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A pilot study on secondary anemia in "frailty" patients treated with Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine: safety of treatment explored by HRV non-linear analysis as predictive factor of cardiovascular tolerability

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Abstract. – OBJECTIVE: Iron deficiency anemia (IDA) in patients with heart disease is correlated with decreased exercise capacity and poor health-related quality of life, and predicts worse cardiovascular outcomes, especially for elderly patients. IDA can worsen cardiac function that can be monitored with Heart Rate Variability (HRV) analysis, providing important information about cardiac health. In a recent study we explored the effect and the tolerability of the administration of Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) in "frailty" patients with secondary anemia and low kidney failure, by analysing the HRV frequency domain. The aim of the present study is the further confirmation of the safety of the already evaluated intervention, by analysing non-linear domain of HRV.

PATIENTS AND METHODS: In this pilot study we enrolled 52 "frailty" elderly patients, with a recent diagnosis of secondary anemia due to iron deficiency, with Class II New York Heart Association (NYHA) hypertensive heart disease, low kidney failure, and atherosclerosis. The patients were divided in 2 groups: Group A (N=23 patients) received oral administration of Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) 2 tabs/day, containing 60 mg of Fe^{3+}, for 24 days; Group B (N=29 patients) received intravenous administration of ferrous gluconate 63 mg/day added to saline solution, while they were hospitalized (15±5 days). We evaluated laboratory values of hemoglobin (Hb) and sideremia levels. Furthermore, we measured ECG signals before and after treatment, using non-linear analysis techniques.

RESULTS: Both intravenous and oral treatments evaluated in this study, were effective and safe about the cardiovascular risk in "frailty" elderly patients, as resulted from non-linear HRV analysis. Efficacy results showed that hemoglobin and sideremia levels after treatments are significantly increased. The HRV non-linear analysis showed that all parameters evaluated, except for the SD1 values in the Group A, were not affected by treatments, confirming the absence of cardiovascular risk of the therapy.

CONCLUSIONS: Non-linear HRV evaluation confirmed that oral administration of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel forte®) did not impact the cardiovascular risk, without causing adverse events typically reported with other iron supplementation therapies, both oral and intravenous.

Key Words:
Cardiovascular risk, Ferric sodium EDTA, HRV, Safety, Iron-deficiency anemia, Non-linear analysis.

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Introduction

Heart rate variability (HRV) is the temporal variation between sequences of consecutive heartbeats, as resulted of a standard electrocardiogram (ECG) measurement. HRV reflects the activity of the Autonomic Nervous System (ANS) that is responsible for the extrinsic regulation of the Heart Rate (HR)[1,2].

HRV is a physiological phenomenon that indicates the degree of psycho-physical adaptability of the individual in response to various factors, such as breathing, exercise, mental stress, anxiety, hemodynamic and metabolic changes, diseases, etc. HRV can be considered as an expression of the totality of several physiological factors modulating the complex cardiac rhythm. Consequently, HRV can give an estimation of the interconnections between the sympathetic and parasympathetic nervous systems. In particular, sympathetic nervous system is activated in response to stressful and dangerous situations, causing a series of effects (acceleration of the heartbeat, pulmonary dilation, increase in arterial pressure, peripheral vasoconstriction, pupillary dilatation, increased sweating), leading to a fight-or-flight response. In contrast, the parasympathetic nervous system controls calm, rest and tranquillity situations, producing a slowing of cardiac rhythm, increasing bronchial muscle tone, dilating blood vessels, decreasing blood pressure, slowing breathing and relaxing muscle[1,2].

A high HRV provides a signal of the good degree of adaptation of the organism to internal and external stimuli and characterizes a healthy individual with efficient mechanisms of regulation of the autonomic nervous system. Conversely, a low HRV is often an indicator of abnormal and insufficient adaptation to external factors with consequent reduction of physiological functions and can provide an index of pathological conditions[3,4].

HRV analysis is able to determine the prevalence of sympathetic or parasympathetic activity and to give information about the kind of nervous system prevalent in unbalanced situations. Overall, HRV is an index to assess cardiac health and state of ANS. The several sympathetic and parasympathetic autonomic activities contribute to the modulation of heart rate intervals of the QRS complex in the electrocardiogram (ECG)[1-4].

A variety of measures can be used for HRV analysis, including 24 h, short-term (ST, ~5 min) or brief, and ultra-short-term (UST, <5 min) measurements, that can be applied to time domain, frequency domain, and more complex non-linear measures. The exploration of time domain provides estimation of variability of the time period between successive heartbeats, named interbeat interval (IBI), while frequency domain allows to evaluate the distribution of power spectral density, by dividing it into four frequency bands: ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands[2,4].

Recently, non-linear methods have proven to be extremely useful as new dynamic methods of HRV evaluation, in order to quantify non-linear fluctuations in HR that would not be measurable otherwise, due to the non-stationary characteristics of HRV signals. It is generally recognized that non-linear techniques are able to describe the processes generated by biological systems in a more effective way[1,5]. Non-linear methods analyse variables that cannot be plotted as a straight line and include determination of the time signal's entropy measuring the randomness in the system and the unpredictability of a time series, which results from the complexity of the mechanisms regulating HRV[2,3]. Entropy measures have been used widely in HRV analysis with encouraging results. Several non-linear methods have been developed, including Approximate Entropy (ApEn), Sample Entropy (SampEn), MultiScale Entropy (MSE), SD1/SD2 of Poincare plot and Detrended Fluctuation Analysis (DFA)[1].

In time series analysis, ApEn provides a measure of the degree of irregularity or randomness within a data set and it is used as a measure of system complexity. ApEn examines the conditional probability of the inherent similarities in the time series based on statistical analyses that appear to be compatible with the general clinical need to distinguish healthy subjects from the abnormal ones. Lower values of ApEn indicate greater regularity, and higher values bring more disorder, randomness and complexity of the system. In particular, ApEn computed on HRV has been shown to have a discrimination power to distinguish patients suffering from Congestive Heart Failure from healthy people. ApEn has higher value in the case of normal subjects and it falls as the R-R variation decreases, as for cardiac abnormal cases, indicating smaller variability in the beat to beat interval. A decreased ApEn value was related to the ability to predict atrial arrhythmias, such as spontaneous or post-operative atrial fibrillation and to differentiate ventricular arrhythmias. ApEn Statistics can be calculated for a relatively short series of data and biological signals[1,2,5-7].

The Poincaré plot of the R-R intervals is a non-linear analysis technique that allows a visual and quantitative characterization of cardiac variability. The analysis using Poincaré Plot allows representing on a graph every interval R-R according to the previous RR interval. In this way, each point corresponds to a couple of consecutive R-R intervals. It is a method of analysis used to quantify heart rate variability (HRV). The analysis allows extrapolating two parameters: SD1 and SD2, which are considered to be indicative of short and long-term cardiac variability, respectively. Short-term variability provides information about the parasympathetic tone, whereas the long-term variability provides information on the sympathetic tone[1,2,5,6].

DFA or fluctuation analysis of the cardiac variability signal allows the calculation of two exponents, the short-term exponent (DFA1 or α-1 <11 beats, sympathetic tone) and the long-term exponent (DFA2 or α-2 >11 beats parasympathetic tone). The DFA analysis is an updated version of the spectral analysis because it is not influenced by external factors, such as breathing or physical activity. The reduction of α1 using DFA appears to be predictive of increased cardiovascular risk[1,2,5,6].

There is a connection between iron-deficiency anemia (IDA) and cardiovascular outcomes, since IDA can worsen cardiac function and increase risk for hospitalization and death, together with a reduction of exercise capacity and a worsening of quality of life, especially for elderly patients[8]. Several studies showed a relationship between cardiac autonomic function and anemia, in various anemic patient populations, such as vitamin B12 deficiency, sickle cell anemia and thalassemia major. However, iron-deficiency anemia (IDA) is more frequent type of anemia worldwide and previous studies demonstrated that patient with IDA have an altered autonomic balance[9].

Furtherly, in patients with chronic heart failure, iron deficiency and IDA can aggravate the underlying disease, with worsening of clinical outcomes and quality of life. For these reasons, the 2016 European Society of Cardiology Guidelines for the diagnosis and treatment of acute and chronic heart failure recognize iron deficiency as a co-morbidity in chronic heart failure and recommend iron status screening in all newly diagnosed patients with chronic heart failure[10].

Previous results have showed that Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) is effective and safe for the treatment of secondary anemia in "frailty" elderly patients, as evaluated by exploring time and frequency domains of HRV[11,12].

The aim of the present study is the evaluation of the safety of the administration of Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) in "frailty" elderly patients with secondary anemia and low kidney failure, by analysing non-linear domain of HRV.

Patients and Methods

In this pilot study we enrolled 52 "frailty" elderly patients, with a recent diagnosis of secondary anemia due to iron deficiency, with Class II New York Heart Association (NYHA) hypertensive heart disease, low kidney failure and atherosclerosis. The patients were divided in 2 groups: Group A (N=23 patients) received oral administration of Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) 2 tabs/day, containing 60 mg of Fe^{3+}, for 24 days; Group B (N=29 patients) received intravenous administration of ferrous gluconate 63 mg/day added to saline solution, during the hospitalization period of 15±5 days. The enrolled patients (79.1±12.9 years old) had as comorbidity low kidney failure with mean creatinine value in Group A: 1.1+0.6 mg/dL and in Group B: 1.4±1.0 mg/dL; according to international scale for laboratory test (creatinine mg/dL).

We evaluated laboratory values of hemoglobin (Hb) and sideraemia levels. Furthermore, we measured ECG signals before and after treatment, using non-linear analysis techniques. For the ECG signal analysis, Cardio CE palm version 2.0 (XAI-Medica) was used to register standard ECG and beat-to-beat ECG for HRV non-linear evaluation. The Observational study protocol respects the Ethical Committee of Alfredo Fiorini Hospital, Terracina, (Latina), Italy, and patients were included after providing written informed consent.

Statistical Analysis

Statistical analysis was performed using Paired T-test with SigmaStat v. 3.5 (San Jose, CA, USA) analysis program. In statistics, a paired difference test is used in order to compare two sets of measurements and to evaluate whether their population means are different. A paired difference test involves additional information about the sample that is not evaluated in a standard unpaired test, either to extend the statistical power, or to scale back the effects of confounders. Commonly, a paired difference test is applied when subjects are measured before and after a treatment. In this way, the repetition of measurements within subjects can give a greater power than an unpaired test, where the measurements are repeated across subjects. The differences were considered significant when $p<0.05$.

Results

The results of this study showed that oral treatment with Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) at the iron dosage of 60 mg (2 tabs a day) for 24 days (Group A) has been well tolerated in "frailty" elderly patients with secondary anemia due to iron deficiency and low kidney failure, with Class II NYHA hypertensive heart disease and atherosclerosis (Table I). No adverse event has been reported during study period.

The HRV non-linear analysis showed that all parameters evaluated, except for the SD1 values, were not affected by the oral treatment, confirming the absence of cardiovascular risk of the therapy. Only the values of the SD1, which is the parameter linked to parasympathetic activity, showed a statistically significant difference ($p=0.028$) (Table I and Figure 1).

Figure 2 shows an example of non-linear indexes before (in the top side) and after (in the downside) the administration of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®).

Similarly, intravenous iron treatment with ferrous gluconate 63 mg/day added to saline solution, during the hospitalization period of 15±5 days (Group B) showed the lack of statistically significant changes of HRV non-linear parameters evaluated (Table II).

Efficacy results are similar to previous one already reported and confirmed the efficacy of both treatments in Group A and Group B (Table III)[11,12]. In particular, hemoglobin (Hb) and sideremia levels significantly increased, after treatment with Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) (2 tabs/day) for 24 days.

Therefore, the therapy with Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) at the dosage of 60 mg (2 tabs a day) represented an effective and safe treatment of IDA without worsening cardiovascular risk in "frailty" elderly patients.

Discussion

According to previous results obtained about the use of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) in "frailty" elderly patients with secondary anemia due to iron deficiency and Class II NYHA hypertensive heart disease, low kidney failure and atherosclerosis, this study furtherly investigated the efficacy and safety of

Table I. Effect of Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel forte®) administered 2 tabs/day, for 24 days (Group A) on non-linear parameters of HRV. The data are expressed as value±SD.

	Baseline	End of treatment	*p*-value
SD1	56.3 ± 30.8	39.1 ± 16.4	0.028*
SD2	89.9 ± 60.9	74.3 ± 74.9	0.523
ALFA-1	0.798 ± 0.334	0.874 ± 0.354	0.536
ALFA-2	0.740 ± 0.415	0.637 ± 0.285	0.382
ApEn	0.705 ± 0.301	0.784 ± 0.289	0.452
SampEn	1.029 ± 0.670	1.041 ± 0.580	0.945
ShanEn	2.610 ± 1.188	3.089 ± 1.008	0.232

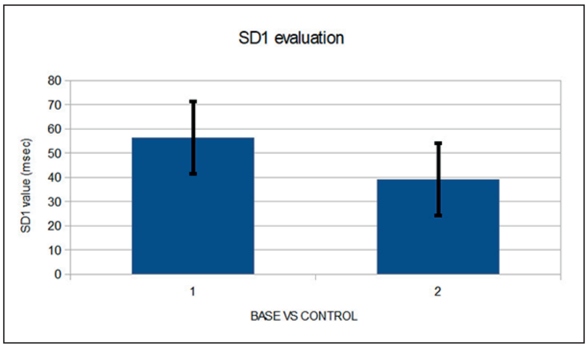

Figure 1. SD1 parameter of HRV evaluation (non-linear domain) at baseline and after the administration of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel forte®) 2 tabs/day, for 24 days (Group A). Data are expressed as mean + standard deviation (SD).

this oral formulation, in particular by analysing non-linear parameters of HRV[11,12].

Non-linear measurements quantify the HRV unpredictability and complexity, including also the non-stationary characteristics of HRV signals and overcoming the theoretical limitations of other HRV methods of analysis, even if all methods confirm their clinical significance[1,5]. The analysis of variability represents a new method for evaluation and treatment of individual patients and therefore a shift from an analytical epidemiological investigation to a continuous analysis of individual variability. Non-linear methods allow evaluating the prevalence of sympathetic or parasympathetic activity in unbalanced situations[1,5].

Several studies[9,13,14] reported that anemia is associated with unbalance of the HRV modulation in ambulatory patients who have stable coronary heart disease and that unbalance of the HRV modulation could potentially mediate the association of anemia with increased cardiac risk. Unbalance of the HRV modulation has been detected also in anemic patients due to vitamin B12 deficiency[15,16], thalassemia[17,18], and sickle cell anemia[19,20].

The elderly population is particularly at risk for developing IDA for multi-factorial causes,

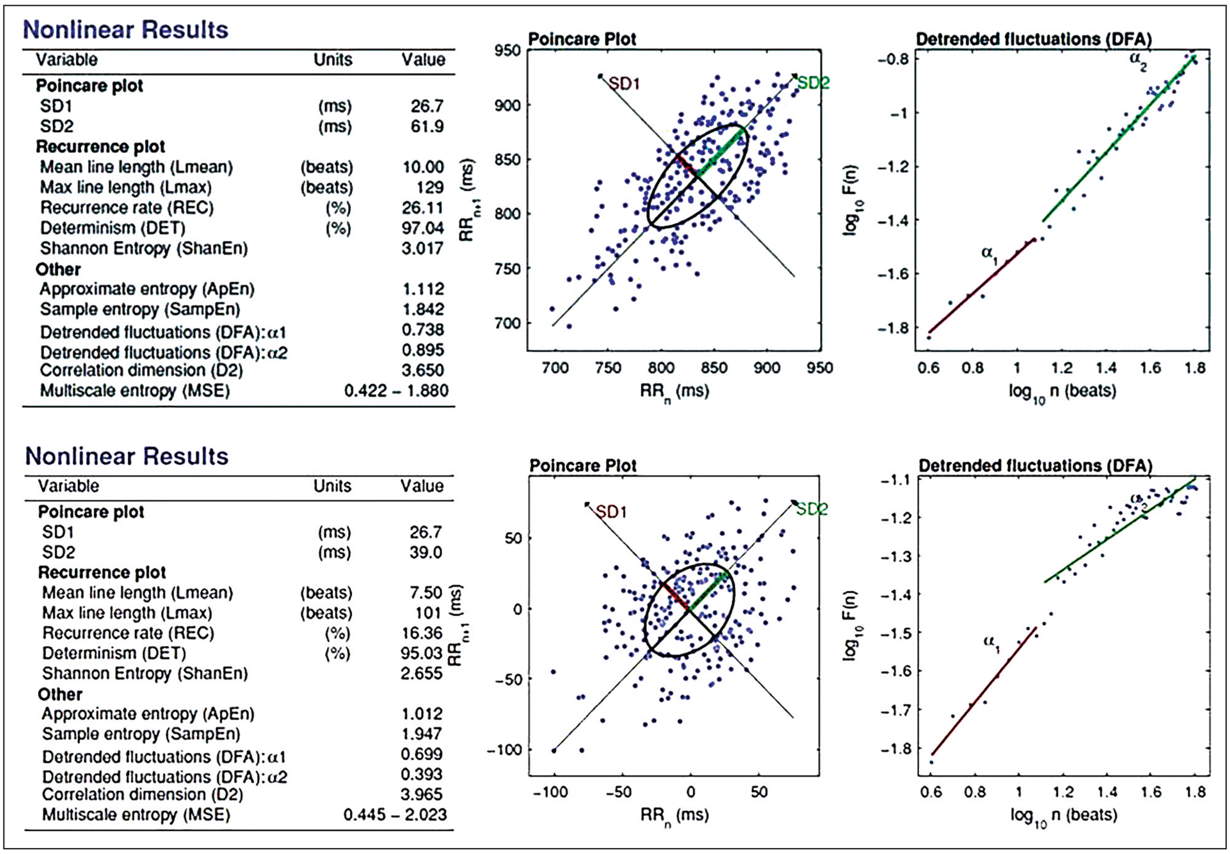

Figure 2. Example of non-linear indexes before (in the top side) and after (in the down side) the administration of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel forte®) 2 tabs/day, for 24 days (Group A).

Table II. Effect of intravenous ferrous gluconate (63 mg/day added to saline solution) administered during the hospitalization period of 15±5 days (Group B) on non-linear parameters of HRV. The data are expressed as value±SD.

	Baseline	End of treatment	*p*-value
SD1	72.997 ± 46.616	71.023 ± 34.574	0.728
SD2	116.653 ± 69.768	137.352 ± 112.252	0.290
ALFA-1	0.787 ± 0.433	0.733 ± 0.266	0.492
ALFA-2	0.776 ± 0.433	0.729 ± 0.464	0.798
ApEn	0.802 ± 0.272	0.795 ± 0.185	0.934
SampEn	1.349 ± 0.645	1.186 ± 0.651	0.384
ShanEn	2.946 ± 0.996	3.167 ± 0.989	0.587

including malnutrition, iron malabsorption and occult gastrointestinal blood loss, which in turn can be a symptom of co-morbidity, such as neoplasms or inflammatory bowel disease. In addition, IDA is a frequent co-morbidity for elderly patients with chronic renal diseases, mainly due to the reduction in the erythropoietin production and to chronic inflammatory state[21]. Patients with chronic inflammatory state show high level of cytokines, such as interleukin-6 (IL-6), which is responsible to induce high levels of hepcidin, a key factor of iron homeostasis. Hepcidin acts by inhibiting the action of ferroportin, an iron channel protein, present on the surface of enterocytes, macrophages and hepatocytes, which allows the introduction of iron into the bloodstream. Hepcidin causes iron retention in macrophages and blocks dietary iron absorption in the duodenum[21]. For these reasons, the patient type here studied, ("frailty" elderly patients with secondary anemia and low kidney failure) is particularly at risk of developing poor quality of life outcomes IDA-related.

In this study, non-linear methods for HRV analysis have been used in order to confirm previous results regarding time and frequency domains[11,12]. In this way, all the domains of HRV have been explored in elderly patients with IDA, notwithstanding the limited sample size of the present study, that cannot allow to generalize results to a broad spectrum of patients with secondary anemia.

The results of this study showed the efficacy and tolerability of oral supplementation with Ferric Sodium EDTA combination, as confirmed by the lack of modification of the non-linear parameters evaluated (Table I), except for the SD1 reduction (Table I and Figure 1), which is indicative of a decreased parasympathetic activity. This result is coherent with previous results detecting an impairment in non-linear HRV indices in patients with IDA, indicating a decreased parasympathetic activity[9].

The use of Ferric Sodium EDTA combination showed no significant differences in HRV parameters before and after treatment, without

Table III. Hemoglobin (Hb) and sideremia levels after oral treatment with Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) 2 tabs/day, for 24 days (Group A) or intravenous administration of ferrous gluconate 63 mg/day added to saline solution, during the hospitalization period of 15±5 days (Group B). The data are expressed as value±SD.

Oral treatment with Ferric Sodium EDTA in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) (2 tabs/day) for 24 days			
Group A (N = 23)	**Baseline**	**End of treatment**	***p*-value**
Hb (g/dL)	9.5 ± 1.3	11.7 ± 1.9	0.001*
Fe^{++} (mcg/dL)	19.5 ± 5.6	53.8 ± 25.9	0.001*
Intravenous administration of ferrous gluconate 63 mg/day added to saline solution, during the hospitalization period of 15 ± 5 days			
Group B (N = 29)	**Baseline**	**End of treatment**	***p*-value**
Hb (g/dL)	8.9 ± 1.5	9.9 ± 1.9	0.001*
Fe^{++} (mcg/dL)	19.6 ± 12.2	37.1 ± 21.9	0.001*

affecting cardiovascular risk and, thus, resulting in a safe therapeutic strategy for elderly patient with secondary anemia, Class II NYHA hypertensive heart disease, low kidney failure and atherosclerosis.

Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) is a dietary supplement formulated to optimise iron absorption and reduce typical side effects of the traditional oral iron formulations. The iron source in this product is Ferric Sodium EDTA, a complex of ferric ion that allows crossing through the stomach without modification and the release of ferric ion in the duodenal tract where it can be absorbed. All other components are added in the formulation in order to improve iron absorption, as broadly reported in literature[22-26].

Conclusions

The use of Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) is an effective and safe treatment in "frailty" elderly patients with secondary anemia and low kidney failure, NYHA Class II hypertensive heart disease and atherosclerosis, as confirmed by non-linear HRV analysis. Non-linear HRV evaluation confirmed that Ferric Sodium EDTA, in combination with vitamin C, folic acid, copper gluconate, zinc gluconate and selenomethionine (Ferachel Forte®) administration is well tolerated and does not cause adverse events typically reported with other iron supplementation therapies both orally and intravenously. Finally, our pilot study underlines that the administration of the formulation of Ferric sodium EDTA (Ferachel Forte®) ensures a statistically significant amelioration of the secondary anemia due to iron deficiency in "frailty" elderly patients with low moderate kidney failure, Class II NYHA hypertensive heart disease and atherosclerosis. Future researches are necessaries for extending the use of technologies based on HRV evaluation to improve patient's outcomes and care.

Conflict of Interest

The Authors declare that they have no conflict of interests. Write informed consent was obtained from all individual participants included in the study.

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