**Materials and Methods**

*Study Cohort*

The study was conducted at the Department of Dermatology, Venerology and Allergology, Wrocław Medical University, Poland, on a group of 74 patients (37 women; the age range was 18–68 years), with HS diagnosed in accordance with well-established standards [14]. Exclusion criteria for this study were: (i) any acute or chronic illness that might influence iron metabolism (including infection, known malignancy, chronic kidney disease, chronic cardiovascular diseases and hematological diseases) and (ii) treatment for anemia or ID in the previous 12 months. None of the patients received any biologic treatment before entering the study. The clinical evaluation of HS severity was made according to the Hidradenitis Suppurativa Severity Index (HSSI) and the 3-degree scale proposed by Sartorius et al. [15] and Hurley [16]. With regard to the HSSI, patients were classified as having mild (0–7 points), moderate (8–12 points) and severe (≥13 points) disease [17]. Clinical characteristics of patients are presented in Table 1.

Thirty-two healthy subjects (10 women, age: 50 ± 5 years) were recruited for the study. The criteria for inclusion were: age >18 years, absence of any acute (occurring in the previous 6 months) or chronic disease and related therapy. The study protocol was approved by the local ethics committee, and all subjects gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

*Hematinics and Biomarkers of Iron Status*

In all HS patients and healthy subjects, venous blood samples were taken in the morning after an overnight fast, following at least 15 min of supine rest. Hematological parameters were made from fresh venous blood with EDTA and clotted blood. After centrifuging, the plasma and serum were collected and frozen at –70°C until further analysis in our laboratory.

The following hematinics were measured using an automatic system ADIVA 120 (Siemens, Healthcare Diagnostics, Deerfield, IL, USA): hemoglobin concentration (g/dL), hematocrit (%), red blood cells (T/L), mean corpuscular volume (fL), mean corpuscular hemoglobin (pg) and mean corpuscular hemoglobin concentration (g/L). Anemia was defined when hemoglobin level <12 g/dL in women and <13 g/dL in men [18].

The following standard blood biomarkers reflecting iron status were measured: serum concentrations of ferritin (μg/L), iron (μg/dL) and total iron-binding capacity (TIBC, μg/dL). Transferrin saturation (Tsat) was calculated as the ratio of serum iron (μg/dL) and TIBC (μg/dL) multiplied by 100 and expressed as a percentage. Serum ferritin was measured using an immunoassay based on electrochemiluminescence with the Elecsys 2010 System (Roche Diagnostics GmbH, Mannheim, Germany). Serum iron and TIBC were assessed using a substrate method with Feren S (Thermo Fisher Scientific, Waltham, MA, USA).

The pool of iron that is being used to fulfill body metabolic needs consists of circulating iron bound to transferrin (expressed as Tsat and reflecting the amount of iron available to cells) and intracellular iron [19]. Serum ferritin reflects body iron storages. Apart from being a major iron storage molecule, ferritin serves as an acute-phase protein, which should always be taken into account when interpreting its levels in the circulation. In the response to inflammatory activation ferritin production is increased, regardless of the amount of stored iron, thereby falsely indicating that iron stores are adequate [20].

Serum soluble transferrin receptor (sTfR, mg/L) was measured using immunonephelometry (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The cellular uptake of transferrin-bound iron is tightly regulated by sTfR, thus it is considered to be a crucial biomarker of unmet tissue needs for iron. In case of iron deficiency levels of circulating transferrin receptor are increased and quantitatively correlate with cellular demand for iron, but not with its body stores [21].

Serum hepcidin (ng/mL) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (BACHEM). This ELISA method was validated as a gold standard for hepcidin assessment, namely liquid chromatography mass spectrometry developed at King’s College London, confirming a strong correlation between the measurements performed using the liquid chromatography mass spectrometry and the BACHEM assay in patients with chronic kidney diseases and healthy subjects. Increased levels of hepcidin may occur in the circulation due to excessive iron stores and/or state of inflammation. Analogically, suppressed production of hepcidin indicates depleted iron stores with or without coexisting anemia [11].

In our study we defined ID based on serum ferritin and Tsat as follows: serum ferritin <100 mg/L, or serum ferritin 100–299 mg/L with Tsat <20%. These definitions were standardized and applied in previous studies in patients with chronic diseases [9, 22]. Depleted iron stores were defined by hepcidin levels lower than 14.5 ng/mL, i.e. the 5th percentile among healthy controls in our laboratory [23].

The serum level of interleukin-6 (IL-6, pg/mL) as a marker of proinflammatory activation was measured using a commercially available ELISA (R&D Systems, Minneapolis, MA, USA) [24]. The upper limits of normal in our laboratory for IL-6 were 1.48 pg/mL. We have prospectively defined HS patients with IL-6 above 1.48 pg/mL as having proinflammatory activation.

*Statistical Analysis*

The normality of the distributions of continuous variables was tested using the Kolmogorov-Smirnov test. Continuous variables with a normal distribution were expressed as means with standard deviations. The remaining continuous variables with a skewed distribution were expressed as medians with upper and lower quartiles. For further analyses, these variables were log-transformed in order to normalize their distribution. The categorical variables were expressed as numbers with percentages. The statistical significance of differences between the groups was tested using Student’s *t* test, the Mann-Whitney U test or the χ2 test, where appropriate. The associations between variables were assessed using the Spearman rank correlation coefficients. All statistical analyses were performed with Statistica 10 (Statsoft, Tulsa, OK, USA). A value of *p* < 0.05 was considered statistically signsificant.