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”THE PLACE OF THE KETODIET IN THE TREATMENT OF CHRONIC DIABETIC KIDNEY DISEASE: METABOLIC AND NUTRITIONAL INVOLVEMENTS, THE CARDIOVASCULAR RISK AND THE KIDNEY SURVIVAL”

ABSTRACT

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THE LIST OF ABREVIATIONS AND SYMBOLS

CKD chronic kidney disease

CDKD chronic diabetic kidney disease

CNAS National Health Insurance Authority

DPCA chronic peritoneal ambulatory dialysis

PCD protein-caloric denutrition

DM diabetes mellitus

eRFG estimated glomerular filtration rate

FSP hand- grip power

HD hemodialysis

HTA arterial hypertension

CCF congestive cardiac failure

BMI body mass index

i- PTH intact parathormone

KDIGO Kidney Disease Outcomes Quality Initiative

KDOQI Kidney Disease :Improving Global Outcomes

MDRD Modification of Diet in Renal Disease (reference study)

nPNA normalized Protein Equivalent-Nitrogen Appearance

PCR reactive C protein

PNA Protein Equivalent-Nitrogen Appearance

PTH parathormone

RAC or **RACu** urinary albumin/creatinine ratio

RFG or **GFR** glomerular filtration rate

SGA subjective global assessment score

RT renal transplantation

Contents

I. GENERAL PART

In the context of the current diabetes mellitus (DM) pandemic, mainly due to the real blast in the cases of type 2 diabetes, when according to estimates by the International Diabetes Federation, the number of diabetics exceeded the psychological threshold of 400 million worldwide (1 in 11 people) and is constantly expanding, it is not surprising that the dynamics of cases of chronic kidney disease follow a similar evolution, the latter being one of the most important chronic complications of diabetes, both in terms of material resources, but also in terms of changes in the quality of life of patients with diabetes [1].

In our country, the PREDATORR study depicts, since 2014, a worrying picture of the prevalence of diabetes - over 11% (of which 2.4% undiagnosed) -, a prevalence that increases with age and is higher in men compared to women. The study also underlines the undersizing of healthcare outside major academic centers, which obviously contributes to underestimating the prevalence of the disease [2].

Type 2 diabetes is responsible in the world for 42% of the total number of cases of chronic kidney disease, only 18% having as etiology hypertension, in an evaluation conducted in 2016 [3]. Up to 6 out of 10 patients with type 2 diabetes may develop CKD and have a 6-fold higher risk of cardiovascular death than developing end-stage renal disease [4]. Decreased renal function, evidenced by estimated glomerular filtration rate (eRFG) and urinary albumin-creatinine ratio (RACu), is associated with increased cardiovascular risk, i.e., dramatic increase in atherosclerotic or non-atherosclerotic cardiovascular disorders, hospitalization, and mortality [5].

Data cited in the literature show that the untreated progression rate of chronic diabetic kidney disease (CDKD) is 9-14 ml / min / year for type 1 diabetes with proteinuria [6] and slightly lower for type 2 diabetes and proteinuria, of about 6 ml / min / year [7]. Fabre et al. noted the earlier onset of proteinuria in type 2 diabetes, but with a slower rate of progression of kidney disease compared to type 1 diabetes [8]. In this respect, all the therapeutic means must be used to slow down the progression of CDKD to the final stage, and the first means of therapeutic approach is of course diet. Without saying that it is the most important, it still remains the cornerstone of any correct therapeutic approach, in any condition, especially in chronic diseases.

The ketodiet is a severely hypoprotein diet, supplemented with a tableted mixture of amino acids and ketoanalogues of tableted essential amino acids, in order to slow the

progression of CKD of any etiology from CKD stage 4. A multitude of studies since the famous MDRD study have shown the usefulness of this type of diet in delaying the progression of CKD, while other studies have challenged such kind of diet, raising the issue of protein-caloric malnutrition, whose risks and implications could outweigh the benefits of a severe hypoproteic diet [9]. However, the costs involved in the process of initiating and maintaining the patient with existing renal replacement methods, as well as solving all the complications that may arise from here are far superior to those costs involved in initiating and monitoring patients on this type of diet. If we compare these two major therapeutic directions refering to the quality of life for the patients, then the balance can tip significantly in favor of the ketodiet, of course, provided that the patient is compliant and adherent in the long term. In the scientific literature there are no studies to doubtlessly prove reliable metabolic and nutritional benefits in diabetic patients with CKD that are on a ketodiet, and in our country, except for a few medical institutions, patients with CDKD are excluded from such diet.

The current study aims to highlight the benefits of using ketodiet in both prolonging renal survival, reducing the decline in glomerular filtration rate and general or cardiovascular mortality, but also in obtaining other metabolic and renal benefits, while maintaining an adequate nutritional balance. Also, as such a diet is difficult to maintain in the long term in diabetic patients, already under a chronic restrictive diet from the diagnosed diabetes, taking into account the cognitive abilities, the psychological and cultural profile, the economic possibilities of each patient, I aim to identify a possible "model" of the patient complying with the ketodiet and a "prediction model" for the decline in the filtration rate according to compliance.

Although the guide of good medical practice, regarding the evaluation and nutritional intervention in chronic kidney disease, drafted by the Romanian Society of Nephrology and published under the auspices of the Romanian College of Physicians in 2007, provided that the very low-protein diet (0.3-0.4g protein / kg body weight / day) supplemented with ketoanalogues of essential amino acids (Ketosteril® 1tb / 5 kg body weight / day), might be indicated to slow the progression of CKD from stage 4 and delay the initiation of renal replacement therapy, in safe nutritional conditions in selected patients, same guide explicitly states diabetes as a contraindication in the diet technique indications [10]. In 2020, the KDOQI (Kidney Disease Quality Outcome Initiative) working group of the Kidney National Foundation and the US Academy of Nutrition and Dietetics published the guide to nutrition in kidney disease, formulating the indication to slow the progression of

chronic kidney disease (CKD stages 3-5 without dialysis) only in patients without diabetes as follows: protein intake 0.28-0.43 g protein / kg body weight / day supplemented with ketoanalogues of essential amino acids or only protein intake of 0.55-0.6 g / kg body weight / day, without other supplements, in metabolically stable patients, without associated diabetes mellitus (grade 2C). For patients with diabetes and CKD stages 3-5 without dialysis there is only a recommendation (opinion), regarding the protein intake to be administered, with 0.6-0.8 g protein / kg body weight / day, to avoid malnutrition and maintain optimal metabolic and glycemic control [11]. However, the debates are ongoing and there have been various studies and meta-analyses looking at the usefulness and safety of ketodiet in patients with diabetes and advanced chronic kidney disease, that prove its benefits in the diabetic kidney disease: it delays the decline of RFG [12, 13, 14, 15, 16, 17, 18], reduces urinary albumin excretion [12, 13, 14, 15, 19] and relieves both hyperparathyroidism and renal osteodystrophy [20, 21, 22, 23, 24, 25]. Moreover, this diet has shown an improvement in insulin sensitivity [26], and such insulin sensitivity could compensate for the excess carbohydrates in the diet, without a negative impact on insulin requirements (19, 26, 27). Recent studies have shown a reduction in the relative risk of terminal CKD and death, while maintaining a good nutritional status in chronic diabetic kidney disease [28, 29, 30, 31].

In 2010, the International Society for Renal Nutrition and Metabolism (ISRNM) organized an expert committee to re-examine the terms and criteria [32] used to diagnose protein-caloric denutrition - "the state in which muscular and fat mass are low" - when at least 3 of the 4 groups of criteria are simultaneously met and at least one test in each category.

The 2020 KDOQI Good Practice Guide on CKD Nutrition explicitly specifies that for all patients with CKD [11] it is recommended that routine nutritional screening be performed at least twice a year to detect patients at risk of protein-caloric denutrition (PCD) and that there is no solid evidence for the use of one means of identifying the PCD to the detriment of another. Thus, the full nutritional assessment must include:

- food intake history (various types of food questionnaires)
- body weight (weight without edema or adjusted weight without edema, etc.) and BMI
- biochemical data (serum albumin, serum cholesterol, etc.)
- anthropometric measurements (skin fold thickness, muscle mass area in the middle third of the arm, arm circumference, clenching force, etc.)

- other clinical and nutrition data (composite scores such as global subjective assessment score, malnutrition-inflammation score, etc.)

CKD-associated metabolic and nutritional disorders include a wide range of changes: reduction of protein and energy intake (anorexia, dietary restrictions, digestive dysfunction, depression), hypermetabolism (excessive energy consumption by cytokines or insulin resistance, metabolic alterations of adiponectin/resistin), complex hormonal disorders, metabolic acidosis, reduced anabolism (reduction of intake of nutrients, growth hormone resistance / insulin-like growth factor 1, testosterone deficiency, thyroid hormone depletion), sedentary lifestyle [33, 34].

The cardiovascular risk factors involved in CDKD are the well-known, traditional factors, but also non-traditional factors, strictly related to the presence and evolution of CKD All these factors finally lead to the onset of ischemic heart disease, heart failure, cerebrovascular disease, the progression of chronic renal disease and cardiovascular-related death [35, 36]. The common mechanisms that contribute to the progression of cardiovascular disease in DM and CDKD are: hypertension, atherogenic dyslipidemia, endothelial dysfunction, hypercoagulability, chronic inflammation and oxidative stress [37]. Cardiovascular complications reported in patients with CDKD vary over a wide pathological range; more specifically, we refer to atherosclerotic diseases such as coronary heart disease, heart failure, ischemic stroke and peripheral arterial disease, as well as to non-atherosclerotic cardiovascular events, such as left ventricular hypertrophy, arrhythmias, sudden death, hemorrhagic stroke and arterial and valvular calcifications [38, 39].

II.SPECIAL PART

4. WORKING HYPOTHESIS AND SPECIFIC OBJECTIVES

The aim of this paper is to demonstrate that patients with CDKD can follow the long-term ketodiet, in nutritional safety conditions, being very important both the strict observance of dietary prescriptions and the number of ketoanalogues tablets. The consequences of compliance with the ketodiet are both prolonged patient survival, reduced decline in glomerular filtration rate and other metabolic and renal benefits.

Inclusion criteria:

- adult patients with type 1 or 2 diabetes

- patients with constant weight in the last 6 months
- eRFG (MDRD equation) ≤ 30ml / min / 1.73m^2
- serum albumin ≥ 3g / dl
- absence of an active infection or severe pathology such as ICC class III-IV, liver cirrhosis or other severe chronic pathology
- stable antihypertensive and antidiabetic treatment in the last 3 months, for at least 3 month on therapy with angiotensin receptor bockers/ angiotensin converting enzime inhibitors
- good digestive tolerance, unchanged in the last 3 months
- patients able to offer written consent and to understand the prescription of ketodiet/patients already on nutritional medical treatment with ketodiet

Exclusion criteria:

- patients under 18, pregnant or nursing women
- hypercalcemia (total serum calcium > 10,2 mg / dl)
- disorders of amino acid metabolism
- oncology patients, patients with digestive or motor disorders who cannot ensure the prescribed food intake
- allergy to any of the components of Ketosteril® ketoanalogue tablets.

- Primary objectives:

1- the formulation of a ketodiet compliance "model".

2- the duration of renal survival until reaching a composite objective, consisting of entering the renal replacement program (hemodialysis, peritoneal dialysis or kidney transplant) or until death of any cause. Determination of cardiovascular and global mortality of patients on ketodiet, at 4 years. Follow-up evaluation of non- / cardiovascular mortality at 8 years, of the need for comparative renal replacement therapy, in patients stratified at 4 years in compliant and non-compliant.

3- the rate of decline in glomerular filtrate expressed in ml / min / 1.73m^2 / year.

- Secondary objectives:

1– assessment of the nutritional and inflammatory status of patients on ketodiet.

2– assessment of additional benefits of the ketodiet, regerding: maintenance of diuresis, blood pressure control, acid-base and ionic balance, phospho-calcium balance, glycemic and lipid control.

5.MATERIAL AND METHOD

Study design

The study was conducted with the recruitment for 30 days of 40 patients (men and women), in the nephrology department of INDNBM "N. Paulescu" and their monitoring during 4 years, between 2013-2017, having a prospective interventional, open-label character and a follow-up for another 4 years of patients (by telephone), to determine renal survival without renal replacement and vital status – the (non) cardiovascular death.

The target population is adult diabetic patients with chronic kidney disease stage 4, having at least 3 months of therapy with angiotensin receptor blockers (BRAs) / angiotensin converting enzyme inhibitors (IECA inhibitors), to whom the medical-nutritional intervention of the ketodiet is proposed; the patients are naive or already following the ketodiet. At time 0 (baseline), the demographic, educational and occupational characteristics of the patients are evaluated, as well as aspects related to the family situation, functional status and associated comorbidities. Ketodiet, the administered nutritional medical intervention, has prescribed a severely hypoprotein diet of 0.4 g protein / kg body weight / day, supplemented with ketoanalogues of essential amino acids accredited by CNAS and the Romanian Society of Nephrology, Ketosteril®, prescribed free of charge. The supplement was administered in the amount of 1tb/5 kg bw/day. For the prophylaxis of protein-caloric denutrition in the monitored patients, at the baseline of study, all patients were hospitalized and evaluated periodically from the nutritional point of view, as well as volume depleted when necessary and the weight taken into account to determine the body mass index BMI (BMI = Wef / H^2) was the weight without clinical signs of hydro-saline retention called the effective weight Wef and H = height in meters. For those who did not achieve this goal, to obtain Wef, the weight estimation formula without edema Wfe, was used as follows:

- Wef = Wfe, if it is between 95-115% of the median of the standard body mass Ws
- Wef = Wfe adjusted = W + 0.25 (Ws-W), if it is outside the mentioned range where W represents the current weight measured on the scale. The tables with the interpretation of standard body masses can be found in Annex II of the guide of good medical practice made by the Romanian Society of Nephrology and published under the auspices of the Romanian College of Physicians [10]. The following strategy was used to calculate the caloric and protein requirement:
- for BMI <30kg / m2 the energy prescription was 30kcal/kgbw/day at age ≥ 60 years and 35kcal / kgbw/day at age <60 years. Two energy requirements were calculated, using the

weight Wef and Wi, respectively, the ideal weight. The highest amount of energy obtained was the recommended one, and based on the weight used in the calculation of the prescribed amount of energy, the prescription of proteins was performed. There were no caloric restrictions for overweight people. The calculation formulas for the ideal weight were as follows:

- Men: 50+ 0.75 (H-150) + (V-20) / 4
- Women: [50 + 0.75 (H-150) + (V-20) / 4] x 0.9

where H = height, V = age

- for BMI ≥ 30kg / m^2 the energy prescription was calculated based on the weight of Wef and with 30kcal/kgbw/day, of which for the ages of ≥ 60 years, i.e. elderly, obese and sedentary, 500kcal were deducted from the total energy obtained. Based on this weight, the protein requirement was also calculated. For both categories of BMI in the severe hypoprotein diet, the daily amount of protein actually administered must be in the range of 0.3-0.5 g/kgbw/day, with the specifications that the lower limit allowed was at least 0.3 g/kgbw/day and the maximum strictly allowed <0.5 g /kgbw/day, and the prescribed amount for patients was 0.4 g/kgbw/day.

Compliance assessment and stratification of patients according to compliance

The evaluation of compliance had as coordinates: on the one hand the evaluation of the food intake, and on the other hand the evaluation of the number of ketoanalogue tablets administered by patient. The number of tablets was evaluated quarterly at each patient's visit, starting with the 3rd month. The compliance with the recommended dietary intake was assessed subjectively at 3 months during the accomodation period and dietary dialogue (the patient had access to dietary and psychological counseling from our specialists whenever necessary). After 6 months from the time of inclusion in the study, after 12 months, then every year, dietary compliance was assessed on the basis of the food questionnaire (protein and calories intake) and the calculation of the protein equivalent of total nitrogen appearance PNA (protein intake) - the formula for calculating PNA (g protein/day). At each visit, patients had access to nutritional and psychological counseling. The overall stratification of patients into compliant and non-compliant was performed at the end of the study (by renal replacement or death). This final compliance was defined as follows: maximum once non-compliance with the number of administered tablets; maximum 1 time exceeding the maximum allowed amount of protein and maximum 1 time not achieving the number of prescribed calories (they were allowed to consume more kcal, but not less), throughout the study.

Renal survival, cardiovascular mortality and the decline of glomerular filtration rate

At the end of each monitoring year, the following are calculated:

- the variation of the estimated filtration rate between the moment 0 of the study entry and the year in which the calculation is made (ΔMDRD 0 -year) in ml/min/1.73m^2
- the survival interval (in months) from time 0 to the year referred to
(R. survival 0 -year)
- the 0-year GFR Decline is calculated i.e. = ΔMDRD 0-year/R.survival 0-year

At the end of the study, i.e. the achievement of the composite endpoint (renal replacement/death), there are calculated:

- ΔMDRD 0-FIN, Survival 0-final, Decline 0-FIN

During the 4 years of study it is quantified:

- if the patient underwent hemodialysis (HD), peritoneal dialysis (DPCA) or RT.
- if there was a cardiovascular (Exit CV) or non-cardiovascular (Exit non-CV) cause of death.

At telephone follow-up after 8 years from the baseline it is recorded if:

- the patient is / is not on dialysis
- there was a cardiovascular or other cause of death

The cases that were lost from the record during the study are specified (the lack of the possibility to monitor the parameters required ending of the study for these patients).

Evaluation of nutritional parameters:

- at baseline and quarterly based on the usual parameters such as weight in kg and BMI in kg/m^2, but also biological parameters such as albuminemia and proteinemia (g/dl).
- at baseline, then annually based on special anthropometric parameters arm circumference (CB), in cm and the clenching force of the fist (FSP) expressed in kgF.
- at baseline and half-yearly based on SGA score (global subjective assessment score).

Evaluation of inflammation parameters:

- at baseline, then quarterly: C-reactive protein (mg/l), fibrinogen (mg/dl), ferritin (ng/ml)
- at baseline and half-yearly - the number of leukocytes (elements/mmc)

Evaluation of other investigated parameters of renal function:

- at baseline and quarterly: diuresis (ml/day), systolic blood pressure (mmHg), glomerular filtration rate (ml/min/1.73sqm), calculated by the formula MDRD4 - to estimate glomerular filtration rate (RFGe), urinary albumin/creatinine ratio (RACu or RAC) in mg/g creatinine, serum bicarbonate (mmol/l), sodium (Na) and potassium (K) in mmol/l, calcium

(Ca) and phosphorus (P) in mg/dl, iPTH in pg/ml, serum hemoglobin (g/dl) and hematocrit (%) respectively.

- at baseline and half-yearly: alkaline phosphatase in UI / l.

Evaluation of metabolic parameters:

- at baseline and quarterly: a jeun glycaemia (md/dl), glycosylated hemoglobin % (HbA1c), total cholesterol (mg/dl)
- at baseline and half-yearly: total insulin dose/day (IU/day), HDL-cholesterol, triglycerides and uric acid, in mg/ dl.

Methods of statistical analysis

Regarding the descriptive statistics, the continuous variables were expressed as mean ± standard deviation (SD), and the categorial ones as a percentage. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. The general linear model for repeated measurements ANOVA was used for dynamic testing of the differences of the means of the parameters to be analyzed. Parametric tests (ANOVA, Independent Samples t-Test) and nonparametric tests for independent variables (Kruskal Wallis, Mann-Whitney U), were applied to test the differences between groups (compliant/non-compliant). Nonparametric tests for repeated measurements were applied to test the differences between parameter values at 12, 24, 36 and 48 months compared to their values at the time of study inclusion. The Cochran test was applied for intragroup testing, in dynamics of statistical differences for category variables. The X^2 test was applied to test the statistical differences between groups (compliant/non-compliant) for non-category variables. Data were analyzed using SPSS 26.0 for Windows, and $p \leq 0.05$ was considered statistically significant. Other particularities of the statistical processing will be detailed in each subchapter of the objectives of interest in the study.

6.REZULTS AND CONCLUSIONS

6.1. Compliance with the ketodiet and the formulation of compliance "models"

6.1.1. Compliance assessment and stratification of patients according to compliance

The evolution of the whole group of patients, of adherence to the prescribed dietary intake and of compliance with the number of prescribed ketoanalogue tablets (Fig.6.1): whether adherence to the number of tablets was obtained faster (over 90% of patients after 12 months) and in a maximum proportion of 100% after 36 months, the same did not happen with adherence to the prescribed diet, which reaches only 80% after 36 months,

and at the end of the 4 years of evaluation at only 90.9%. The weakest compliance on all criteria was at 6 months, which means that this moment from the initiation of the ketodiet is essential to be logistically supported, in the sense of providing nutritional and psychological counseling to patients in order to obtain long-term benefits.

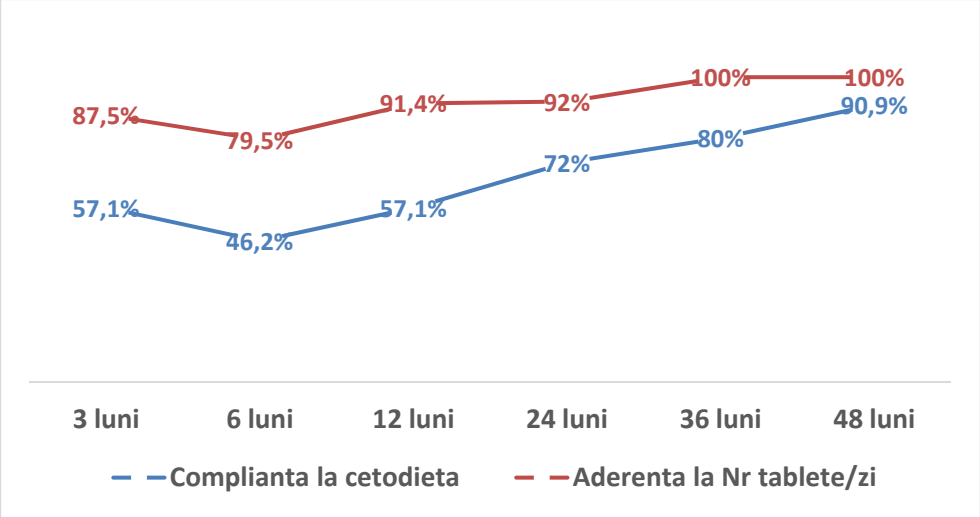

Fig. 6.1.Procentage % of compliant patients on ketodiet (blue) and adherence to precribed number of tablets/day (red) throughout monitoring (months timeline).

In the whole group of recruited patients, we notice that the protein intake determined by the food questionnaire was maximum in the first 6 months of the study, later it was significantly reduced in the first year (48.0 ± 19.9 g/day vs. 48.5 ± 20.3 g/day, p <0.05); the same decreasing trend was maintained annually, up to 36 months of study (at 2 years 40.8 ± 11.6 g/day vs. 48.0 ± 19.9 g/day, p <0.05 and at 3 years respectively 38.2 ± 12.8 g/day vs. 40.8 ± 11.6 g/day, p <0.05). The same happened with the protein intake resulting from the PNA formula, maximum in the first 6 months and significantly reduced in the first year (49.4 ± 19.4 g/day vs. 50.4 ± 20.3 g/day, p <0 .05), then significantly lower in the 2nd year compared to the first (43.8 ± 14.1 g/day vs. 49.5 ± 19.4 g/day, p <0.05), not in the third year.

If we refer to the food caloric intake, the food questionnaire highlights a single area of significant caloric reduction, from year 2 to year 3 of the study (2252.9 ± 475.1 kcal/day vs. 2319.9 ± 409.5 kcal/day, p <0.05), preceded and followed by the absence of significant oscillations of the average calories ingested.

The first statistically significant differences between compliant and non-compliant patients in terms of dieting appeared after 6 months. At 6 months the protein food intake is statistically significantly lower in compliant patients, it can be seen both in the food

questionnaire (compliant 35 ± 5.6 g / day vs. non-compliant 60.1 ± 21.3 g/day, p <0, 05), and by determining the PNA (compliant 36.6 ± 4.8 g/day vs. non-compliant 62.2 ± 62.2 g/day, p <0.05). At 12 months, there are significant differences in food intake, both in terms of protein - detected by the food questionnaire (compliant 35.9 ± 5.7 g/day vs. non-compliant 60.8 ± 21.7 g/day, p < 0.05) and by determining the PNA (compliant 37.6 ± 4.5 g/day vs. non-compliant 61.8 ± 21.5 g/day, p <0.05) - as well as from the caloric point of view (compliant 2440.4 ± 366.6 kcal/day vs. noncompliant 2120.1 ± 459.3 kcal/day, with p <0.05). These statistically significant differences were maintained at 24 months.

After 36 months, the differences begin to fade, so that only after determining the PNA, it can be noticed a significant difference in protein intake between the two categories of patients (compliant 35.9 ± 6.1 g/day vs. non-compliant 55.1 ± 8.5 g/day, with p <0.05).

However, after resorting to the final stratification, at the end of the entire follow-up period, only 45% of the participants were compliant (18 out of 40), taking into account at the same time all the compliance criteria (the two criteria simultaneously observed throughout during monitoring: C = compliance with diet and simultaneously NT = compliance with the number of ceoanalog tablets). Finally dividing patients into compliant and non-compliant, in the time interval between study entry and final (a renal replacement therapy/exitus/ study exit), it is noted that there are no statistically significant differences except for the average protein intake achieved, as shown in the table 6.1.

There were statistically significant differences between compliant patients and noncompliant patients throughout the study only in terms of average protein intake, so the intake assessed on the basis of the food questionnaire indicates in compliant 35.4 ± 5.1 g/day vs. non-compliant 60.0 ± 19.1 g/day, with p <0.05, and based on the PNA determination, an average value of protein intake of 36.9 ± 4.6 g/day vs. non-compliant at which 61.7 ± 18.9 g/day is obtained, with p <0.05.

Table.6.1.Characteristic parameters of compliance with the ketodiet

Characteristic parameters of compliance to ketodiet	Total		General compliance (C+NT)			
			Yes		No	
	Average	DS	Average	DS	Average	DS
Average protein intake/questionnaire (g/day)	48,7	18,9	35,4#	5,1	60,0	19,1
Average protein intake/PNA (g/day)	50,2	18,8	36,9#	4,6	61,7	18,9
Average prescribed protein intake (g/day)	30,1	3,6	30,7	3,9	29,6	3,3
Average caloric intake/questionnaire (Kcal/day)	2271,6	438,8	2422,3	375,0	2142,3	456,4
Average prescribed caloric intake (Kcal/day)	2344,7	335,6	2389,2	378,3	2308,2	300,4
# p<0,05 for compliants vs. non-compliants						

Graphically representing those specified in the table above, we can see in figure 6.2, the difference between the prescribed amount of protein and the achieved one (objectified by the protein questionnaire or PNA formula), being obviously significantly higher in the case of noncompliant patients compared to compliant ones. The same is not the case for caloric intake (Figure 6.3), both categories of patients manage to comply with the overall dietary caloric intake, in similar proportions.

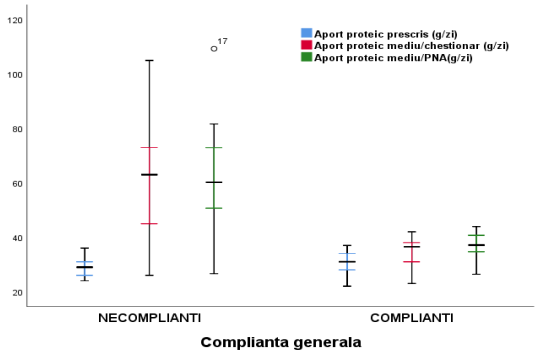

Figure 6.2.Characteristics of average protein intake (g/day) in compliants and non-compliants (compared to prescription- in blue)

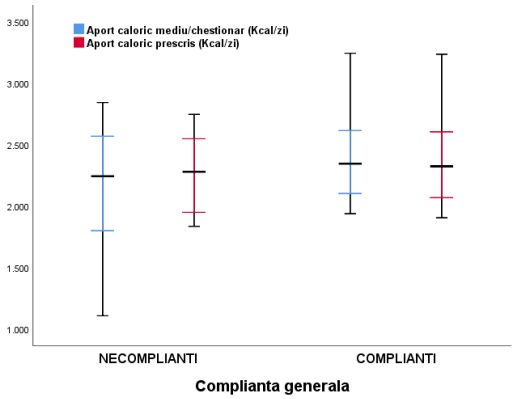

Figure 6.3.Characteristics of average caloric intake (kcal/day) in compliants and non-compliants (compared to prescription- in red)

6.1.2. Possibility to estimate compliance “models”

6.1.2.1. Analysis of patients' characteristics at the beginning of the study - compiling a "model" for the compliant patient.

At the time of inclusion in the study (baseline), patients were analyzed both in terms of demographic, family, socio-professional, functional characteristics and in terms of associated comorbidities. At the end of the study, after allocating patients in the two

categories - compliant and non-compliant - the characteristics above were compared: statistical relevance with p <0.05 (*) had higher education and the existence of another person in care, as significant characteristics for the group of compliant patients. At the limit of statistical significance with p = 0.054 were obesity and congestive heart failure, with a slight preponderance in the group of non-compliant patients, a clinically sustainable finding, as obese patients generally have a low adherence to any type of diet, all the more so to ketodiet, and patients with heart failure generally achieve inadequate caloric intake. In order to have relevant statistical power, a larger number of recruited patients is needed, which could give relevance to these findings and allow extrapolation to the entire population of patients with CDKD.

6.1.2.2.Bagplot charts for formulating the profile of the compliants according to food intake.

Because the analysis of compliance with the ketodiet clearly shows that the main challenge is not to observe and maintain the number of daily ketoanalogue tablets, but mainly to achieve and maintain a correct daily intake of protein and calories, I will focus on these last potentially predictive elements for a positive outcome of the patients. The bagplot graph represents bivaried extensions (two-dimensional box & whiskers), analogous to the one-dimensional box plot (univariate box plot) and allows a quick visualization of location, spread, skewness and outliers data. It represents a method of exploratory data analysis.

The variables discussed are the average amounts of protein (g / day) from the estimates of the food questionnaire and respectively from the PNA determination, as well as the average amount of kcal (kcal / day), resulting from the food questionnaire. Any line drawn through the depth median (Tukey's highest depth point) divides the plane into two halves where an equal number of points are found. The equation of the line is "aX + bY + c = 0", where X = kCal average/questionnaire, Y = Pr.average/questionnaire, and a, b, c are parameters established by discriminant analysis.

The dark blue zone contains the points that are in the interquartile range (range between the first and third quartile; its length is denoted by IQR). IQR = value of quartile 3- value of quartile 1, i.e. the length of the interval in which 50% of the ordered values of the variable are found.

The light blue area represents the points that are in the range LL = first quartile - 1.5 x IQR and UL = third quartile + 1.5 x IQR; the rest of the points are considered possibly aberrant.

The size of the bag shows the skewness of the densities that generated the data.

As it can be seen in Figure 6.4, the line drawn through the midpoint suggests that the two cohorts, compliants and non-compliants, can be separated with a small error by this line.

This means that a modeling of the profile of the two cohort members can be successfully continued, using only the variables kCal average/questionnaire and Pr.average/questionnaire, thus: the values of the two variables of the individual are replaced in the equation of the line, and if the result is a positive number, then the individual is non-compliant (represented in red), and if the result is a negative number, then the individual is compliant (represented in green). The orientation of the bag indicates a positive correlation, if the bag is oriented upwards; in our case both the representation of the Pr.average questionnaire vs. kCal average/questionnaire, as well as Pr. average/ PNA vs. kCal average/questionnaire have a positive orientation, with spread, skewness and similar aberrant values, so that they are comparable as a way to predict the (non) compliance of patients by entering the values mentioned at some point during the evolution of patients.

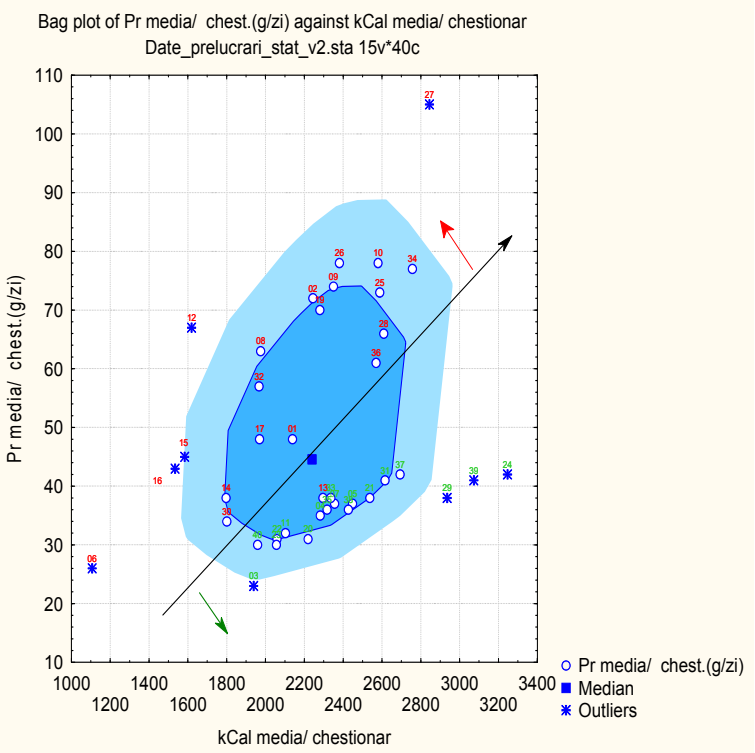

Figure 6.4.Bagplot representation of (non) compliants depending on Pr average/questionnaire(g/day) and kCal average/questionnaire(kcal/day)

Obviously we obtain a similar graph if we correlate Pr. average/ PNA = Y with kCal.average/ questionnaire =X, thus we obtain the following bagplot from figure 6.5.

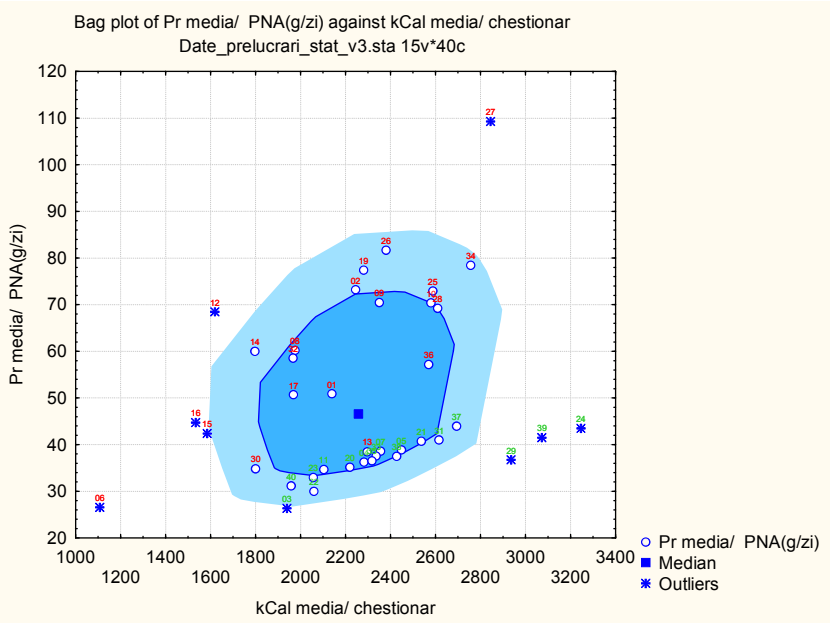

Figura 6.5. Bagplot representation of (non) compliants depending on Pr average/PNA(g/day) and kCal average/questionnaire (kcal/day)

The determination of the average protein intake by means of the food questionnaire is equivalent to that performed by its determination in PNA, fact demonstrated by the linear aspect of the bag obtained if X = Pr. average/questionnaire and Y = Pr. average/PNA (Figure 6.6), which means that the two variables are redundant.

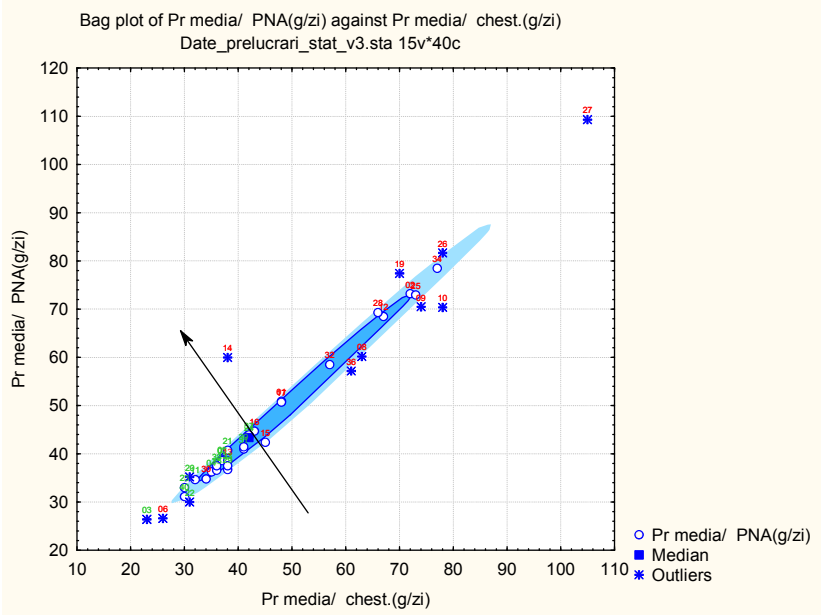

Figura.6.6. Bagplot representation of (non) compliants depending on Pr average/PNA (g/day) and Pr average/questionnaire (g/day)

6.2.Renal survival until entering the renal replacement programm (HD, DPCA or RT) and the (non) cardiovascular mortality.

During the 4-year follow-up period, out of the 40 patients included in the study, 27 patients (73.0%) entered the renal replacement program (hemodialysis in 11 patients, peritoneal dialysis in 9 patients or kidney transplantation in one patient), and 9 patients died of cardiovascular disease (of which 3 patients died after initiating dialysis).

In patients complying with the ketodiet, in the 4 years of clinical-biological follow-up, there was a significantly lower percentage of deaths or inclusion in the renal replacement program (40.0%) compared to non-compliant patients (95.5%). The same significant differences were maintained at the last telephone visit 8 years after inclusion in the study (50.0% of deaths and renal replacement therapy in compliant patients vs. 85.7% of deaths and renal replacement therapy in non-compliant patients; $p < 0.05$). Compliant patients had a significantly lower death rate compared to non-compliant patients, both during the 4 years of study (6.7% vs 36.4%, $p < 0.05$) and at 8 years after inclusion in the study (17.6% vs 78.6%, $p < 0.05$).

During the first 4 years, the total mortality of non-compliants (100%, i.e. 8 out of 8 deaths) was represented by cardiovascular causes, of which 2 hemodialysis patients and 6 patients without renal replacement, as in the case of compliants, the only registered death was also of cardiovascular cause. There was a equal number of hospitalizations for major cardiovascular events in both compliant and non-compliant patients (2 cases each group).

The last visit made by telephone at 8 years highlights in the group of non-compliant patients 7 out of 11 (63.63%) deaths from cardiovascular causes, all patients being on dialysis (5 in hemodialysis and 2 in peritoneal dialysis); in the group of compliant patients, however, out of the 2 new deaths, 1 death (50%) had a cardiovascular cause, in a patient on hemodialysis.

In order to demonstrate the advantages of compliant patients over non-compliant ones on the main objectives of renal survival and mortality, in order to generalize the observations made on the monitored cohort to the entire population, we chose the following methods of mathematical statistics:

- *Survival analysis* to highlight the importance of ketodiet in prolonging the *duration of renal survival until entering the renal replacement program/death*;
- *Dispersion analysis* to highlight the importance of ketodiet in decreasing the *rate of decline of glomerular filtrate*.

The statistical processing below was done with the *StatSoft, Inc. software package (2007). Statistics (data analysis software system), version 8.0.* www.statsoft.com

Survival analysis

Exploratory testing and hypothesis testing — survival analysis techniques — include descriptive methods that estimate the distribution of survival times in a group, methods of comparing survival in two or more groups, and techniques for adapting linear or nonlinear regression models to survival data. The defining features of survival analysis usually cover mortality table, survival distribution, estimation of Kaplan-Meier survival function and additional techniques for comparing survival in two or more groups. "Survival" means in this context the duration of renal survival until entry into the renal replacement program or until death of any cause; this duration, measured in months, is specified by the variable " *Surviv 0-fin*" in the input data. In this study *Surviv 0-fin* represents selection values on the *T* variable. The censorship to the right is specified by the binary variable "*CENSOR*" from the input data and is coded with "*NO*" if the patient has finished the observation period without entering the renal replacement program or has not died and with "*YES*" otherwise. In order to generalize the observations made on the monitored cohort to the entire population, the patients of the cohort were monitored in terms of compliance with the recommended ketodiet. The monitoring was coded by the binary variable "*Compliant*" from the input data; the variable is coded with "*YES*" if the patient followed the recommended protocol and with "*NO*" otherwise. For a more in-depth analysis of the link between dietary compliance and the number of recommended/prescribed tablets, the discrete variable "*Comp + NT*" was introduced with the following values: 0 if the patient did not follow the diet and the number of tablets; 1 if the patient didn't follow the diet, but did take the prescribed number of tablets; 3 if the patient followed the diet and took the prescribed number of tablets. The variable *Compliant*, respectively the variable *Comp + NT* divide the cohort into two, respectively three, subgroups allowing the comparative study of the survival functions of the two/three subgroups.

Non-parametric estimation of survival function by the Kaplan-Meyer method

The Kaplan-Meyer method is the non-parametric reference method for estimating the survival function; it is a non-parametric method because the survival function is estimated without any hypothesis on the instantaneous mortality rate, the hazard ratio.

Figure 6.7. represents the graph of the survival function, for the entire cohort, estimated with the Kaplan-Meyer method. It is observed that, for this cohort, the probability of survival at half of the study, i.e. at 28 months, is 60%.

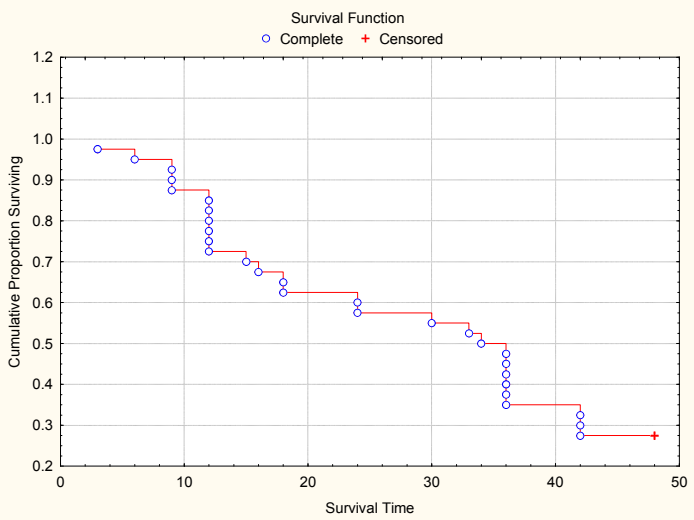

Figure 6.7.The survival function for the entire cohort studied (survival time- months)

The survival function for the two groups given by the variable *Compliant* is presented in Figure 6.8. In the case of dietary compliance, it is observed that the probability of survival at 48 months (the end of the study) is 55%, in the non-compliant group, this decreases to under 10%. The non-compliant group has a survival of 60% only at 12 months, i.e. in the first quarter of the study period.

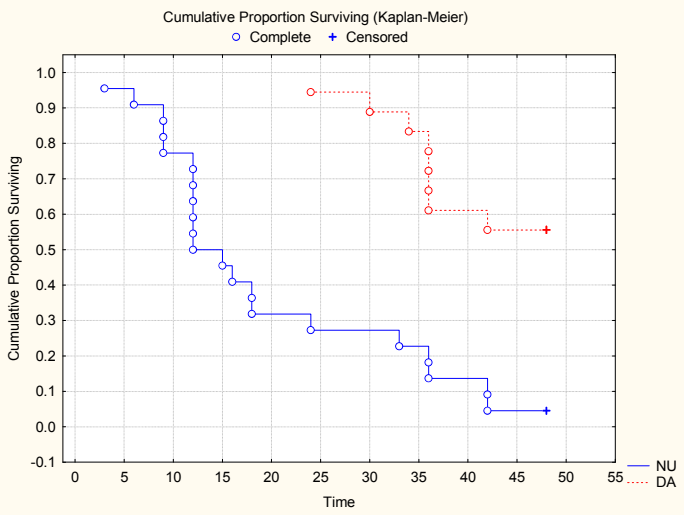

Figure 6.8.The survival functions for the 2 groups of the cohort: NO(blue)= non-compliants; YES(red)= compliants

The survival function (months of living) for the three groups given by ***Comp + NT*** is shown in Figure 6.9. The figure shows that the probability of 55% survival at the end of the study is reached only by those who followed the diet and medication, whilst the patients who followed only the number of ketoanalogue tablets have a probability of survival at the end of the study of 10%, whilst those who did not follow neither diet nor the medication have 0 probability of survival, in fact this probability is reached at 35 months, i.e. after approx. 2/3 of study time.

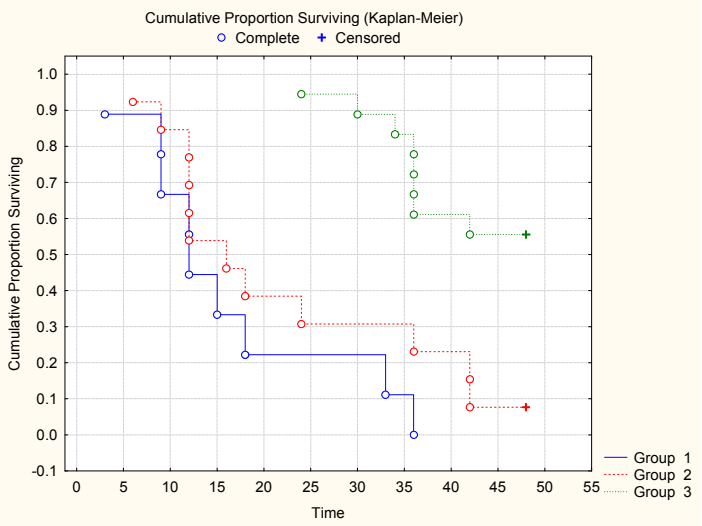

Figure 6.9.The survival studies for the 3 subgroups in the cohort:
Group 1((blue)= completely non-compliants; Group 2((red))= partially compliants; Group 3 (green)= totally compliants

Groups 1 and 2 have a comparable survival function, with no significant differences, up to 33 months, shortly after which the entire contingent of the non-compliant group is quickly depleted. The compliant group 3 has of course the best survival function, significantly different from both groups mentioned, up to 42 months, after which the contingent of compliant patients evolves apparently parallel to group 2, that of patients who follow only the correct amount of ketoanalogue tablets, but not the recommended food intake (Figure 6.9)

The graphs in Figures 6.10. and 6.11 show the proportion surviving of the subgroups shown in the graphs 6.8. and 6.9. The novelty that stands out, compared to the graphs above, is that they show the stability of the number of survivors (almost all) in the group of compliant patients. A suggestion for further research would be to find out the

cause of the decrease in the proportion of compliant patients to 60% in 35 months from the start of the study, at approx. 2/3 of study time.

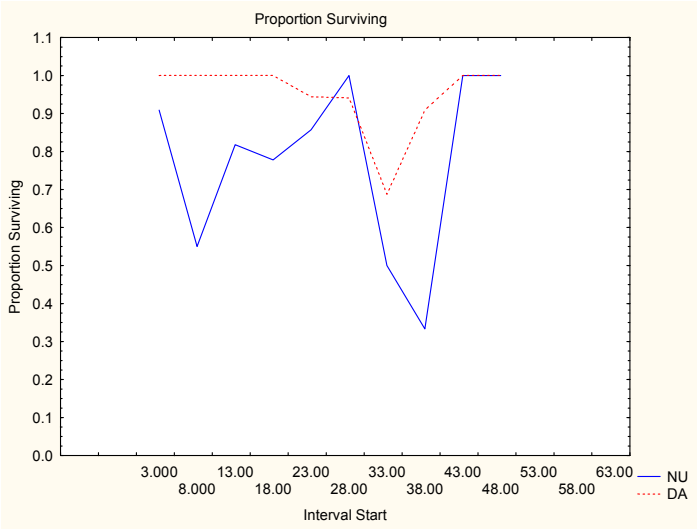

Fig.6.10. Proportion surviving for the 2 subgroups of the cohort: NO(blue)= non-compliants; YES(red)=compliants

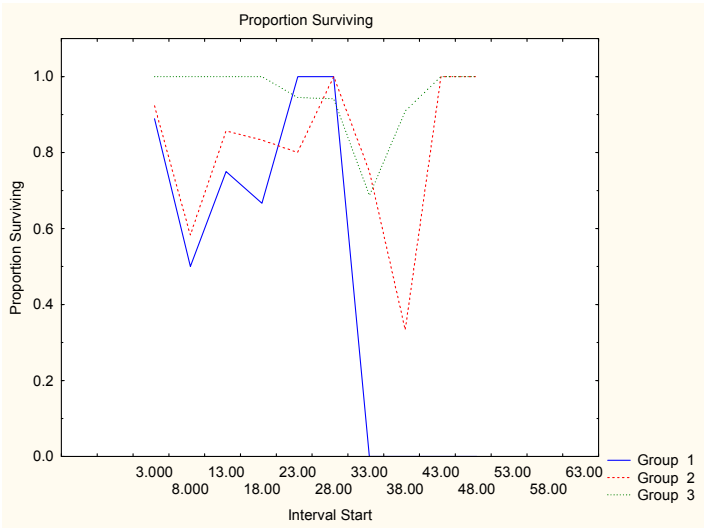

Figure 6.11.Proportion surviving for the 3 subgroups of the cohort:

Group 1= Compliance with diet=0 (no) and compliance with tablet number NT=0 (no);

Group 2= Compliance with diet=0 (no) and compliance with tablet number NT=1(yes);

Group 3= Compliance with diet=1(yes) and NT=1(yes);

The descriptive statistics summarized in table 6.2. numerically supports the observations in the graphs. It is obvious that patients with complete compliance (median 48 months, with a mean of 41.88 ± 7.8 months) have a better survival than non-compliant patients

(median of 12 months, with a mean of 16.33 ± 14.58 months, p <0.05), even if the number of censored cases is higher in the group of compliant patients (10 out of 18) versus non-compliant patients (0 out of 9). The situation of partially compliant patients (do not follow the diet, but only the number of prescribed ketoanalogue tablets), with a number of 1 censored case out of 13, describes a similar evolution of the survival function with that of non-compliant patients (median 16 months, with a mean of 22.23 ± 14.58 months).

Table 6.2

Descriptive statistics for eachgroup (Date_prelucrari_stat_v2.sta)

	Median	Mean	Std.Dv.	No.uncsd	N.censrd	Total N
0	12.00000	16.33333	11.13553	9	0	9
1	16.00000	22.23077	14.58397	12	1	13
3	48.00000	41.88889	7.80565	8	10	18
Total	35.00000	29.75000	15.67907	29	11	40

As can be seen from Figure 6.11., the survival rate decreases immediately after 3 months for groups 1 and 2 and is minimal in the range of 30-40 months for all three groups, with the exhaustion of the entire contingent of non-compliant patients (group 1) at 35 months. After 45 months, the proportion surviving for the two groups of compliant patients group 3 and partially compliant patients group 2 are perfectly superimposable. These data suggest the particular importance of a correct intake of ketoanalogues on the cumulative survival and proportion surviving of patients, even if dietary requirements in the severe hipoprotein domain are not strictly observed. The main element is the correct administration of ketoanalogues, a fact supported by the national study, conducted by Chen in 2021 [30].

Comparison of survival functions

Figure 6.8. shows the graphs of survival functions obtained, based on observations on the cohort investigated in this study. The natural question is: *is the visible difference between the charts a random one or a significant one, in the sense that it is specific to the entire population with chronic diabetic kidney disease*?

The answer to this question is obtained, among other things, with the *logrank test*- a non-parametric test, because survival times are not Gaussian distributed. The logrank test is based on ordering the ranks of survival times (rankordering); it compares the rank (order) of death times in the two groups. The null hypothesis H_0 is that the rank of death times are

randomly distributed between the two groups as they occur. The differences between the observed deaths and those expected in the H_0 hypothesis are then calculated until the last death. The sum of the differences is divided by the standard deviation of this amount: this is the logrank test statistic. The result for the graph in Figure 6.8 is given by the value $p \ll 0.05$ obtained, more precisely $p = 0.00003$, which shows that the null hypothesis is rejected with a probability of approx. equal to 1, i.e. the two curves differ significantly.
So, the compliance with the ketodiet procedure + tablets (ketodiet) is significantly effective for patients with CDKD.

6.3. The decline in glomerular filtrate rate (expressed in ml/min/1.73m^2/year)

The multivariate analysis of the decline in glomerular filtration rate after 1 year and until the end of the 4-year study reveals a series of particularly interesting results.

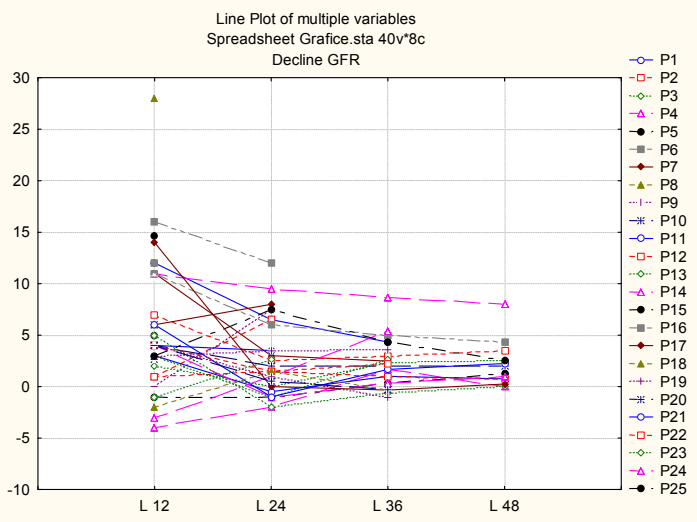

Figure 6.12.The GFR decline (ml/min/1,73m^2/year)- on abscissa, left side - for the patients (P1-P25) who survived ≥ 24 months (L24)

Figure 6.2 shows that of the 25 patients (P_1-P_{25}) who survived without renal replacement for at least 24 months (L24), 7 were non-compliant patients (28%) and 18 were compliant patients (72%), and after 36 months the decline of the GFR stabilizes between 5 and 0 ml/min/1.73m^2/ year for the most represented patients, these being 78.5% from the group of compliant patients (11 out of 14) and 21.5% of the non-compliant group (3 out of 14).

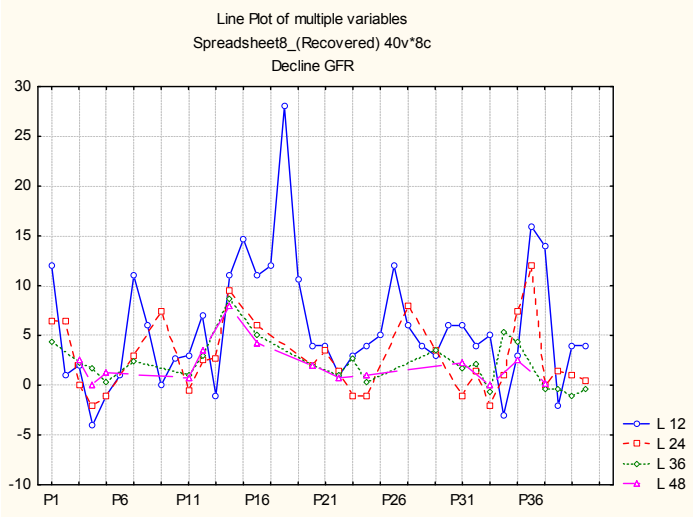

Figure 6.13.Means of the GFR decline (ml/min/1,73m^2/year) in the whole cohort of patients, at 12 months (L12), 24 months (L24), 36 months (L36) and 48 months (L48)

Figure 6.13 shows the largest decline in GFR in the first year of study for most patients studied in the whole group. As the years go by, the decline in GFR decreases, so that at the end of the 4th year the decline in GFR is for all alive participating patients, between 0 and 10 ml/min/1.73m^2/ year. Relevant is the fact that after the 3rd year, the patients remained alive without renal replacement are mostly compliant patients (as revealed by the survival analysis).

Bi-factorial ANOVA analysis

The purpose of analysis of variance (*ANOVA*) is to test for significant differences between averages by analyzing variances. Specifically, by fragmenting the total variation on different sources (associated with a different effect in design), we will be able to compare the variance due to inter-group variability with that due to intra-group variability. Under the null hypothesis (that there are no differences between the averages of the groups in the population), the variance estimated based on intra-group variability should be about the same as the variance estimated from inter-group variability. The decline of the RFG (ml/min/1.73m^2/ year) is compared in the two compliant vs. non-compliant groups during the monitoring months - figure 6.14.

The effect of the "Compliant" factor is significant, whilst the effect of the "Month" factor and the interaction of the two factors is insignificant. This also explains why, in the subsequent analysis, the selection averages for "Compliant/non-Compliant" will be significantly different as opposed to the averages on "Months" which will not be different.

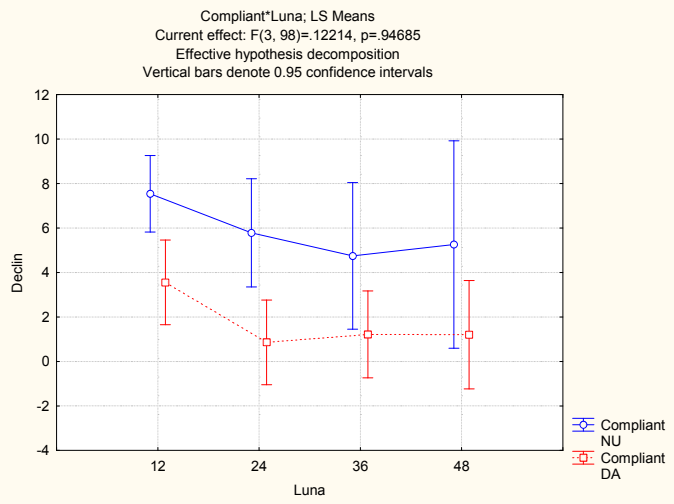

Figure 6.14.Decline of GFR (mean values in ml/min/year), on abscissa, depending on: compliance (YES/NO) and evaluation moment (months from baseline), on ordered

Indeed, following in Figure 6.14. and Table 6.3, we note a statistically significant difference (p = 0.00004) in the mean rate of decline of the RFG in the group of compliant patients (1.77 ± 0.35 ml / min/1.73m^2/year) versus non-compliant (6, 52 ± 0.84 ml/min/1.73m^2/year).

Table 6.3.Decline of GFR depending on compliance

Compliant; Weighted Means (Rata declin v3.stw) Current effect: F(1, 98)=18.333, p=0.00004 Effective hypothesis decomposition

	Compliant	Decline GFR Mean	Decline GFR Std.Err.	Decline GFR -95.00%	Decline GFR +95.00%	N
1	NO	6.521905	0.847280	4.810787	8.233022	42
2	YES	1.772969	0.359014	1.055536	2.490401	64

Table 6.4. Decline of RFG depending on months of survival

Months; Weighted Means (Rata declin v3.stw) Current effect: F(3, 98)=2.5803, p=0.05786 Effective hypothesis decomposition

	Months	Decline GFR Mean	Decline GFR Std.Err.	Decline GFR -95.00%	Decline GFR +95.00%	N
1	12	5.749500	0.971833	3.783782	7.715218	40
2	24	2.729655	0.690582	1.315061	4.144249	29
3	36	2.140000	0.485726	1.132665	3.147335	23
4	48	2.073571	0.572661	0.836413	3.310730	14

In contrast, Table 6.4 does not show any statistically significant difference in the decline of the RFG from one year to another, except from year 1 to 2 years (5.74 ± 0.97 ml/min/ 1.73m^2/year versus 2.72 ± 0.69 ml/min/ 1.73m^2/year, p = 0.057).

An essential hypothesis in the analysis of variance (ANOVA and the t test for mean differences) is that the variances within the different groups are similar (homogeneous). In any case, it is important to realize that the assumption of homogeneity of variants is usually not crucial for ANOVA like other hypotheses, especially in the case of balanced (n equal) designs and the fact that these tests are not so robust in themselves.

6.3.1. Statistical tests for equality of filtration decay averages on independent samples given by the variable Compliant

If we analyze the averages of the decline of the glomerular filtration rate at different moments of evolution, after 1 year, 2 years, 3 and 4 years respectively between the two cohorts, compliant and non-compliant, we observe a statistically significant difference (p <0.05) in all these moments, but the strength of the test differs depending on the inhomogeneity of the samples.

The Levene test (homogeneity of dispersions). If the Levene test is statistically significant, then the hypothesis of homogeneous dispersions is rejected.

F-ratio test (F-ratio - Variances). The dispersion due to variability between groups/ dispersion due to variability within the group. The statistic will be a higher value if the intergroup variability is large compared to the intra-group variability, which is unlikely to happen if the averages of the populations in the groups all have the same value.

An average GFR0-1year decline of 3.55 ± 4.14 ml/min/1.73m^2/ year in compliant vs. 7.54 ± 6.98 ml/min/1.73m^2 for non-compliant, p=0.039 has been recorded. The mediocre power of the test =0.6847 is due to the very inhomogeneous samples (p– Variances = 0.033 and p– Levene = 0.028). Similarly, comparing the averages of the decline GFR0-2 years, in compliant with 0.861 ± 2.4 ml/min/1.73m^2/year vs. non-compliant with 5,787 ± 3.5 ml/min/ 1.73m^2, we obtain statistically significant p= 0.0001 and an excellent test power = 0.985 (p-Variances = 0.163 and p-Levene = 0.134). The averages of the RFG decline at 3 years are 1,218 ± 1.55 ml/min/ 1.73m^2/year for compliant vs. 4.75 ± 2.25ml/min/ 1.73m^2/year, with a p = 0.00035, statistically significant and an excellent test power = 0.9432, as in the previous situation at 2 years (p-Variances = 0.231 and p-Levene = 0.545). The analysis at 4 years of the average GFR0-4 decline shows in compliants 1.204 ± 0.967 ml/min/1.73m^2/year vs. non-compliant 5.26 ± 2.404 ml/min/ 1.73m^2/year, with p = 0.0005 and a very good test power = 0.9675, even if the samples are

inhomogeneous (p-Variance = 0.0357 and p- Levene = 0.0151), a situation due to the small selection volumes (3/11). The latter makes the statistical significance questionable. Likewise, for the analysis of the glomerular filtration rate decline RFG0-Fin over the baseline - primary endpoint interval, statistically significant differences are displayed between the RFG decline averages for compliants and non-compliants. Thus, the compliant ones have an average of the decline of 1.32 ± 1.4 ml/min/1.73m^2/year, compared to the non-compliant ones with 7.55 ± 5.87 ml/min/1.73m^2/year and p = 0, 000087, but having very inhomogeneous samples (p-Variances = 0.0000 respectively p-Levene = 0.00606), so debatable as test power.

6.3.2. Statistical tests for equality of filtration decline averages on independent samples given by the variable "renal replacement".

Statistical tests performed for the equality of the mean of the RFG decline on the independent samples given by renal replacement showed that between the decline of the glomerular filtration rate RFG0-Fin of those who reached the final goal without renal replacement (Renal repl.= NO), by 2.2 ± 3.61 ml/min/1.73m^2/year and those with renal replacement (Renal repl.= YES) with 7.06 ± 5.79 ml/min/1.73m^2/year, there is a statistically significant difference, p = 0, 00326. The same can be observed at 2 years (1.3 ± 2.84 ml/min/1.73m^2/year vs. 4.6 ± 4.05 ml/min / 1.73m^2/year, p = 0.015), at 3 years (1.0 ± 1.55 ml/min/1.73m^2/year vs. 4.1 ± 2.26 ml/min/1.73m^2, p = 0.0008), respectively at 4 years (1,2 ± 1.19 ml/min/1.73m^2 vs. 4.1 ± 2.77 ml/min/1.73m^2/ year). There is no statistically significant difference at 1 year of follow-up, suggesting that overall long-term benefits are felt, in terms of delaying renal replacement, after at least 1 year of diet.

In conclusion, we are witnessing a significant impact of the ketodiet on the delay of the initiation of renal replacement, from one year to another, only in the situation of compliant patients and after the first year of therapy. The effect is sustained at comparable rates thereafter, throughout the monitoring period of up to 4 years.

6.4. Prediction model for the glomerular filtration rate decline curves. The comparative study of the decrease slopes for non-compliant and compliant patients

The general polynomial regression model of the "Glomerular filtration rate decline" curves that best approximates the evolution of the glomerular filtration rate decline is represented by the equation D (t) = a0 + a1 * t + a2 * t ^ 2. The set for estimating the coefficients a0-a2 is of the form {t, m (t)}, with t = 12, 24, 36, 48 months and m (t) is the average rate of decline at time t. The equation of the tangent to D (t) is D '(t) = a1 + 2 * a2 * t and the

angle of ascent / descent of the decline curve in the last two moments of time is arctan [D' (36)], arctan [D ' (48)] with D '(t) considered trigonometric degrees.

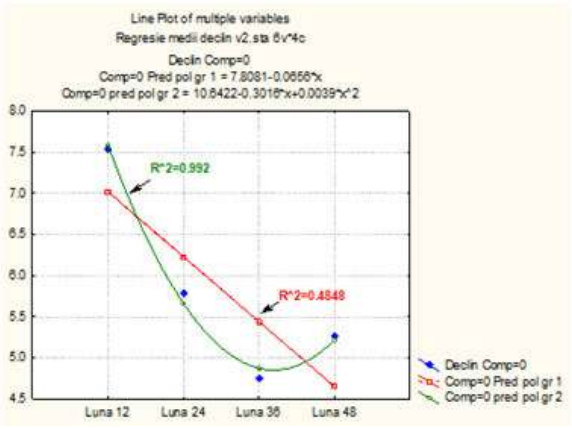

Fig.6.15.RFG decline/decline slope- non-compliants

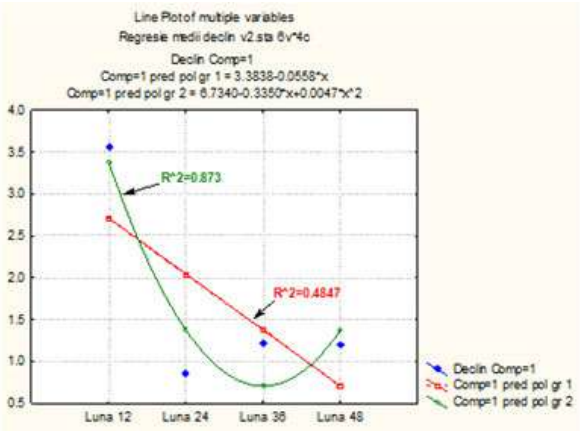

Fig.6.16.RFG declie/decline slope- compliants

This way the coefficients are for:

Group 1 non-compliants	Group 2 compliants
a0= 10.64	6.73
a1= - 0.301	- 0.335
a2= 0.004	0.005
D'(36)= - 0.013	0.03
arctan = **-8 (172)**	**-2 hexadecimal grades**
D'(48)= 0.083	0.15
arctan = **5**	**9 hexadecimal grades**

In this way, at any time t we can predict the slope of the rate of decline of glomerular filtration, distinct for compliant and non-compliant patients. Thus for t = 36 months and t = 48 months, respectively, the slope is different for non-compliant (-8 and 5 hexadecimal grades) versus compliant patients (-2 and 9 hexadecimal grades).

6.5. Nutritional evolution of patients

During the study, no deterioration of the nutritional status was found for the ***entire analyzed group***. In the group of compliant patients, there were statistically significant improvements in nutritional status compared to baseline: at 2 years regarding arm circumference (33.2 ± 5.8 cm vs. 31.3 ± 4.3 cm, p <0, 05) and respectively at 4 years if we evaluate BMI (30.0 ± 5.5 kg/m2 vs.28.5 ± 3.9 kg/ m2, p <0.05). In the group of noncompliant patients there is a significant increase at 6 months (4.4 ± 2.6 points) vs. baseline (3.8 ± 2.2 points) of the SGA score (p <0.05), which could reflect the difficulties of compliance and adherence to their diet.

If we compare the compliants with the non-compliants, we note how the compliants have statistically significantly higher values ($p < 0.05$) of albuminemia at 3 months (4.4 ± 0.3 g/dl vs. 4.0 ± 0.4 g/ dl), at 1 year (4.4 ± 0.3 g/dl vs. 4.1 ± 0.3 g/dl) and at 3 years (4.3 ± 0.4 g/dl vs. 3.9 ± 0.3 g/dl), as well as proteinemia at 3 months (7.3 ± 0.5 g/dl vs. 7.0 ± 0.4 g/dl), and subsequently for the most part of the 3rd year, at 30 months (7.2 ± 0.5 g/dl vs. 6.9 ± 0.2 g/dl), at 33 months (7.2 ± 0.5 g/dl) dl vs. 6.7 ± 0.5 g/dl) and 36 months, respectively (7.1 ± 0.4 g/dl vs. 6.6 ± 0.3 g/dl). All these data lead to the confirmation of nutritional safety in the long-term use of ketodiet in patients with CDKD. The compliance with the ketodiet in the strictest sense, with the correctness of food intake and supplementation of ketoanalogues, may even improve any protein nutritional deficiencies that have occurred in CDKD, before ketodiet has started.

6.6.Evolution of inflammation

There were no statistically significant differences neither comparing compliant with non-compliant, nor at the comparison between baseline with the moments 1, 2, 3 or 4 years of all parameters, except in the group of compliant patients. In compliant patients, the fibrinogen increased significantly compared to baseline at 3 and 4 years (475.5 ± 116.8 mg/dl and 554.0 ± 128.4 mg/dl, respectively, vs. baseline 421.5 ± 84.9 mg/dl, $p < 0.05$), but not accompanied by an increase in VSH or PCR. The significance of this increase is not known.

6.7.Evolution of other renal parameters

The whole group of patients had an increase in hemoglobin and hematocrit from one visit to another in years 3 and 4, as well as a decrease in blood pressure values from one visit to another in the 2nd and 4th year, still without gaining statistical significance compared to the average values at the baseline. Diuresis and potassium did not undergo statistically significant changes throughout the study, at various times, neither compared to baseline, nor on the two cohorts - compliants and non-compliants, which confirms once again that an eminently vegetarian diet does not cause increases in potassium in patients with CDKD (normal K= 3,6-5,2 mmol/l). With a statistically significant difference from baseline ($p < 0.05$) we observe in ***compliant patients***:

- higher bicarbonate: at 1 year 26.3 ± 4.8 mmol/l and respectively 25.9 ± 3.8 mmol/l at 3 years, vs. 23.9 ± 4.4 mmol/l, mean at baseline.
- higher serum calcium, but maintained in the normal range (8,6-10,2 mg / dl): at 2 years 9.8 ± 0.5 mg/dl, respectively 9.8 mg/dl at 3 years, vs. 9.3 ± 0.5 mg/dl, mean at baseline.

- lower i-PTH: at 2 years 75.1 ± 42.1 pg/ml, at 3 years 77.3 ± 42.4 pg/ml and at 4 years 74.3 ± 27.2 pg/ml vs. mean at baseline value of 128.3 ± 83.2 pg/ml.
- higher alkaline phosphatase in the 4th year: at 42 months 95.4 ± 28.2 IU/l and at 48 months 92.7 ± 24.3 IU/l vs. at baseline mean value of 84.1 ± 30.1 IU/l (but within normal limits!), probably a pattern of increased bone metabolic activity (normal alkaline phosphatase = 35-104IU/l).

With a statistically significant difference from baseline ($p < 0.05$) we note in ***non-compliant patients***:

- higher serum calcium only at 2 years (but within normal limits): 9.6 ± 0.5 mg/dl vs. baseline 9.3 ± 0.5 mg/dl
- lower Ht% at 3 years: 32.0 ± 7.4% vs. baseline 35.9 ± 4%, a sensitive witness for the increase in blood circulating volume, not accompanied by a parallel decrease in serum hemoglobin.

If we compare the groups of compliant versus non-compliant patients, we will notice that there were statistically significant differences from baseline in urinary RAC, higher in non-compliant patients (2432.5 ± 1824.2 mg/g creatinine vs. 932.6 ± 1477, 5 mg/g creatinine at baseline, $p < 0.05$), differences that were preserved, losing statistical significance only after the 3rd year (3036.2 ± 3075.3 mg/g creatinine non-compliants vs. 512.6 ± 565.1 mg / g creatinine in compliants, $p < 0.05$), due to the reduction of urinary RAC in non-compliant patients, pattern maintained until the end of the 4 years of follow-up. The significance of this fact consists in the efficiency of a hypoprotein diet on the hyperfiltration and urinary excretion of albumin in general, but also in the reduction of RFG, greater in non-compliant than in compliant patients.

Continuing to compare compliant and non-compliant patients, we note:

- significantly lowered serum phosphorus (normal range=2,7-4,5 mg/dl): at 9 months with a mean value of 3.8 ± 0.6 mg/dl vs. 4.4 ± 0.8 mg/dl and $p < 0.05$; at 15 months with a mean of 3.7 ± 0.4 mg/dl vs. 4.3 ± 0.8 mg/dl and $p < 0.05$. The differences disappear in the 3rd year, as adherence of the whole group to ketodiet increases, as found in the compliance analysis, namely - at 36 months the adherence to the number of tablets is 100%, and compliance with food intake is 80%, so that at 48 months to reach 90.9% food compliance and also 100% adherence to ketoanalogues.

6.8.Metabolic evolution of the patients

All patients in the study group had improved HbA1c from one visit to another in the second half of the study from 21 to 45 months. All patients in the group had lower a

jeun glycaemic values from one visit to another in the first 6 months. If we compare compliant patients vs. non-compliant patients, we notice statistically significant differences:

- a jeun blood glucose at 3 months (121.3 ± 41.1 mg/dl vs. 169 ± 56 mg/dl, $p < 0.05$), at 39 months (133.9 ± 47.5 mg/dl vs.242.7 ± 69.5 mg/dl, $p < 0.05$) and at 42 months (115.8 ± 23.3 mg/dl vs. 288.3 ± 126.8 mg/dl, $p < 0.05$).
- HbA1c% lower in the compliant group: at 9 months (6.7 ± 1.0% vs. 7.5 ± 1.4%, $p < 0.05$), at 15 months (6.8 ± 1.0% % vs. 8.2 ± 1.8%, $p < 0.05$), at 18 months (6.8 ± 1.1% vs.8.3 ± 1.1%, $p < 0.05$), at 21 months (7.0 ± 1.3% vs. 8.3 ± 1.0%, $p < 0.05$), at 24 months (6.8 ± 1.1% vs. 8.4 ± 1.1 %, $p < 0.05$), at 27 months (7.0 ± 1.2% vs 8.2 ± 1.1%, $p < 0.05$), at 30 months (7.0 ± 1.1% vs.8.6 ± 1.6%, $p < 0.05$) and at 42 months (6.9 ± 1.3% vs.8.6 ± 0.1%, $p < 0.05$), so throughout the study, at most instances of analysis, glycemic control of compliant patients was better.

Regarding total cholesterol, compliant patients had lower values compared to non-compliant ones already after the first 3 months, but the statistical threshold of $p < 0.05$ was reached only at 42 months (142.3 ± 36.2 mg/dl vs 220.7 ± 24.6 mg/dl, $p < 0.05$). Only compliant patients had a statistically significant decrease of total cholesterol on long term compared to baseline: at 2 years and 4 years of treatment (136.2 ± 33, 1 mg/dl and 138.8 ± 39.3 mg / dl respectively vs baseline with 163.6 ± 53.4 mg/dl, $p < 0.05$).

Compliant patients also had a significant reduction in serum uric acid at 2 years vs.baseline (5.3 ± 1.0 mg/dl vs. 6.2 ± 1.8 mg/dl, $p < 0.05$). In contrast, nonc-ompliant patients experienced a statistically significant reduction at 2 years in HDL-cholesterol compared to baseline (44.4 ± 6.8 mg / dl vs. 47.3 ± 9.2 mg/dl, $p < 0.05$) and also at 2 years they required a higher insulin dose versus baseline (60.7 ± 59.2 IU/day vs. 46.8 ± 37.6 IU/day, $p < 0.05$), proving a more difficult metabolic control in the long run.

7. THE IMPORTANCE OF OBTAINED DATA, PERSONAL CONTRIBUTIONS AND FUTURE APPROACHES

In the light of the data obtained, it can be firmly stated that the ketodiet can be put into current practice and successfully implemented in patients with CDKD, without nutritional risks, with an improved nutritional profile compared to baseline, if strictly followed, and the careful evaluation of all demographic, social, familial and professional characteristics, comorbidities and functional status of those to whom we want to offer this

type of diet is as important as the ongoing assessment of the caloric and protein intake for the success of nutritional therapy. Further studies with larger cohorts of patients will confirm more other "successful/derogatory characteristics" of patients, along with those identified in this study (see chapter 6.1.2.1).

The weakest compliance with the ketodiet was observed at 6 months (46.2% for the correct dietary intake of calories and protein and 79.5% for adherence to ketoanalogue tablets), a timepoint representing a good predictor for the future evolution of patients (the patients compliant at 6 months remain in their most part compliant throughout the study). Considering this evidence it is justified to propose and support the mandatory providing of a educational session with the patient, at least at 6 month after stating ketodiet, toghether with a complex team (diabetologist, nefrologist, dietetician/ dietetician assistant and psychologist), necessary for formulating adequate solutions considering the individual profile. Based on data offered by the food questionnaire and the PNA calculation we can perform a prediction model to determine at any time if the target patient is a compliant one or a non-compliant. This can be easily achieved, on quarterly or monthly basis, by the nutrition assistant or by a physician trained in appling the food questionnaire- the way it was presented in chapter 6.1.2.2.

Although it is obtained and maintained 100% after 3 years in all patients, easier than the severe hypoprotein diet, the correct and sufficient administration of ketoanalogue is at least as important as the strict compliance with the diet, a fact proven by the survival analysis of the comparative survival rates, in compliant patients, partially compliants only with the supplement of ketoanalogues and non-compliant patients respectively, in chapter 6.2. Ketoanalogues added to a hipoprotein diet can make the difference between life and death in all patients studied, determining a cumulative proportion of the survival after 45 months of treatment similar to that of perfectly compliant patients, but still retains a lover survival proportion in the 30-40 month range, placed between the compliant and non-compliant groups. All these findings reinforce and support the conclusions stated in the Taiwanese national study published in 2021 by Chen et al., which shows the association of the ketoanalogue use with the lower mortality rates of any cause, lower need for permanent hemodialysis and a lower rate of cardiovascular complications [30]. Moreover the present study shows that adding ketoanalogues to a hipoprotein diet- not necessarily severe hipoprotein diet- clearly proves to be superior in renal/overall survival, when compared to a standard hipoprotein regimen, even to the severe hipoprotein diet, but without ketoanalogues (chapter 6.2).

Only compliant patients had a significantly lower rate of RFG decline than baseline throughout the study, more pronounced from the first to the second year, had lower mortality, and statistically significantly lower renal replacement required than non-compliant patients, where all deaths were caused by cardiovascular events. A GFR decline graph can be generated that could accurately predict the evolution of GFR at any time depending on the (non) compliance of the investigated patient, in the way stated in chapter 6.4.

Remarkable and new is the objective finding of improved metabolic parameters: far more than better a jeun blood glucose and clearly improved HbA1c values throughout the study, the compliant patients had also statistically significant greater values of serum albumin and serum proteins than non-compliants, after the first 3 months of treatment and only in the compliant group there were significant improvements of BMI and mid-arm circumference noticed. Only the compliant patients have statsitically significant decreases compared to baseline in terms of not only serum uric acid, but also the serum cholesterol.

The main study limitation consists in the low number of patients included in the follow-up; at the same time the study isn't randomized, and the patients following standard ketodiet are compared with a group containing patients with insufficiently reduced protein intake, as well as patients who do not accomplish enough intake of calories or do not take adequate number of ketoanalogue tablets (for ethical reasons the non-compliant patients were not excluded from the administration of ketoanalogues).

The clear conclusion is that a properly administered ketodiet brings to these patients with CDKD benefits similar with those proven so far in non-diabetics, but also additional metabolic and glycemic benefits can be achieved, with a favorable impact on the evolution of associated diabetes and cardiovascular risk it involves, without the association of protein-caloric denutrition. Due to ketodiet applied in CDKD the renal survival increases, life of quality obtained is prolonged for the patient, but also public health costs are reduced. Monthly costs for renal replacement are: 7293 RON for conventionally HD and 4832 RON for standard DPCA, so further gain of more months without renal replacement means a significant economy in the budgetary expenses of the public health system [40, 41]. Mobilization and reorganization of all available human and material resources is necessary for the support and surveillence of ketodiet in these patients, by means of complex and efficient teams, consisting of nephrologist, diabetologist, nutritionist/ nutrition assistant and psychologist, the way we have accomplished in INDNBM ”Prof.Dr.N.Paulescu”.

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