**Purpose / Objectives**

Erythroferrone was discovered in 2014 as a regulator of hepcidin synthesis and is synthesized by erythroblasts. Erythroferrone main function is to suppress hepcidin synthesis during inflammation, thereby regulates iron homeostasis. We aimed to establish erythroferrone reference interval for Bulgarian population using validated ELISA method.

Potential role of erythroferrone comes down to modulation of hepcidin synthesis in cases of iron accumulation in hemochromatosis and restriction of trace element in anemia of chronic inflammation through changes of signalling pathways and inflammatory cytokines. One of the approaches might be suppression of erythroferrone in patients with beta-thalassemia or conditions with iron accumulation during non-effective erythropoiesis. Other therapy can be expressed with increment of erythroferrone which may influence on anemia in chronic inflammation, especially in patients with unbalanced therapy inflammatory process.

**Materials & Methods**

151 healthy volunteers were included. In all included participants intima-media thickness (IMT), ankle-brachium index (ABI), and blood pressure were measured; body mass index (BMI) were calculated. Biological specimen (venous blood) was taken in order to evaluate total red blood cells count, erythrocyte indices – MCV, MCH and MCHC, hemoglobin, hematocrit, high sensitive CRP, serum iron and TIBC; serum transferrin, ferritin, haptoglobin, CRP, LDH, CPK, ASAT, ALAT, creatinine, glucose, lipid profile (total and HDL, LDL-cholesterol, triglycerides), hepcidin, TNF-α, IL-6. Included volunteers were divided into two age borders – a) from 18 to 50 years old and b) above age of 50. The distribution was as follows: males – total number 103, below age of 50 - 59 (57.3%), females – total number 114, below age of 50 - 69 (60.5%).

**Results**

Used program for statistical processing of collected data and for establishment of reference values was according to IFCC/CLSI document C28-A3 from march 2008. Parametric evaluation of reference interval for erythroferrone for total group of clinically healthy males and females, n=200 - 95% reference interval showed values from 6.3 ng/ml to 15.7 ng/ml. Gaussian distribution was established for serum erythroferrone in control group of healthy volunteers from Bulgarian population.

**Summary/Conclusion**

Before established the reference ranges, we validated ELISA method for serum erythroferrone quantification. During the steps of this process we found wide range of quantification, very low limit of detection and analytical variation; high diagnostic sensitivity and CV in recovery of the method. All these analytical characteristics showed that selected ELISA method for serum quantification is reliable for routine laboratory application in diagnosis and monitoring of therapy in different diseases, that involves dysregulation of iron homeostasis. We used program for statistical processing of collected data and for establishment of reference values according to IFCC/CLSI document C28-A3 from march 2008. Parametric evaluation of reference interval for erythroferrone for total group of clinically healthy males and females, n=151 - 95% reference interval showed values from 6.3 ng/ml to 15.7 ng/ml. Gaussian distribution was established for serum erythroferrone in control group of healthy volunteers from Bulgarian population.

Acknowledgments. We kindly appreciate financial support of Medical University – Sofia; as this work is part of grant No J1-7/2013.

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**Reference ranges for serum erythroferrone in Bulgarian population**


1Dept. of Clinical Laboratory, Medical University-Sofia, Bulgaria; 2Clinical laboratory Ramsa, Sofia, Bulgaria; 3Dept. of Haematology and Immunology, Medical University-Sofia, Bulgaria; 4Dept. of Internal Diseases, Medical University-Sofia, Bulgaria; 5Dept. of Neurology, Medical University-Sofia, Bulgaria