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Efficacy and tolerability of FerroGUNA versus iron sulfate in the treatment of iron deficiency anemia in pregnancy: non-inferiority controlled clinical trial

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SUMMARY

A condition of more or less pronounced anemia is very frequent in pregnancy (up to 20-30% in some cases). An oral supplementation with iron salts can be considered a real therapy, although side effects that occur in the gastro-intestinal tract always reduce the compliance of these salts. Iron sulfate is often used in iron therapy thanks to its low cost and good bioavailability. However, an oral supplementation of this salt often induces nausea, vomiting, epigastric heaviness and poor gastro-intestinal tolerability depending on the concentration of ionized iron in the upper gastro-intestinal tract.

This multicentric, open, randomized, controlled, parallel-group trial has been conducted to demonstrate a non-inferiority and a higher tolerability of FerroGUNA compared to iron sulfate in pregnant women after week 12 who needed iron supplementation.

This trial was conducted in Italy on 49 patients aged over 18 years enrolled according to well-defined criteria, treated for 12 consecutive weeks with 525 mg/day of iron sulfate heptahydrate (Ferrograd®) or with 2 sachets/day of FerroGUNA. The hematochemical and biophysical parameters were assessed on a monthly basis.

After treatment, the increase in hemoglobin that occurred in both groups was clinically relevant (>0.5 g /dl) compared to screening.

Therefore, this clinical trial showed the non-inferiority, higher tolerability

and ease of administration of FerroGUNA compared to Ferrograd® in the treatment of iron deficiency during pregnancy.

KEY WORDS: Anemia - Pregnancy - Iron Deficiency - Iron sulfate.

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Introduction

Iron deficiency anemia

Anemia comes from the Greek word αναιμια, anaimia meaning "lack of blood". It is defined as the decrease of the total amount of hemoglobin or the number of red blood cells. Anemia, intended as iron deficiency, is due to inadequate concentration of iron necessary for the formation of the haem group and, consequently, of red blood cells.

Iron is an essential element for life because of its key role in the transport of oxygen and as enzyme cofactor in many metabolic reactions. Except for specific diseases, the concentration of iron in the body is the result of a dynamic balance between nutritional intake and physiological loss. The absorption of iron is *up*-regulated by lack of ion or increased erythropoiesis, and is *down*-regulated in inflammatory states or iron surplus. This constant regulation is performed through physiological homeostatic mechanisms (iron homeostasis), in which a specific protein - hepcidin - plays a key role. Hepcidin is synthesized in the liver and exerts a regulation action by binding to another protein, ferroportin, a *carrier* glycoprotein which is located in the basolateral membrane of the enterocytes and is responsible for regulating the exit of iron from the intestinal mucosa cell to the blood. When hepcidin is present, it binds to ferroportin and limits its functions by hindering the exit of iron in the blood. When hepcidin is absent, the inverse happens. Furthermore, hepcidin also prevents the macrophages - the cells responsible for recycling the iron contained in the red blood cells that reached the end of their lifespan - from releasing iron. A dys-regulation of hepcidin and/or ferroportin may result in various diseases connected with iron homeostasis (Fig. 1) (1).

Diet can contain two forms of iron : non-heme or heme iron. Non-heme iron is present as ferric ion (Fe^{+++}) or as ferrous ion (Fe^{++}), the latter being better absorbed than its oxidized form. The acid environment of the stomach, ascorbic acid and luminal reductases increase the bioavailability of iron from food because they act by reducing the ferric ion (Fe^{+++}) to ferrous iron (Fe^{++}).

Heme iron is found primarily in meat and is well absorbed; pancreatic enzymes release it from the heme group of hemoglobin in the intestinal lumen. Later on, it is absorbed by the cells of the intestinal mucosa as metalloporphyrin, which is degraded by heme-oxygenase-1 with release of ferrous iron that can follow two pathways.

It can be stored as ferritin, which forms the actual body iron stores, or it can pass through the cell, flowing into the bloodstream through ferroportin, which has the task - just like ceruloplasmin and hephaestin - of facilitating the re-oxidation from ferrous ion to ferric ion, a process required for transport in the blood (1).

Ferroportin is present in the mucosa of the proximal tract of the small intestine, in macrophages, in hepatocytes and the syncytiotrophoblasts of the human placenta.

Once in the blood, iron is transported by transferrin to the bone marrow for the hemoglobin synthesis and the incorporation of the latter into the erythrocytes. Transferrin possesses high affinity for ferric ion, and binds all the ion present in the plasma. The bound between iron ion and transferrin is mediated by apo-transferrin and by its receptor *pathway* (2).

The average life span of erythrocytes is about 120 days, after which they are degraded in the spleen and the liver by the macrophage and the reticuloendothelial systems.

The recycling of iron from senescent red blood cells is a primary source of iron for erythropoiesis, giving the bone marrow a ion quantity equal to 40-60 mg/day (Fig. 2).

In adults there are about 3-4 g of iron. Generally the diets of Western countries provides a ion intake of approximately 7 mg per 1,000 Kcal. Nevertheless, only 1-2 mg are normally absorbed every day. The Recommended Daily Allowance (RDA) is based on age, pregnancy or breast-feeding. During pregnancy RDA is 27 mg/day (3).

Iron deficiency anemia occurs when the availability of iron in the body is insufficient for a proper hemoglobin synthesis

(hemoglobin <14 g/dl in males and <12 g/dl in females).

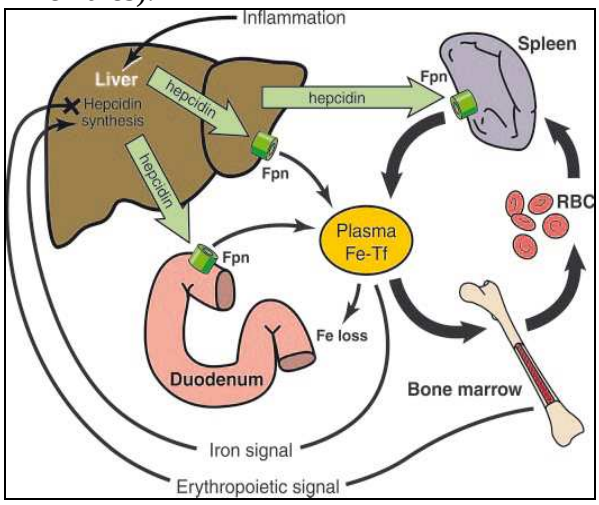

Fig. 1 - Role of hepcidin in normal iron homeostasis: through the regulation of ferroportin, hepcidin is able to control the entry of iron into plasma. The most important movement of iron, which is regulated by hepcidin-ferroportin interactions, includes the release of iron from macrophages that recycle iron in the spleen and other organs, dietary ion absorption in the duodenum, and the release of iron from liver stores.
The concentration of extracellular iron can stay within normal limits thanks to a feedback that induces the synthesis of hepcidin and the mobilization of liver stores when the concentration of plasma iron must become saturated. The synthesis of hepcidin is modulated by signals from the erythropoietic activity, ensuring a sufficient iron supply to the bone marrow in the event of increased demand from the erythrocytes.
During inflammation, hepcidin production is stimulated and the entry of iron into plasma is inhibited, thus causing hypoferremia, and anemia secondary to inflammation. Figure and caption translated into Italian by Josh Gramling-Gramling Medical Illustration, Woodstock, GA.

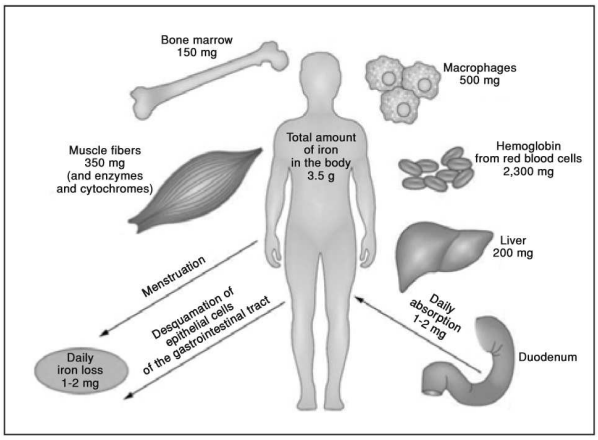

Fig. 2 - Iron homeostasis in healthy conditions. Approximately 3.5 g of iron are stored by the human body. Most of the iron in the body is contained in the hemoglobin of red blood cells (2,300 mg). About 10% is contained in muscle fibers (myoglobin) and other tissues (enzymes and cytochromes) (350 mg). The remaining iron is stored in the liver (200 mg), in macrophages (500 mg) and in the bone marrow (150 mg). A daily amount of 1-2 mg is lost through the desquamation of skin cells, through the gastrointestinal tract, the bile ducts, the urinary tract, and through the menstrual cycle. Figure and caption were translated into Italian by Stein J. et Al. Diagnosis and management of iron deficiency anemia in patients with IBD. From Nature Reviews Gastroenterology and Hepatology 7:559-610 (November 2010).

Typical signs are decreased mean corpuscular hemoglobin concentration, as well as decreased volume of red blood cells (4) and hematocrit (5).
The clinical picture is usually non-specific (paleness, fatigue, easy fatigability) (6).
Iron deficiency anemia is more common in females due to constitutional and dietary factors, and above all, due to menstrual cycle loss and past pregnancies.
The iron loss that occurs during the menstrual cycle is almost equal to the dietary monthly intake; it is so frequent, that iron stores are never "replenished"(7).

Among women of childbearing age, who are not pregnant, about 40% show ferritin levels ≤ 30 μg/l, which correspond to scarce or absent iron stores (8).
Prophylaxis is justified only in patients showing other risk factors for iron deficiency (for example poor diet) and is appropriate even in cases of malabsorption, menorrhagia, pregnancy, and gastrectomy (4).
The treatment of iron deficiency anemia consists in supplementing three types of ferrous iron salts, basically via the oral route: iron fumarate, iron sulfate and iron gluconate. These 3 preparations are different due to a different concentration of elemental iron.
Iron fumarate contains 33% of elemental iron; iron sulphate contains 20% of elemental iron; iron gluconate contains 12% of elemental iron.

Anemia in pregnancy

As mentioned before, iron is an essential micronutrient for human life. It is necessary for the synthesis of hemoglobin in the erythrocytes, but also of myoglobin in myocytes, as well as for the function of a great number of iron-dependent enzymes. In pregnant women an adequate iron balance is a basic requirement for a good pregnancy, a normal development of the fetus and the maturity of the newborn (8).
A more or less pronounced anemic status is very common during pregnancy (up to 20-30% of women during the third trimester in some cases) as a result of physiological

hemodilution, either because pregnancy, with its increased metabolic demands, easily implies a latent disease state, or because the increased needs of a pregnant woman are not supported by a proper diet.
Just to simplify, a pregnant woman is considered as anemic when her hemoglobin levels (Hb) drop below those levels listed below, taking into account that these are not ideal values, even if they are used as discriminative indices for epidemiological studies.

Week of gestation	Hb g/100 ml
0-16	11.2
17-24	10.6
25-40	10.0

The most common forms of anemia in pregnancy are:

acquired:

1. iron deficiency anemia
2. post-hemorrhagic anemia
3. anemia due to infectious processes
4. megaloblastic anemia
5. acquired hemolytic anemia
6. aplastic anemia

hereditary:

1. thalassemia
2. hemoglobinopathy
3. hereditary hemoglobin anemia without hemoglobin abnormalities.

There are no specific forms of anemia strictly connected with pregnancy, also because the so-called gestational megaloblastic anemia is actually a folic acid deficiency anemia, whose onset is identified with an increased need (5).
For this purpose, it is necessary to emphasize that Italian gynecologists have been sensitized for many years to prevent this form of anemia, and are used to prescribe a supplementation of folic acid from the very early stages of pregnancy (9), indeed even in view of a planned pregnancy. This has especially occurred since when striking data have been published about the role of this substance in preventing the development of neural tube defects in fetus (4).

Iron-deficiency anemia in pregnancy

It accounts for over 95% of anemia cases in pregnancy, and is often underestimated because the changes in haematological values to be considered are not always well-known.
WHO figures identify anemia in pregnancy with levels of Hb < 10 g/dl (5, 7).
However, any patient with a level of Hb < 11-11.5 g/dl in early pregnancy should be treated as anemic, because hemodilution that occurs during gestation reduces the level of Hb up to typical values of an anemic state (5-7).
Indeed, despite there is a heated controversy about the need for a regular iron supplementation during pregnancy, an iron supplementation is usually recommended for the majority of pregnant women, even if the level of Hb in early gestation is normal (7).
This prophylactic measure prevents the depletion of iron stores and anemia that may result from abnormal bleeding or subsequent pregnancy. Unless the nutritional intake is implemented through the diet, pregnant women at term will show iron deficiency (7).
Moreover, we also must consider the fact that, although iron is preferentially transported through the placenta and the hematocrit (Hct) of the newborn is usually normal in spite of the mother's anemia, her/his total reserves of iron will generally be reduced, thus requiring an early iron supplementation through diet (7).
Using standard therapies on the market, Hb levels should increase (in non-pregnant women) by 100-200 mg/100 ml daily or 2 g/100 ml in 3-4 weeks.
When Hb has reached normal levels, treatment should be continued for another 3 months to restore the iron stores (7).
Although iron supplementation is recommended from the beginning of pregnancy, it is often started after the 12^{th} week of amenorrhea, when the neurodegenerative disorders typical of this period naturally diminish and will not be added to the known side effects related to an iron supplementation, such as nausea, vomiting, heartburn, constipation, abdominal pain and diarrhea (10).
In conclusion, we can say that, in addition to a recommended intake of folic acid from the

earliest stages of pregnancy (if not even before the onset thereof), the finding of a hemoglobinemia < 11.2 g/dl at the 12th week of amenorrhea may force a gynecologist to prescribe an iron supplementation.
The rationale of this study was to evaluate the effectiveness and tolerability of FerroGUNA, a food supplement with iron fumarate, copper citrate, L-ascorbic acid and Baobab dried fruit pulp in pregnant women beyond the 12th week of amenorrhea who need iron supplementation. This food supplement was compared with the effectiveness of the product mostly prescribed in Italy against this deficiency.

Materials and methods

The purpose of this study was to evaluate the effectiveness and safety of FerroGUNA in pregnant women beyond the 12th week of amenorrhea who showed levels of Hb < 11.2 g/dl. This study was a multicenter, open, randomized, controlled, parallel-group study and lasted total 20 months. The product under study, FerroGUNA, is a food supplement with iron fumarate (14 mg/dose), copper citrate, L-ascorbic acid, enriched with Baobab (*Adansonia digitata*) dried fruit pulp.
Its pharmaceutical form is orodispersible granules with sweeteners in single-dose sachets. The recommended dosage is one or two sachets daily.
In this study, since the patients enrolled were pregnant, and so with increased need, the recommended daily dosage was the contents of two sachets daily, one in the morning, and the other in the evening, between meals, to be dissolved directly in the mouth.
The reference drug was Ferrograd® in tablets containing 525 mg of ferrous sulfate heptahydrate (corresponding to 105 mg of elemental iron) to be taken between meals. The prescribed dose was one tablet daily.
The patients included in the study were enrolled and assigned at random to either treatment, they had to be at least 18 years old, and they had to meet the inclusion and exclusion criteria as per the experimental protocol. The patients had to be pregnant at the 12th week of amenorrhea and show an hemoglobin value of ≤ 11.2 g/dl, besides having signed and dated a written informed consent. We excluded all patients with *Body Mass Index* (BMI) > 30, affected by gastrointestinal diseases that might reduce the absorption of the products of the study, accentuate the side effects of these products, or have contraindications against iron supplementation (e.g. gastritis, diverticulosis of the colon, celiac disease, Crohn's disease, etc.), subjects affected by forms of anemia different from those due to iron deficiency (e.g. Mediterranean anemia), subjects who were taking drugs that could reduce the absorption of the products of the study (e.g. antacids with aluminum) or drugs whose absorption could be inhibited by the products of the study (e.g. L-thyroxine for the treatment of hypothyroidism), twin pregnancy, multiparity > 4 and cigarette smoking > 10/die. The patients were evaluated monthly for three months (T0 - T3) by performing blood tests and measuring vital parameters. Moreover, the compliance with the dosage and possible appearance of side effects were monitored.
The primary *outcome* was the increase in Hb blood (by considering an increase of at least 0.5 g/dl as clinically relevant). The secondary *outcomes* were the increase of Hb within the period established and the tolerability of the product. The levels of serum iron and ferritin were also assessed. At the end of treatment, subjects treated with FerroGUNA were asked to assess if taking the product was easy, its palatability, pleasantness, digestibility and their overall perception of the product. The data were collected in an electronic *Case Report Form* (e-CRF) by SAS Pheedit.

Results

49 patients enrolled completed the study. All patients showed values of Hb ≤ 11.2 g/dl at the time of enrollment. The average level of Hb at the time of *screening* was 10.8 g/dl.
Among the 49 patients who completed the study, 37 were treated with FerroGUNA and 12 were treated with Ferrograd®.

Concerning the 37 patients who took FerroGUNA, some measurements of Hb values in the first (3 patients), in the second (2 patients) and in the third month of treatment (5 subjects) were missing (Fig. 3).
The two groups were homogeneous in terms of hematocrit values, serum iron, ferritin, age, and BMI. The primary *outcome* was to assess the Hb increase in the blood, considering a difference of at least 0.5 g/dl of Hb as clinically relevant in the whole period of investigation (T0 - T3), with an average increase of 1.4 g/dl for the group treated with Ferrograd® and 0.8 g/dl for the group treated with FerroGUNA (Fig. 4).
In both groups a clinically relevant (> 0.5 g/dl) and a statistically significant (*p-value <0.05) increase for both Ferrograd® and FerroGUNA during the 3 months of the study (T0 - T3) (Fig. 5) was highlighted.
Although the increase of the values of Hb in the blood was statistically significant *(p-value*

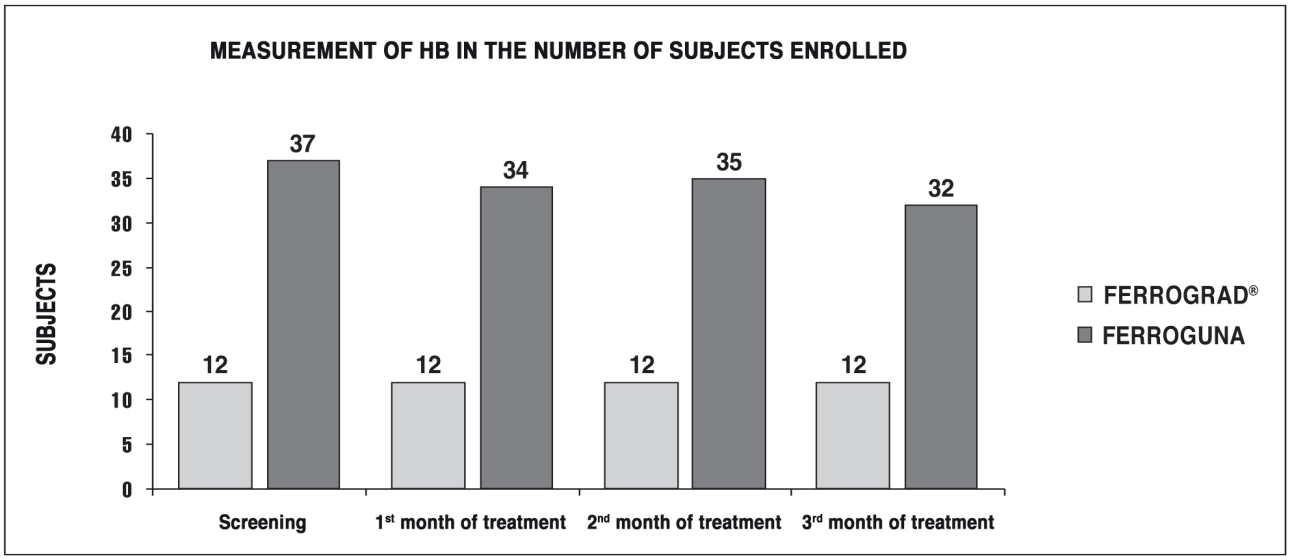

Fig. 3 - The diagram indicates the number of subjects enrolled in the two experimental groups during the evolution of the clinical study.

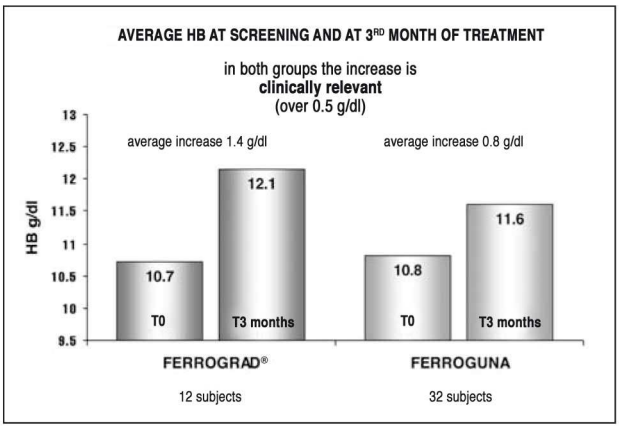

Fig. 4 - Assessment of hemoglobin (Hb) levels in the period of study. The diagram shows Hb average concentration in the two experimental groups at the beginning of the study (T0) and after 3 months (T3) of treatment. A clinically relevant Hb increase was found both in the group treated with Ferrograd® and that treated with FerroGUNA (>0.5 g/dl).

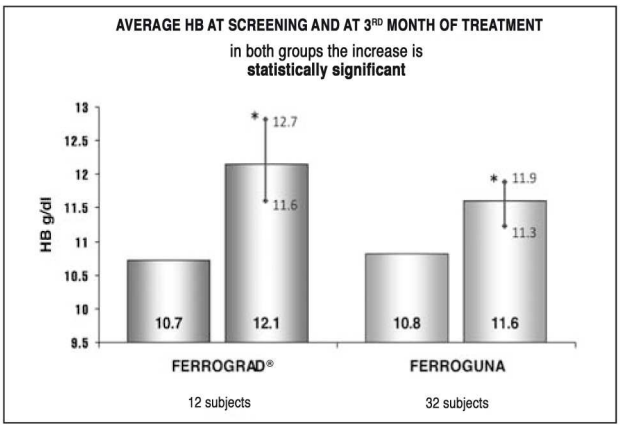

Fig. 5 - Assessment of hemoglobin (Hb) levels in the period of study. The diagram shows average Hb levels in the two experimental groups at the beginning of the study (T0) and after 3 months (T3) of treatment. A statistically significant Hb increase was found both in the group treated with Ferrograd® and that treated FerroGUNA (p-value<0.05).

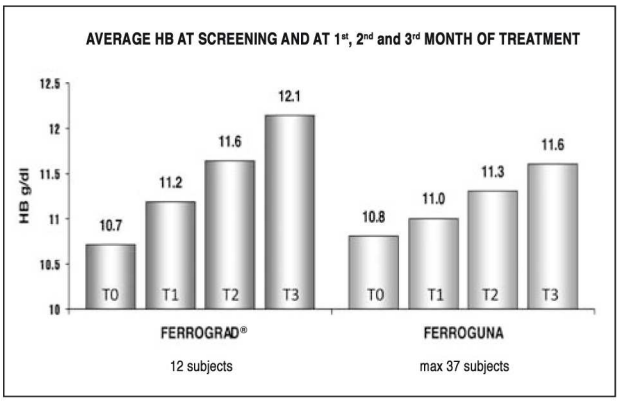

Fig. 6 - The diagram shows the average hemoglobin (Hb) levels found in the two experimental groups in all the periods of the clinical study.

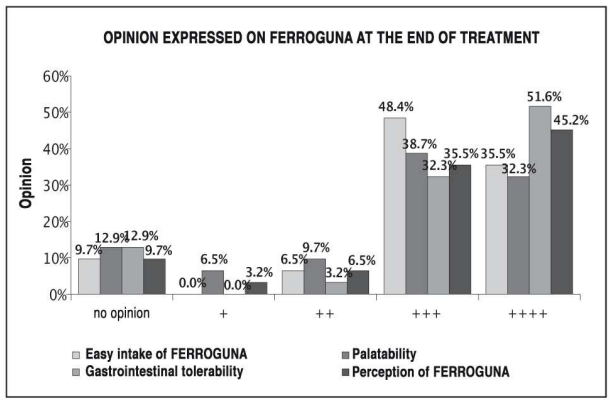

Fig. 7 – Opinion expressed by the subjects treated with FerroGUNA in terms of tolerability of the product. As we can see in the diagram, the subjects expressed a positive opinion about easy intake of the product, palatability, gastrointestinal tolerability, overall perception of the product, and gave high scores based on a scale from one to four (+ / + + + +).

< 0.05) in all periods, a clinically relevant and statistically significant (*p-value <0.05*) increase was reached for both products of the study between the *screening* and the T3 periods (Fig. 6).

In the first two months of treatment if you evaluate the variation of hemoglobin levels between subjects treated with Ferrograd® and those treated with FerroGUNA, there is no statistically significant difference. This shows a non-inferiority of FerroGUNA compared to the reference drug (Fig. 6), whereas at time T3 the treatment with Ferrograd® showed a greater capacity of restoring Hb levels. As expected, the evaluation of Hb within the single periods has shown that in the group treated with FerroGUNA the increase was more gradual. As FerroGUNA is a dietary supplement, it restores normal values in a more physiological way.

Both serum iron and ferritin levels showed a comparable increase in both groups, at the end of a three month treatment (T0 - T3).

Regarding the assessment by the subjects treated with FerroGUNA with respect to the tolerability of the treatment, especially easy intake, palatability, gastrointestinal tolerability, and overall perception of the product, the results were positive. Most of the patients assigned high scores (Fig.7).

At the end of the study the adverse events were analysed and included in the e–CRF. Eight subjects reported side effects during treatment. 25% of these subjects belonged to the group treated with Ferrograd® and 6.7% to the group treated with FerroGUNA. Concerning Ferrograd®, the following events were reported: in one patient a mild constipation, while in two patients moderate to severe diarrhea episodes lasting one or two days. In one case, it was necessary to discontinue the drug for one day.

Regarding FerroGUNA, one case of mild gastric hyperacidity, one case of mild constipation and, in another subject, one episode of abdominal pain were reported. These patients, however, expressed their positive opinion about the treatment and assigned a score of + + / + + + + based on a scale from one to four (+ / + + + +). Two subjects reported short-duration headache, probably not due to the product intake.

Conclusions

This study showed a non-inferiority of FerroGUNA, food supplement with ferrous fumarate iron, L-ascorbic acid, copper citrate, and Baobab dried fruit pulp, compared to Ferrograd® in the treatment of iron deficiency anemia in pregnancy.

The primary *outcome*, i.e. the increase of Hb in the blood by at least 0.5 g/dl in the treatment period T0 - T3 months was achieved.

FerroGUNA increases the hemoglobin concentration in the blood in a clinically relevant, i.e. > 0.5 g/dl (average 0.8 g/dl after 3 months), and statistically significant (*p-value* <0.05) way, during a T0 - T3 month period.

A non- inferiority of FerroGUNA compared to Ferrograd® is statistically proved even within those single periods analyzed (except for Ferrograd® vs. FerroGUNA at T3 months).

In the group treated with FerroGUNA, the increase of Hb values was more gradual and the normal values were restored in a more physiological way.

The blood levels of ferritin and serum iron showed a comparable increase in both groups at the end of the 3 month treatment.

Also the opinion expressed by the subjects belonging to the group treated with FerroGUNA with respect to their overall perception of the product was very positive (high assessment in a point system) (Fig. 7).

A low incidence and mild intensity of the adverse events resulting from the administration of FerroGUNA showed a greater tolerability of the latter compared to Ferrograd®.

Based on the above mentioned data we can conclude that the food supplement FerroGUNA is capable of increasing the concentration of Hb in the blood in a way that is absolutely comparable to Ferrograd®.

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