

**BLOOD TRANSFUSIONS AND IRON METABOLISM IN PREMATURELY BORN INFANTS  
(PREMATURITY AND IRON HOMEOSTASIS)**

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Accepted 6<sup>th</sup> October, 2013

**ABSTRACT**

**PURPOSE:** To investigate the impact of blood transfusions before 28<sup>th</sup> postnatal day (early blood transfusions) on the iron metabolism indices in prematurely born infants.

**MATERIAL AND METHODS:** 102 infants, born before 33<sup>rd</sup> gestational week were investigated. They are divided in two groups: group 0 – without, and group 1 – with early blood transfusions. The serum level of ferritin, transferrin, soluble transferrin receptors, iron, total iron binding capacity and transferrin saturation were examined.

**RESULTS:** Group 1: The start ferritin levels and iron levels in 30<sup>th</sup>, 33<sup>rd</sup> and 36<sup>th</sup> week postconceptual age (PCA) were higher; the levels of soluble transferrin receptors throughout monitored period and total iron binding capacity in 33<sup>rd</sup> and 36<sup>th</sup> week PCA were lower. Group 0: The mean transferrin levels rose sharply after 33<sup>rd</sup> week PCA and the differences were significant in 36<sup>th</sup> and 39<sup>th</sup> week PCA. Examining saturation of transferrin, the iron overload samples predominate in 30<sup>th</sup>, 33<sup>rd</sup> and 36<sup>th</sup> week PCA and are three times often at the term according to the non-transfused infants.

**CONCLUSION:** The early blood transfusions lead to serious disorders of iron status and high risk of iron overload at term in infants, born before 33<sup>rd</sup> gestational week.

**KEYWORDS:** blood transfusion, iron metabolism, iron overload, prematurity

**INTRODUCTION**

Iron (Fe) is an essential microelement that takes part in many biological processes: oxygen transport, cellular breathing, DNA-synthesis, cell cycle and many others. [1, 2]. It is essential for early brain growth and development in humans. [3]

Transplacental iron supply increases with gestational age in parallel with the increasing iron needs of the fetus and reaches its maximum in the third trimester between 36<sup>th</sup> and 40<sup>th</sup> gestational week (GW).

However, free Fe can have harmful effects. It triggers the oxidative stress by catalyzing the generation of highly reactive cytotoxic hydroxyl radicals. The latter damage the macromolecular components of the cell, including its nucleic acids and proteins, and lead to cellular destruction. [3, 4] Premature infants are at an increased risk of anemia when compared to their full term counterparts. According to its pathogenesis, anemia in the newborns can be grouped into several categories: posthemorrhagic, hemolytic, hypoplastic and anemia of critical illness. The etiology of anemia of critical illness is multifactorial. It repeats the etiology of the other types of anemia and also includes infection, immune dysfunction and metabolic disturbances. [5, 6].

The prophylactic and therapeutic opportunities in anaemias of premature infants include:

- Blood losses minimizing through blood samples restraint and using of micromethods and non-invasive examination tests;
- Iron supplementation - the means about administration rout, intakes number and dosing of the iron drugs are still controversial [7, 8];
- Vitamin supplementation - B12, folic acid, A, E [9, 10, 11];
- Prophylactic and therapeutic application of recombinant human erythropoietin (rHuEPO) [12, 13, 14, 15];
- Erythrocyte transfusions. Each ml of erythrocyte concentrate is worth 1 mg Fe. The rising of unbound to transferrin Fe and plasma ferritin levels is detected in the premature infants after erythrocyte transfusions [16], which may lead to realization of oxidative stress diseases, or necrotizing enterocolitis particularly [6, 17]. According to some authors [18, 19] the transfusion

iron accessibility may be greater in the transfused than in non-transfused infants.

The different pathogenic types of anemia correspond to the different postnatal periods. Hemorrhagic and hemolytic anemias are typical of the neonatal period, while hypoplastic anemias like anemia of prematurity and anemia of critical illness occur later in infancy. Because of this, the corrective blood transfusions (BTs) during the neonatal period are called early; while those after this period are known as late.

Despite the progress made in the clinical care for infants with very low and extremely low birth weight (ELBW), many BTs are still required during the hospital stay. These frequent BTs lead to iron overload in prematurely born infants. This happens because of physiological insufficiency of the binding proteins, immaturity of antioxidant systems and suppressed secretion of erythropoietin (EPO) [20, 21]. It is only logical to assume that the mechanisms of iron metabolism and respectively the ability of the organism to manage with its disorders mature with postnatal age.

#### PURPOSE:

To investigate the impact of BTs in the neonatal period on the iron metabolism in premature infants.

#### MATERIAL AND METHODS

**Study Design:** The study is prospective and controlled in accordance with the ethical standard (confirmed by the Ethical Committee of the Medical University of Pleven, Bulgaria).

102 prematurely born infants were enrolled in this trial for a three-year period. They were divided into two cohorts: a control group 0 which includes non-transfused infants (n 35) and a case group 1 consisting of infants transfused during the neonatal period (n 67). The eligible babies were required to: be born before 33<sup>rd</sup> GW, be older than one week of postnatal age at the time of the enrollment (that is, to have survived the early neonatal period), have no postoperative complications, show good tolerance to enteral feeding by the end of the second postnatal week (formula intake of 100 ml/kg/day), be systematically assessed until discharge or until term.

The restrictive transfusion policy accepted in the study department was applied whenever patients needed blood products. All children were administered prophylactically iron and vitamin supplementation as follows: vitamin B12 50 mcg/week i.m. beginning from the second postnatal week until discharge, folic acid 0.04 mg p.o., iron as ferric hydroxide polymaltose complex – 4 mg/kg/day p.o. Folic acid and iron were included after oral feeding had been well tolerated.

**Monitored indices:** Serum levels of ferritin (Ferr), transferrin (Tf), soluble transferrin receptors (sTfR), iron (Fe), total iron binding capacity (TIBC) analyzed at 27<sup>th</sup>, 30<sup>th</sup>, 33<sup>rd</sup>, 36<sup>th</sup> and 39<sup>th</sup> week PostConceptual Age (PCA). The samples were collected in serum test tubes Primavette® (KABE Labortechnik). The serum Fe were analyzed by

calorimetric assay, and Ferr, Tf, sTfR – with reagents of company Roche by biochemical analyzer COBAS INTEGRA 400. The transferrin saturation (SatTf) was calculated additionally by the formula:  $SatTf [\%] = (Fe / TIBC) \times 100$ . Normal ranges for the indices: The published ranges of normal values of all the indices of iron homeostasis, except for Ferr, are for term newborns one month old. [22]. The ranges of Ferr are applicable for low-risk premature children. [23].

**Statistical analysis:** The data obtained from infants in both case and control group were analyzed using Student unpaired t-test to find statistical significance. Analysis was performed using STATGRAPHICS plus for Windows 2.1, Microsoft Office Excel 2010 and Windows XP Professional. The p-value < 0.05 is considered significant.

#### RESULTS:

The infants who received BTs by the 28<sup>th</sup> day represent two thirds of all premature babies included in the study (Table 1). They were more immature and had lower birth weight. They often suffered from perinatal infections but not from nosocomial infections, and rarely required late BTs. The patients from control group 0, despite their relative maturity and higher mean birth weight, frequently suffered from nosocomial infections. They required more late BTs, which significantly prolonged their hospital stay.

Patients from group 1 showed higher levels of Ferr at birth (Fig 1). These values decreased with postnatal age but still remained above the 95<sup>th</sup> percentile. The measurement of maximal Ferr value was made in the 33<sup>rd</sup> week PCA. The difference between the mean values of Ferr became bigger towards term despite the higher frequency of late BTs in group 0.

The mean levels of Tf rose sharply in patients from group 0 and the differences were significant in the 36<sup>th</sup> and 39<sup>th</sup> week PCA as they exceeded the upper limit of the normal ranges (Fig 2). In group 1 the values of this indicator remained on the lower side of the range.

The measured serum levels of sTfR showed higher mean values (above the 95<sup>th</sup> percentile) in the non-transfused children for the whole period of the study. There were two distinguished drops of the curve: the first appeared between 30<sup>th</sup> and 33<sup>rd</sup> week PCA and the second was between 36<sup>th</sup> and 39<sup>th</sup> week PCA (Fig 3).

Infants who had early BTs showed significantly higher mean values of Fe during the 30<sup>th</sup>, 33<sup>rd</sup> and 36<sup>th</sup> week PCA, but, towards term, the values of the two cohorts became equal (Fig 4). The mean values of Fe in both groups were below the normal range.

The TIBC levels measured between 33<sup>rd</sup> and 36<sup>th</sup> week PCA in haemotransfused infants were significantly lower (34.4 vs. 39.1 mcmol/l – p 0.02; 32.4 vs. 36.6 mcmol/l – p 0,01). This correlated with the higher values of Fe in the same group. Towards term the mean values of TIBC in both groups became equal.

## DISCUSSION

The biological iron which BTs bring with the adult hemoglobin can be potentially reutilized. This possibility is at the hearth of the notion that the restrictive BTs policy applied for infants with ELBW leads to development of iron deficiency at term. On the other hand, the presence of concomitant factors that influence the mechanisms of iron metabolism helps the organism tolerate the iron overload. Ferr, Tf, STfR, Fe and TIBC are biomarkers of the iron status. Ferr is the major protein that stores iron. Nuclear Ferr serves to detoxify iron which otherwise may catalyze the oxidative DNA injury. [23]. The factors that influence Ferr concentration at birth are gestational age, sex, maternal iron status and conditions that prevent the normal transplacental iron exchange. [23].

Low concentrations of Ferr can be detected only when there is iron deficiency. High concentrations of Ferr are observed in cases of neonatal haemochromatosis, excessive intake of iron, or after BTs. The serum Ferr levels also rise with any infection and neoplasm. In these conditions, Ferr is an acute phase reactant and masks iron deficiency [23].

The lowest value of the normal range of serum Ferr in prematurely born infants has not been established yet. Clinicians use a cut off value of Ferr which is between 60 and 100 mcg/l in infants and adults whose erythropoiesis is stimulated by recombinant human EPO [22, 24]. Normal levels range from 100 to 800 ng/ml. In critically ill patients, these values can be different. [5, 25] There are no published standards showing the minimal normal value of Ferr for infants born before the 30<sup>th</sup> week as there are not enough clinical trials on the subject. According to Siddappa et al [23], the lowest accepted value for Ferr measured from umbilical cord samples of low-risk prematurely born babies is 35, while the highest is 267 mcg/l.

Even though the patients from group 1 were more immature and had lower birth weight, which can lead to lower iron storage, the levels of Ferr remained high during the whole study period. The values of Ferr in group 0 were lower and the peak in the 33<sup>rd</sup> week PCA can be explained with superimposing of nosocomial infection and the role of Ferr as an acute phase protein. Despite the higher frequency of late BTs in the same group, the levels of Ferr tended to decrease toward the end of pregnancy. This demonstrates greater maturity of the iron regulating systems with the advance in postnatal age.

The Tf-level reflects the biochemical capacity for binding and transport of endogenous Fe. All non-hem Fe in blood circulation is normally bound to Tf but only 30% of Tf-binding sites are occupied by Fe. [26] Where there is iron overload, unbound Fe can be found in the body. The serum levels of Tf of premature infants are lower than those of adults, and can be highly saturated with Fe. [27].

In the present study, Tf-levels of patients from group 0 rose sharply after the 33<sup>rd</sup> week PCA. This fact proves the excess of free Fe which the iron homeostasis aims to neutralize by binding it to Tf.

Despite the reports that TfR are reflectors of iron status in prematurely born infants, some authors [28] claim that they

are correlated with the stimulation of erythropoiesis rather than to iron status.

In the present study, the levels of sTfR were higher in non-transfused children during the whole study period, which is explained with the hypoxia-induced erythropoiesis. During the 33<sup>rd</sup> and 39<sup>th</sup> week PCA, two drops in the sTfR-levels were observed. These periods were also characterized with nosocomial infections and a need for late BTs, both of which depress the erythropoiesis significantly. The serum iron levels can be influenced by daily variations and conditions, such as inflammation, infection, neoplasms, and liver diseases. Therefore, it is rarely used as a sole indicator for the etiology of anemia. Levels below 9 mcg/l indicate an iron deficiency. [22, 25]

The iron levels of our transfused patients remained significantly higher until the 36<sup>th</sup> week PCA. This fact, interpreted in the context of higher Ferr and lower Tf and TIBC, suggests an accumulation of free Fe, which significantly exerts iron homeostasis mechanisms. At the same time, the natural way of binding Fe by including it in the molecule of the hem of hemoglobin is suppressed, which is demonstrated by the lower values of sTfR.

Transferring saturation is an indicator which gives a generalized evaluation of the iron status and cannot be influenced by a systemic inflammatory reaction. Serum Fe and SatTf give information about the quantity of Fe readily available for the erythropoiesis. According to the National Kidney Foundation [29], the recommended values of SatTf are 20-50%. SatTf below 20% indicates a reduced access to iron and, depending on the clinical presentation, a need for iron supplementation. Conversely, SatTf above 55% suggests possible iron overload. Functional iron deficiency is suspected in critically ill patients with high Ferr and low SatTf.

The iron status can be illustrated more clearly if the patients are split up in 3 subgroups: patients with iron deficiency (SatTf  $\leq$  20%), patients with normal iron status (SatTf 21-50%) and patients with iron overload (SatTf  $\geq$  51%) (Fig 5).

According to the study's data, iron deficiency predominates in samples taken from the non-transfused patients from group 0 during the 30<sup>th</sup> week PCA. With the growing of the postnatal age, a tendency towards an increase of the samples with normal SatTf was observed. For group 1, iron overload was observed in more than one third of the patients who had early BTs and it occurred 3 times more often towards term when compared to non-transfused children as the difference is significant in the 30<sup>th</sup>, 33<sup>rd</sup> and 36<sup>th</sup> week PCA.

## CONCLUSIONS:

In the present study, early blood transfusions lead to serious disturbances of iron metabolism. The mechanisms of regulation of iron metabolism were significantly exerted in the patients, and this can be demonstrated by the rise of the mean values of ferritin (the iron-storage protein), the serum iron and the saturation of transferrin. This leads to an iron overload at term in the transfused patients as 45% of them were affected.

In the non-transfused group, the iron homeostasis reacted adequately to the changes in the serum levels of free iron by increasing the iron-binding protein transferrin. Besides, the erythropoiesis, which was additionally stimulated by the birth process, inserted free iron where it belonged – into the molecule of the hem where the synthesis of hemoglobin is carried out. Therefore, iron overload in patients at term were 3 times less likely to occur than in patients who had early blood transfusions.

The decision when and how to supplement prematurely born infants with iron will depend on the individual case, taking into account the prenatal history and clinical presentation of the patient, as well as the dynamics of the biochemical indices of iron homeostasis.

It was accepted a SatTf<50% as a standard measure for the start of iron supplementation in a clinically stable prematurely born infant.

### RECOMMENDATIONS:

There is a need of ongoing monitoring of the hematological status in prematurely born infants after discharge, continuing until the stabilization of bone marrow function and overcoming the risk of anemia in infancy. The iron supplementation depends wholly on the patient's needs and has to be determined in every case individually.

### ACKNOWLEDGEMENTS:

The research team wishes to thank to Ass. Prof. Petkana Hristova, MD, PhD for her main contribution in performing the statistical analyzes. Much gratitude goes to the staff and Heads (Dr. V. Simov, MD and Dr. D. Georgieva, MD) of the Department for Nurture of Premature Infants. Acknowledgement goes to Mrs. P. Dilova, E. Parvanova, V. Hristova and Mr. V. Yordanov for their contribution in the collection and examination of the blood samples.

### SUMMARY BOX:

The biological iron which BTs bring with the adult hemoglobin can be potentially reutilized. This possibility is at the hearth of the notion that the restrictive BTs policy applied for infants with ELBW leads to development of iron deficiency at term. On the other hand, the presence of concomitant factors that influence the mechanisms of iron metabolism helps the organism tolerate the iron overload. In the present study, early blood transfusions lead to serious disturbances of iron metabolism in infants, born before 33<sup>rd</sup> gestational week. This resulted in a high risk of iron overload. The decision when and how to supplement prematurely born infants with iron will depend on the individual case, taking into account the prenatal history and clinical presentation of the patient, as well as the dynamics of the biochemical indices of iron homeostasis.

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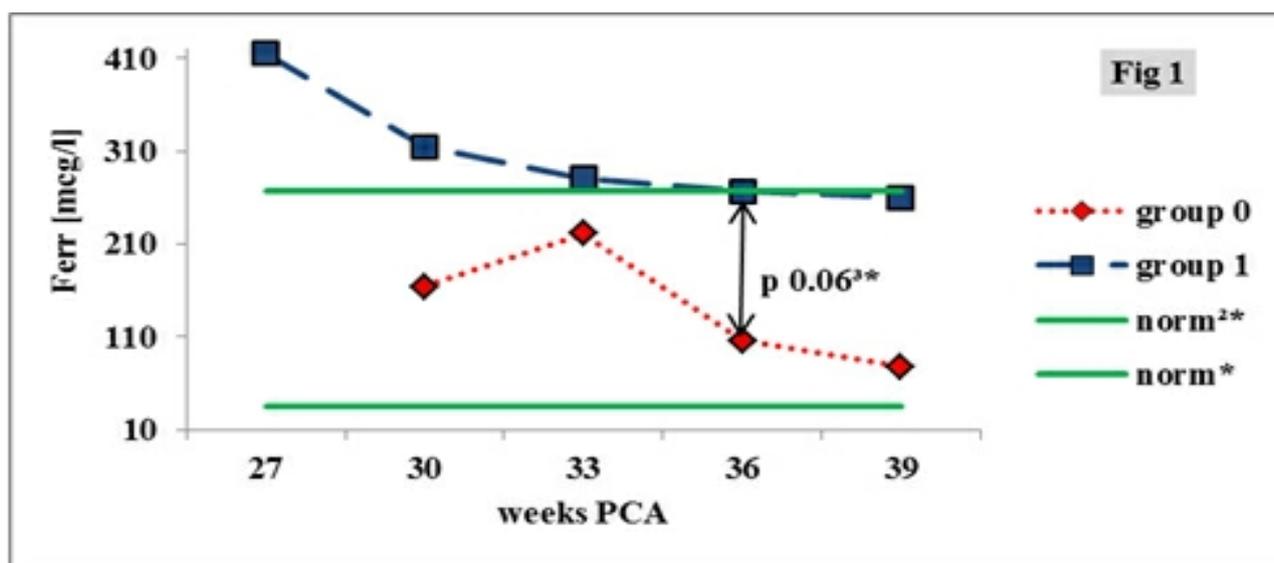
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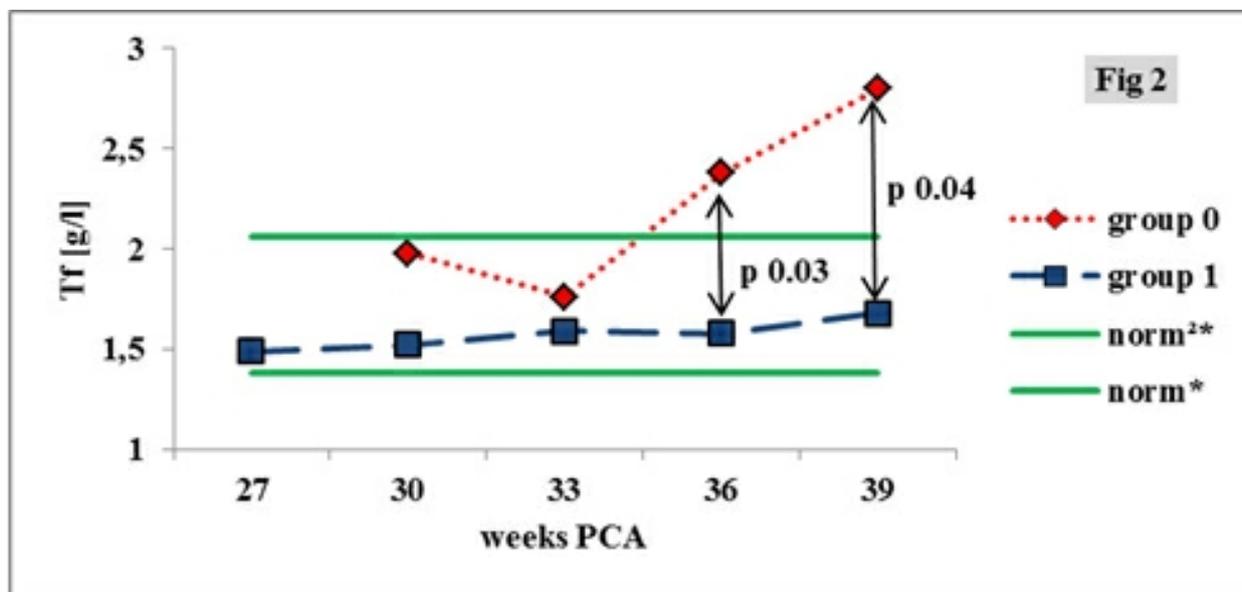
## Tables and Figures

**Table 1** Characteristics of the groups (group 0 - without BTs before 28th day; group 1 - with BTs before 28th day)

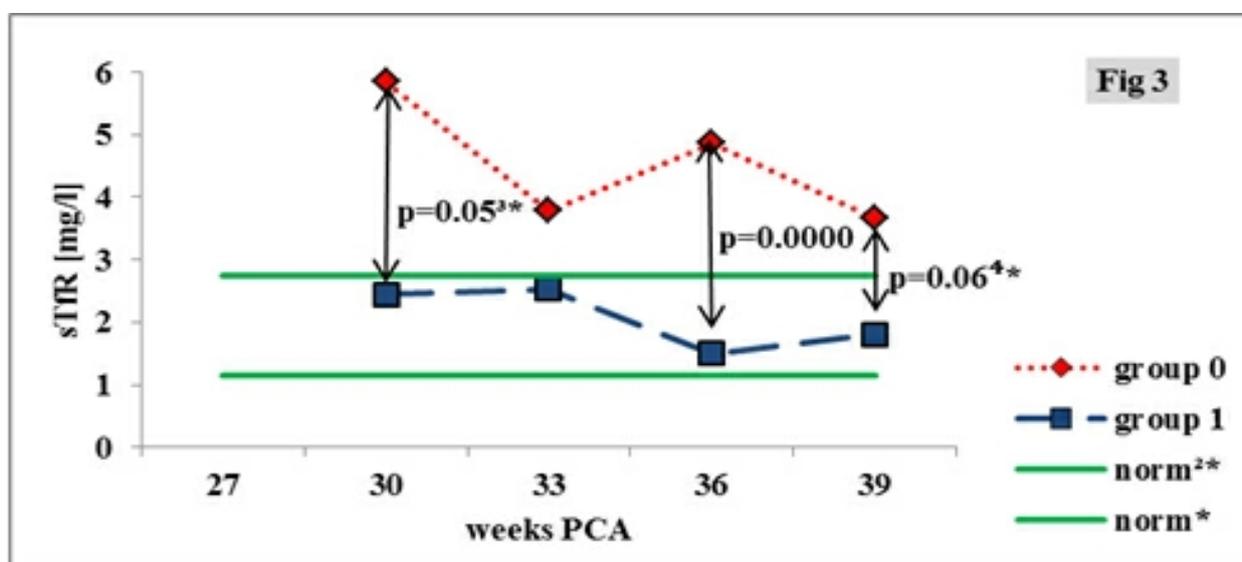
Characteristics	Group 0	Group 1	p
n	35	67	-
Mean gestational age (GWs)	30,0±1,5	28,4±2,3	0,0003
Mean birth weight (g)	1415±298	1220±325	0,004
Frequency of intraamniotic infections (%)	25	40	NS
Frequency of nosocomial infections (%)	37	3	0,0001
BTs after 28th postnatal day (per patient)	0,9±0,9	0,3±0,4	0,0004
Hospital stay (days)	57±25	40±22	0,0009



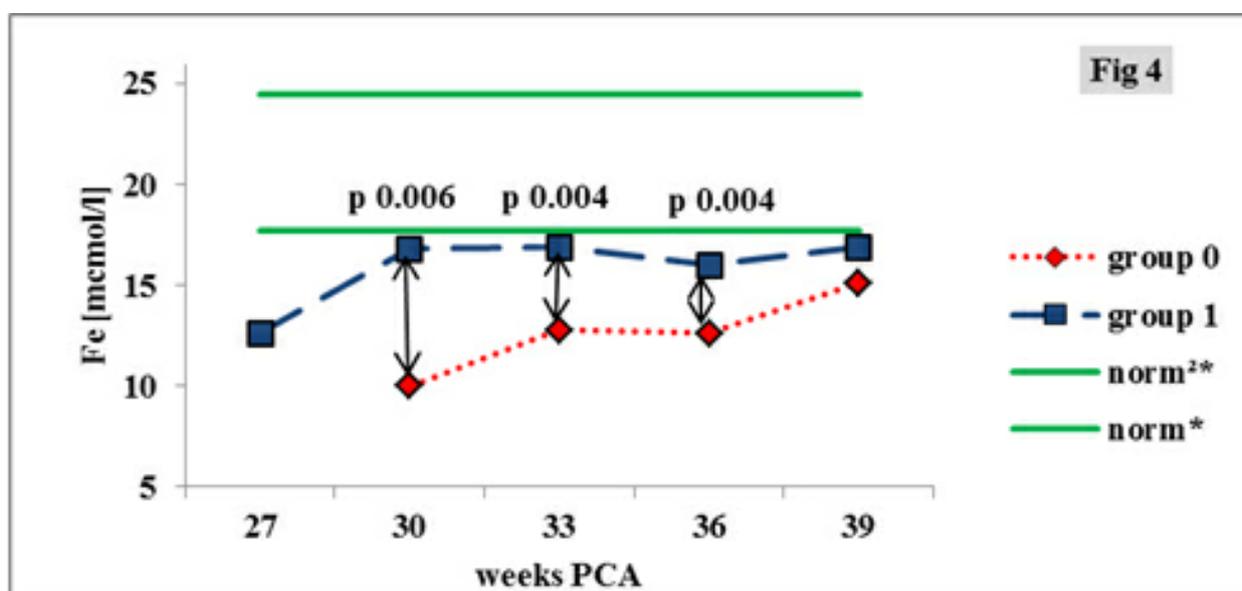
**Fig 1** Ferritin dynamics [mcg/l] in patients without – group 0, and with early BTs – group 1 (\*norm – lower limit; <sup>2</sup>\*norm – upper limit; <sup>3</sup>\*p (Kruskal-Wallis test) = 0.02)



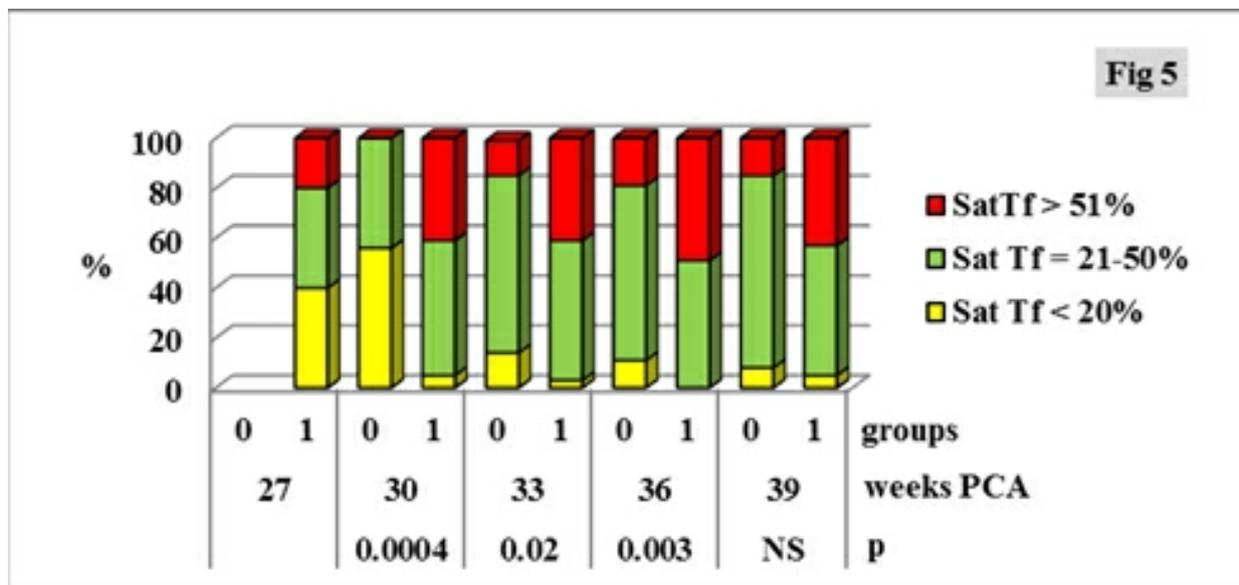
**Fig 2** Transferrin dynamics [g/l] in patients without – group 0, and with early BTs – group 1 (\*norm – lower limit; <sup>2\*</sup>norm – upper limit)



**Fig 3** Dynamics of sTfR [mg/l] in patients without - group 0, and with early BTs - group 1 (\*norm - lower limit; <sup>2\*</sup>norm - upper limit; <sup>3\*</sup>there is a significant difference according to all statistical tests; ?\*there is not a significant difference according to all statistical tests)



**Fig 4** Dynamics of Fe [μmol/l] in patients without - group 0, and with early BTs - group 1 (\*norm - lower limit; <sup>2\*</sup>norm - upper limit)



**Fig 5** Comparative characteristics of the SatTf [%] between group 0 (without early BTs) and group 1 (with early BTs).