA) IV - 55%, clinical congestion - 73%], undergoing treatment with HF-specific medication adapted to the clinical particularities of each patient.

5.2.2 PREVALENCE OF ERYTHROCYTE SERIES CHANGES

The erythrocyte profile characteristic of IC patients studied in the age group 45-64 years is characterized by significantly lower values of red blood cells (RBC), hemoglobin concentration and hematocrit (Ht) in men as well as significantly lower mean corpuscular volume (MCV) both in women and men with HFs studied compared to the healthy population of the same age and sex. In contrast, patients with HF studying over 64 years have significantly lower values of erythrocyte counts, Hb, Ht, compared with corresponding healthy population with the same age and sex. The number of erythrocytes, hemoglobin and hematocrit decreases progressively, and MCV values increase slightly as HF patients progress to elderly. Lower blood cell count, Hb and Ht with age is more pronounced in men with HF study compared to healthy men of the same age. Female sex is associated with significantly lower erythrocyte

counts, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and higher values of red cell distribution width (RDW-CV).

History of HTN and estimated glomerular filtration rate (GFR) $<60\text{ml} / \text{min} / 1.73\text{m}^2$ on admission was significantly associated with lower values of the number of RBC, Hb and Ht; decreased GFR is associated with a corresponding decrease in the number of erythrocytes, the Hb and Ht. Diabetes is an important predictor of lower values of Hb, MCH and MCHC. In the group of patients with atrial fibrillation, the RDW-CV values are significantly higher. The presence of COPD as well as smoker status has been associated with a significant increase in erythrocyte count, Hb and Ht.

NYHA functional class IV, the presence of clinical congestion, systolic blood pressure (BP) $<90\text{ mmHg}$ and $\text{LVEF} \leq 35\%$ are accompanied by a erythrocyte profile characterized by: lower blood cell count, Hb, Ht, MCHC and an increase of RDW-CV values.

Treatment with furosemide at home was associated with lower Hb and Ht values and administration of angiotensin converting enzyme (ACE) inhibitors was associated with significantly higher Ht values compared to those without ACE or angiotensin receptor blockers (ARB).

5.2.3 PREVALENCE OF LEUKOCYTE SERIES CHANGES

The leukocyte profile characteristic of patients with IC studied is associated with significantly lower values of white blood cell counts and basophils compared to the corresponding healthy population as age and sex. The number of lymphocytes and leukocytes progressively decrease as patients with HF get older. The female sex is associated with significantly lower values of the number of monocytes.

The presence of $\text{GFR} <60\text{ml} / \text{min} / 1.73\text{m}^2$ at admission is characterized by lower lymphocyte counts, monocytes and a significantly greater number of neutrophils. The decrease in GFR is associated with a proportional decrease in lymphocyte count. Diabetes mellitus is an important predictor of the higher leukocyte and neutrophil count in patients with severe HF. The lymphocyte, eosinophil and basophil counts are significantly lower in patients with atrial fibrillation. Smoking is accompanied by a significant increase in the number of leukocytes and monocytes.

The NYHA IV functional class is accompanied by a change in the leukocyte profile (decreased lymphocyte, eosinophil and basophil counts with neutrophil counts and approximately similar leukocyte counts), the presence of systemic congestion is associated with a significantly lower number of lymphocytes, systolic BP values $<90\text{ mmHg}$ are accompanied

by significantly lower lymphocyte counts and significantly higher monocytes, and LVEF<35% is associated with significantly lower white blood cell counts and lymphocytes.

Treatment with ACE inhibitors is associated with significantly lower leucocyte and monocyte counts and statins and beta blockers are associated with higher lymphocyte counts than those without these drug classes in the treatment regimen. The number of lymphocytes is lower in patients with antiplatelet therapy, the number of leukocytes is lower under oral anticoagulant therapy and the number of basophils is lower in patients without antiplatelet or oral anticoagulant therapy.

5.2.4 PREVALENCE OF THROMBOCYTE CHANGES

Although 14.5% of the patients studied had mild thrombocytopenia, and patients over 75 years of age had significantly lower platelet counts, however, the platelet count was not influenced by the presence of HF. Statin therapy is associated with significantly elevated platelet counts and lower platelet distribution (PDW) compared to those without statins in the treatment scheme, while the use of betablockers is associated with a significantly higher number of thrombocytes compared to patients without beta-blocking therapy.

5.2.5 THE PRESENCE OF ANEMIA

The prevalence of anemia in hospitalized patients for HF decompensation is high, with 38.5% of patients being anemic and increasing with age regardless of gender. Anemia in severe IC is associated with older age (75.1 vs. 72.3 years), female sex, more frequent comorbidities (61% of anemic patients with renal dysfunction) and a more severe clinical picture at presentation (NYHA functional class, the presence of clinical congestion, lower systolic BP values) and lower LVEF. Anemia has been indirectly correlated with GFR, clinical congestion, lower body mass index (BMI), total serum cholesterol, lymphocyte count, inflammatory markers [erythrocyte sedimentation rate (ESR) and fibrinogen]. The presence of anemia was not directly influenced by the administration of specific HF treatment.

Patients with anemia and HF have changes in the red cell line (reduction in the number of erythrocytes, hemoglobin, hematocrit, MCV, MCH and MCHC and increasing RDW-CV values), changes in leukocyte line (decrease in the number of leukocytes and lymphocytes) and the platelet line (PDW decrease), suggesting generalized bone marrow dysfunction. The association between anemia, a lower number of lymphocytes, a lower BMI and lower total serum cholesterol may suggest malnutrition as one of the causes of anemia in patients with HF's studied.

Predictors of anemia are: female sex, presence of comorbidities (HTN, diabetes mellitus), severe clinical manifestations (NYHA functional class, congestion, systolic BP), the values of BMI, serum cholesterol, and spironolactone and beta-blocker treatment.

5.2.6 MORPHOLOGICAL TYPES OF ANEMIA

Normochromic normocytic anemia was morphologically most common type of anemia (68.6%). 1 of 10 patients have a form of hypochromic anemia (normocytic and microcytic) and 3.02% have macrocytic anemia. Hypochromic anemia (microcytic and normocytic) are more common in women (75%) and patients with diabetes (63.8%). 50% of patients with anemia could have iron deficiency (RDW > 14.5% associated with MCV < 98fl). Anemia of chronic disease could be present at the 42.48% of the patients with anemia and RDW-CV ≤ 14.5% and MCV < 98fl.

5.3 DISCUSSIONS

Demographic, clinical and imaging characteristics of patients included in the study are similar to those described in the literature for patients with advanced HF. Hematological changes may be determined by the hormonal profile characteristic of the two sexes, the influence of heart failure and associated comorbidities on the hormonal profile and the bone marrow synthesis ability and are similar to the data in the literature [15-27].

CHAPTER 6

STUDY II – IMPACT OF HEMATOLOGICAL CHANGES ON THE PROGNOSIS OF PATIENTS WITH HEART FAILURE

6.1 PURPOSE AND SPECIFIC OBJECTIVES OF STUDY II

The purpose of the research was to identify haematological changes that may be prognostic factors for the progression of heart failure with reduced LVEF.

To analyze the time elapsed until a new rehospitalization for heart failure decompensation or death, all patients included in the study were followed for a period of three years from the baseline. The mean follow-up was 920.4 ± 410 days (minimum 3 days and maximum 1460 days).

The study objectives were: description of demographic parameters, clinical and laboratory features associated with rehospitalization and death in patients with heart failure as well as identification of predictive haematological parameters for the risk of rehospitalization for worsening heart failure in one year and risk of death in three years.

6.2 RESULTS

6.2.1. ANALYSIS OF REHOSPITALIZATION AT 1 YEAR

HF decompensation readmission rate for the first year after discharge is 1 to 3. Rehospitalisations are more common in women, valvular HF, those with renal dysfunction, atrial fibrillation, smoking and severe clinical picture of HF (NYHA functional class IV, the presence of congestion, systolic BP <90 mmHg), LVEF <35%, those who are receiving oral anticoagulation and those without treatment with beta-blockers and spironolactone. PDW values > 13% are predictive of increased risk of rehospitalization only in the presence of higher Ht values. CHEM values > 33.05 g / dl are predictive of the risk of decrease rehospitalization, regardless of the presence / absence of anemia. The absence of anemia was associated with an increased risk of rehospitalization along with a decrease in LVEF. The value of hematocrit has an insignificant individual influence and high Ht values could contribute to increasing the predictive role of PDW.

6.2.2. ANALYSIS OF MORTALITY AT 3 YEARS

A percentage of 24.7% of HF patients died in the first 3 years of inclusion in the study. Death was associated with the presence of COPD, atrial fibrillation, severe clinical picture of HF (NYHA IV functional class, congestion and systolic BP <90 mmHg) , LVEF <35% but also rehospitalization history. Anemia was more common in patients who died (31.4%), but it was not a predictor of mortality in this study. The most common morphological type of anemia encountered in deceased patients was normocytic normochromic anemia (65.6%).

Although MCHC, hematocrit and platelet counts were included in the predictive mortality model, these haematological parameters have low individual predictive value, the risk of death being higher only if lower MCHC values are associated with a lower platelet count and higher Ht value, similar to LVEF decrease.

6.3 DISCUSSIONS

Both the rate of rehospitalisation and death are consistent with the published literature. PDW is a platelet index associated with increased risk of rehospitalization similar to results of other studies ^[28]. There are no clinical studies that have analyzed the impact of MCHC values on the risk of rehospitalization in HF patients. Although in the descriptive analysis of our study, anemia was associated with a more severe clinical status, however, it was not a predictor of rehospitalization as it has been in several clinical trials published so far ^[28-30]. This study did

not show a correlation between Ht and the number of days to rehospitalization, most studies showing the association between lower Ht values and increased risk of rehospitalization [31, 32].

MCHC lower values are predictors of mortality in this study, similar to the literature [33]. Although Ht has been included in the predictive mortality model, it still has a much lower predictive value compared to the studies reported by several clinical studies that demonstrated the significant predictor role for mortality of Ht [31, 32]. In our study, the number of platelets has a very low influence on survival, data from literature showing that a lower number of platelets has been associated with increased mortality in patients with LVEF <40% [34].

CHAPTER 7

FINAL CONCLUSIONS OF THE THESIS

1. The haematological profile characteristic of patients with HF studied is characterized by significantly lower values of erythrocyte, hemoglobin, hematocrit, leukocyte and basophil count compared to the corresponding healthy population as age and sex.
2. Decreased erythrocyte count, Hb and Ht with age is more pronounced in men with HF studied compared to healthy men of the same age, similar to the results of other studies.
3. Decrease in GFR is associated with a proportional decrease in erythrocyte count, hemoglobin, hematocrit and lymphocyte count.
4. Diabetes mellitus is an important negative predictor for lower hemoglobin, HEM and CHEM, as well as for the higher leukocyte and neutrophil count in patients with severe HF.
5. Haematological changes associated with worsening HF on the one hand, refers to a decrease in the number of red blood cells, along with other parameters (red blood cell Hb, Ht, MCH, MCHC) confirming anemia and the presence of lymphopenia, and on the other hand is found higher values of RDW-CV, results consistent with the published literature.
6. Furosemide treatment was associated with lower hemoglobin and hematocrit values, similar to the results of other studies.
7. The prevalence of anemia in patients hospitalized for decompensation HF is 38.5%, normochromic normocytic anemia being the most common (68.6% of the anemia), similar to results of other studies.

8. Anemia in HF is associated with more advanced age, female gender, more frequent comorbidities, and a greater severity of heart failure (NYHA functional class, clinical congestion and lower systolic BP), lower LVEF, similar with other studies.
9. PDW values > 13% are positive predictors of the risk of rehospitalization only in the presence of higher Ht values while MCHC values > 33.05 g / dl are negative predictors of the risk of rehospitalization, regardless of the presence / absence of anemia.
10. Both MCHC, Ht and the number of platelets are very poor mortality predictors.

STUDY LIMITS

Although the present study performs an integrative analysis of all haematological cell lines (erythrocyte, leukocyte and platelet), affecting a hematologic parameter and influencing other blood chemistry parameters, there are some limits regarding the following aspects:

- I had no information on sideremia, serum ferritin, transferrin saturation, vitamin B12, folate, serum erythropoietin levels or inflammatory markers such as CRP, proinflammatory cytokines (TNF- α , IL-1, IL-6) or direct plasma volume measurement or erythrocyte mass so I could not differentiate the etiology of anemia and identify the subclinical iron deficiency,
- hematological parameters were evaluated once at the time of inclusion so we can not comment on the importance of their change over time,
- it is a unicentric study, and although it involved a significant number of patients with severe HF, it was relatively low compared to some of the above-mentioned clinical trials, which may be sources of error in interpreting the results.

PERSPECTIVES

The results of the current study could be supported in the future by multicentre studies and /or regional registries or a national register, including more patients and prospective character, for a better characterization of the hematologic profile of HF patients in the Oltenia region and / or in Romania, similar to the world records registers.

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