MEDICAL UNIVERSITY - PLEVEN

FACULTY OF MEDICINE

DEPARTMENT OF NEPHROLOGY, HEMATOLOGY AND GASTROENTEROLOGY

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POSSIBILITIES FOR CORRECTION OF SOME ABNORMALITIES OF CALCIUM-PHOSPHATE AND BONE METABOLISM IN PATIENTS WITH CHRONIC RENAL FAILURE

ABSTRACT

of a dissertation for awarding the educational and scientific degree PhD

Scientific speciality "Nephrology."

Scientific supervisor:

Prof. Dr. Vasil Velichkov Todorov, DSc

Pleven, 2023

The dissertation contains 162 pages, of which: introduction, aim and objectives - 2 pages; literature review - 50 pages; material and methods - 5 pages; own results - 56 pages; discussion - 23 pages; conclusions - 3 pages; contributions - 2 pages; references - 16 pages. It is illustrated with 35 tables and 48 figures. The list of references includes 284 sources, of which 7 are in Cyrillic and 277 - in the Latin alphabet.

The dissertation was discussed at an extended departmental council meeting of the Department of Nephrology, Hematology and Gastroenterology of the Medical University - Pleven and was directed for public defence before a scientific jury.

The scientific jury is composed of members appointed by Order No. 2915/26.Sep.2023 of the Rector of Medical University - Pleven:

Members internal to MU - Pleven:

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1. Prof. Borjana Petrova Delijska, DSc

2. Prof. Valentin Hristoforov Ikonomov, DSc

3. Prof. Raina Teodosieva Robeva, PhD

The public defence of the dissertation will be held at 11/22/2023 from 11.30 hours in the Ambroise Pare Hall, in the TELEC of MU-Pleven.

The defence materials are available for interested persons on the website of the Medical University of Pleven: http://www.mu-pleven.bg/index.php/bg/

ABBREVIATIONS

ABD - Adynamic bone disease.

BMI - Body mass index;

CKD - Chronic kidney disease;

CRF - Chronic kidney failure;

CT – Computed tomography;

DOPPS - Dialysis Outcomes and Practice Patterns Study;

DXA - Dual-energy x-ray absorptiometry;

HD – Hemodialysis;

IHD - Ischemic heart disease;

i-PTH - intact Parathyroid hormone;

KDIGO - Kidney Disease Improving Global Outcomes;

LCx - circumflex branch of the left coronary artery;

MBD - Mineral and bone metabolism disorders;

NKF-KDOQI - National Kidney Founadation-Kidney Disease Outcomes Quality Initiative;

RCA - Right coronary artery;

REIN - Renal Epidemiology Information Network;

RRT - renal replacement therapy;

SHPT - Secondary hyperparathyroidism;

t-ALP - total Alkaline phosphatase;

URR - Urea Reduction Ratio;

VDRA - Vitamin D receptor activator;

INTRODUCTION

Chronic kidney diseases have ranked among the most significant social diseases in recent decades. Affecting approximately 10% of the world population, they are associated with an increasing risk of cardiovascular morbidity, bone fractures and premature death.

Abnormalities in mineral and bone metabolism are a major contributor to the risk health profile of patients with chronic kidney disease. Occurring early with impaired renal function, they are almost universal in the stage of chronic renal failure. Defined as part of a complex clinical syndrome, hypercalcemia, hyperphosphatemia, secondary hyperparathyroidism, renal osteodystrophy, and extraskeletal vascular calcifications are associated with adverse health events and outcomes in the dialysis patient population. Despite significant advances in theoretical knowledge, new medications, and rules for successful therapy, mortality among patients with chronic renal failure remains high. Achieving therapeutic goals for all patients in actual clinical practice is unachievable, raising the question of the factors responsible for the discrepancy between global rules and local practices and outcomes. Therefore, the continuous monitoring of mineral and bone metabolism disorders concerning the characteristics of the studied population, the clinical and laboratory manifestation, the complex evaluation of the administered therapy, the possibilities of their correction, the achieved results and available reserves for their improvement on a global and regional scale remains a topical problem. It helps maintain the vigilance and aspiration of medical professionals to improve the health and well-being of individual patients and society.

I. AIM AND OBJECTIVES

The present study aims to comprehensively evaluate mineral and bone metabolism disorders and the possibilities of correcting some of its characteristic parameters in patients with chronic renal failure (chronic kidney disease stage 5D) in actual clinical practice.

STUDY OBJECTIVES:

1. To investigate the peculiarities in the characteristics of the studied cohort of dialysis patients that are relevant to the clinical and laboratory profile of mineral and bone metabolism disorders and the possibilities for their therapy.

2. To investigate the significance of age below and above 65 years as a factor determining significant differences in mineral and bone metabolism and their disorders in dialysis patients of different age groups.

3. To investigate the influence of a patient's age on treatment for mineral and bone metabolism abnormalities.

4. To analyze the results and emerging trends in controlling mineral metabolism and bone metabolism, comparing them with the available rules and world trends.

5. To outline the reserves and new opportunities for therapeutic management of mineral and bone metabolism disorders in the patient cohort studied.

II. MATERIALS AND METHODS

1. Study object.

The study was single-centre, longitudinal, and ambispective. Data regarding mineral and bone metabolism disorders (MBD) of 145 patients with stage 5D chronic kidney disease (CKD) on hemodialysis (HD) were analyzed. All patients were over 18 years of age, had had at least three months of dialysis treatment, and had at least one parameter studied.

2. Study site and period.

The study was conducted at the Clinic of Nephrology and Dialysis of Dr Georgi Stranski University Hospital - Pleven. The study covered five years period, from 01.01.2017 to 31.12.2021.

3. Administration of the study.

The Ethics Committee of MU - Pleven approved the questionnaire prepared for the study. The documentary data was handled by the principal investigator and supervised by the research supervisor.

4. Methods used.

4.1. Documentary method.

A detailed analysis was made of data from medical records of the hemodialysis patients, including case histories from hospital stays, laboratory test results, and imaging study results - radiographs, CT scans, echocardiograms and coronarographies. Demographic data on age, sex, body mass index (BMI), and underlying renal disease cause of terminal chronic kidney failure were collected. Comorbidities known at the time of patient inclusion in the study were summarized and categorized into 7 significant diseases: diabetes mellitus, hypertension, ischemic heart disease, peripheral vascular disease, and cerebrovascular disease. Data were also collected on new events of relevant comorbidities occurring during the course of the study. Available information on fracture experiences (upper, lower extremity, vertebral bodies, etc.) known at study inclusion and new ones occurring during the study period were analyzed. Similarly, data on the presence of calcifications in the wall of blood vessels (abdominal aorta, iliac arteries, femoral arteries, radial artery, etc.) were collected from imaging studies. When echocardiographs and coronarographies were performed, they were analyzed for calcifications in the heart valves (mitral and aortic valves) and in coronary vessels when the patients were included in the study and during the follow-up period.

The duration of dialysis treatment was defined as the number of months of hemodialysis treatment from the first HD session to the start of the study. Patient compliance was assessed based on analysis of data on medication taken/refused provided by the Dialysis Unit for the treatment of CKD-MBD disorders. Physical independence was evaluated according to the patient's ability to ambulate without assistance. Data were collected on vascular access for hemodialysis and intake of other medications known to have a negative effect on CKD-MBD.

Monthly data on ongoing therapy for CKD-MBD disorders regarding treatment with vitamin D receptor activators, phosphorus-reducing drugs, and calcimimetics were processed. The 2009 KDIGO recommendations, updated in 2017, were adopted as assessment criteria.

Patients were followed until completion of the study period, transfer to peritoneal dialysis or death. Causes of death in the study population were analyzed.

4.2. Laboratory studies.

Blood samples from the patients were collected using the standard technique at the beginning of the hemodialysis session at the Central Clinical Laboratory of Dr Georgi Stranski University Hospital. Automated and standardized methods of biological sample processing were used. The analyzed laboratory parameters were: total serum calcium, serum phosphate (monthly in 2017 and every second month in the period 2018-2021), total alkaline phosphatase (t-ALP), serum albumin, creatinine level before dialysis session (at 4 months according to accepted standards for hemodialysis treatment), intact parathyroid hormone (i-PTH) (at 1 to 6-month intervals according to prior values, expected dynamics, and ongoing treatment), serum magnesium. An electrochemiluminescence method with an Elecsys analyzer (Roche) was used to measure serum total vit. D levels.

URR% was calculated using the formula for Urea Reduction Ratio = 100 * [1 - (Urea Post HD/Urea Pre HD).

4.3. Imaging methods.

Data from conventional abdominal, pelvic, and lumbosacral spine radiographs (anterior and lateral) with visualization of the abdominal aorta, iliac and femoral arteries, and hand (palm) and forearm radiographs visualizing the arterial vessels were analyzed. Data from computed tomography scans of the same areas performed with 16-slice CT were also used, as well as CT angiographies of the lower extremity vessels and data from coronarography and echocardiography. A specialist in imaging diagnostics, nephrologist, or cardiologist performed reporting of vascular and other calcifications.

4.4. Statistical methods.

The study data were processed using the IBM SPSS Statistics 25.0 statistical software and MedCalc Version 19.6.3. and Office 2021 Excel.

In the analysis of the results, the following were applied:

- ✓ Descriptive analysis of the frequency distribution of the considered traits;
- ✓ Graphical analysis and presentation of the obtained results;
- ✓ Comparison of relative proportions;
- ✓ Fisher's exact test, Fisher-Freeman-Halton exact test, and Chi-square test ($\chi 2$) - to test hypotheses about the presence of dependence between categorical variables;
- ✓ Kolmogorov-Smirnov and Shapiro-Wilk non-parametric test - to test the distribution for normality;
- ✓ Student's t-test (Student's t-criterion) - to test hypotheses about differences between the means of two independent samples;
- ✓ Mann-Whitney non-parametric test - to test hypotheses of difference between two independent samples;
- ✓ Correlation analysis - to test hypotheses about the existence of a relationship between quantitative traits.

The significance of results, conclusions and inferences were determined at a significance level of $p<0.05$.

III. RESULTS

1. Demographic data

The mean **age** of the study cohort was 60.9±13.1 years, age range 25-83 years. Ninety-one (62.8%) of the subjects were male, and 54 (37.2%) were female (Fig. 1).

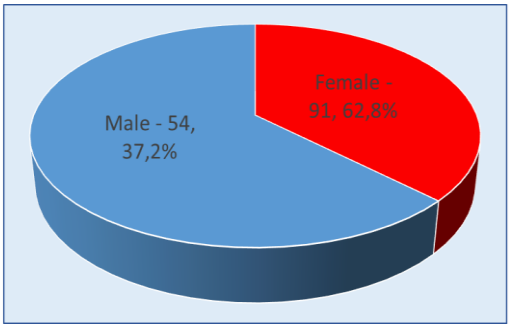

Figure 1. Distribution of patients by gender (n=145).

The age characteristics of the study cohort of patients varied according to **gender**. The largest age group in males was 50-59 years - 31 (34.1%), and in females, the predominant age group was 70-79 years - 16 (29.6%) - Fig. 2.

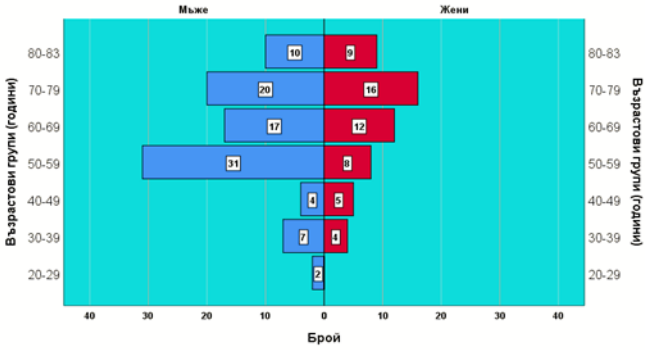

Figure 2. Distribution of patients by age and sex, men (n=91, in dark blue), women (n=54, in red), on the x-axis – patients, n, on the y-axis – age in years.

Due to the data accumulated in the last decades on significant differences in the clinical presentation and treatment of CKD-MBD according to age, two age groups - under and over 65 years - were distinguished for the purpose of the present study. The patients over 65 years of age accounted for 70 (48.3%) of all individuals studied, and 75 (51.7%) of the patients fell into the young and middle-aged groups (Fig. 3).

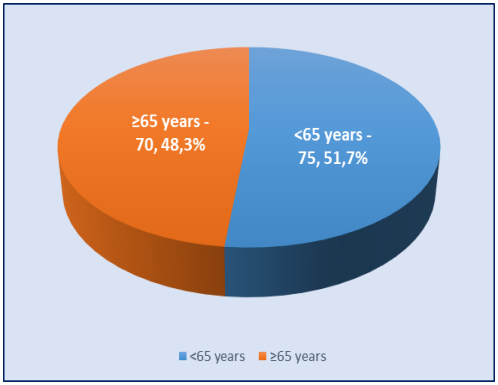

Figure 3. Distribution of patients by age under and over 65 years (n=145).

The mean age in the younger age group was 50.5±9.3 years, and in the elderly group, it was 72.1±4.7 years. The two age groups were not significantly different by gender (p=0.122).

The mean number of patients in the total cohort and the number and relative proportion of subjects in the two age groups remained relatively constant during the study period (Fig. 4).

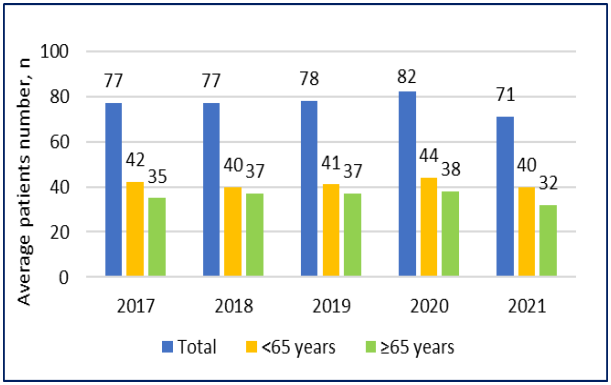

Figure 4. Distribution of patients by age for the study period.

The **body mass index (BMI)** for the entire study group had a mean value of 25.04±5.4 kg/m2. We found no significant difference in BMI according to age (Fig. 5). The highest proportion of patients with normal weight (BMI 18.5-24.9 kg/m2) was 51.7% and 53.1% in both age groups, respectively.

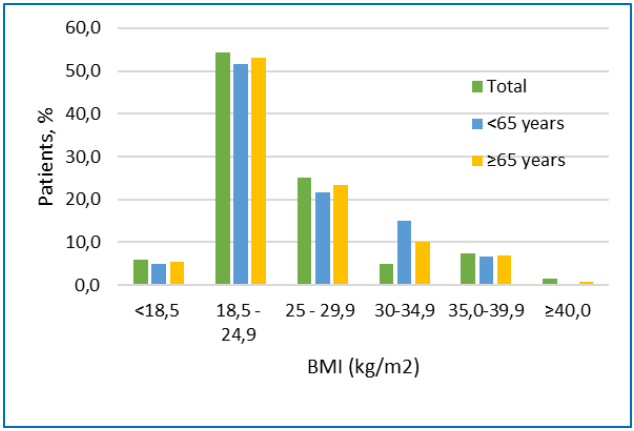

Figure 5. Distribution of patients by body mass index (BMI).

The most common **nephropathies** leading to end-stage chronic kidney disease (ESRD) in the whole cohort were chronic glomerulonephritis (41.3%), hypertensive (18.7%) and diabetic nephropathy (16.0%), accounting for 76.0% of all cases. In the two age groups, they were ranked in order of frequency: hypertensive nephropathy (32.9%), chronic interstitial nephritis (25.7%), and diabetic nephropathy (22.9%) in patients aged <65 years and hypertensive nephropathy (25.5%), chronic glomerulonephritis (24.1%), and diabetic nephropathy (19.3%) in the ≥65 years age group, respectively (Fig. 6). Statistically significant differences were found only concerning chronic interstitial nephritis, which was a more frequent pathology in young patients, and chronic glomerulonephritis, more frequent in older patients.

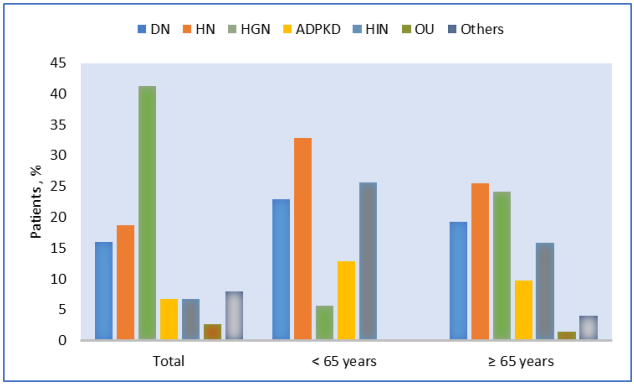

Figure 6. DN - diabetic nephropathy, HN - hypertensive nephropathy, CGN - chronic glomerulonephritis, ADPKD - autosomal dominant polycystic kidney disease, CIN - chronic interstitial nephritis, OU - obstructive uropathy.

Almost all patients studied (98.6%) had at least one significant **comorbidity**. In general, and in both age groups, patients most often had one, two or three comorbidities, with a borderline statistically significant difference by age (p=0.051). The hypertensive disease was present in 96.6% of all patients, and diabetes mellitus in 28.3%. The two significant diseases were more frequent comorbidities in elderly patients ≥65 years compared with the younger age group <65 years, 98.6% and 34.3%, compared with 64.7% and 22.7%, respectively, although there was no significant difference (Table 1).

Table 1. Characteristics of patients according to comorbidities.

Concomitant diseases	Total		< 65 years		≥ 65 years	
	n	%	n	%	n	%
Without CD	2	1.4	2	2.7	0	0.0
With 1 CD	56	38.6	36	48.0	20	28.6
With 2 CD	45	31.0	17	22.7	28	40.0
With 3 CD	33	22.8	16	21.3	17	24.3
With 4 CD	5	3.4	3	4.0	2	2.9
With 5 CD	4	2.8	1	1.3	3	4.3
Diabetes	41	28.3	17	22.7	24	34.3
Arterial Hypertension	140	96.6	71	94.7	69	98.6
Ischemic heart disease at study entry	54	37.2	21	28.0	33	47.1
MI	12	8.3	6	8.0	6	8.6
Chronic IHD	42	29.0	15	20.0	27	38.6
Rhythm disorders	11	7.6	2	2.7	9	12.9
Unstable angina pectoris	4	2.8	1	1.3	3	4.3
Ischemic heart disease – new cases	19	13.1	11	14.7	8	11.4
MI	5	6.7	3	4.3	8	5.5
Chronic IHD	6	8.0	5	7.1	11	7.6
Rhythm disorders	2	2.7	2	2.9	4	2.8
Unstable angina pectoris	0	0	1	1.4	1	0.7
Ischemic heart disease - total number of cases	70	48.3	30	40.0	40	57.1
Peripheral vascular disease at study entry	14	9.7	7	9.3	7	10.0
Peripheral vascular disease - new cases	8	5.5	4	5.3	4	5.7
Peripheral vascular disease - total number of cases	22	15.2	11	14.7	11	15.7
Cerebrovascular disease at study entry	20	13.8	10	13.3	10	14.3
Cerebrovascular disease - new cases	13	9.0	5	6.7	8	11.4
Cerebrovascular disease - total number of cases	33	22.8	15	20.0	18	25.7
Other comorbidities	43	29.7	25	33.3	18	25.7

CD - Concomitant diseases

Patients with evidence of different clinical forms of ischemic heart disease (IHD) at inclusion in the study were 54 (37.2%), with a significantly higher prevalence in the older age group - 33 (47.1%) compared to the younger age group 21 (28.0%),

p=0.021. At the end of the follow-up period, the incidence of IHD had increased to 70 (48.3%) in the whole population, with the higher incidence among elderly patients over 65 years persisting (p=0.047). Two of the clinical forms of IHD, chronic IHD and rhythm disturbances, were again significantly more frequent pathology in patients aged 65 years and older, 27 (38.6%) compared with 15 (20%), p=0.014, of patients with chronic IHD aged 65 years and younger and 9 (12.9%) compared with 2 (2.7%), p=0.021, of cases with rhythm disturbances recorded in both groups. There were no significant differences concerning the newly registered cases of IHD recorded during the study period in the two age groups.

Cases of peripheral vascular disease and cerebrovascular disease at the start of the study and during the follow-up period showed no significant difference in the two study groups.

Dialysis treatment. In the whole study cohort, 76 patients (52.4%) had hemodialysis treatment before inclusion in the study, with a mean duration of 37.8 ± 63.0 months. There were 69 (47.6%) patients newly started on hemodialysis treatment at the time of study inclusion. In the age group ≥65 years, the relative proportion of patients starting hemodialysis treatment was higher, while in the younger age group, the majority of patients were already "dialysis experienced" (p=0.408). The duration of prior HD treatment was not significantly different (p=0.400) in both age groups.

The efficacy of the hemodialysis therapy performed was assessed by URR% with a mean value of 67±8% for the whole patient population and showed no age dependence. For both age groups, the mean URR was 66±8%.

In addition to the BMI value, the serum albumin and serum creatinine levels at the beginning of the hemodialysis session provide information about the nutritional status of the patients (Tables 2 and 3). Serum albumin and serum creatinine at the start of dialysis had higher mean values in the young age group compared with the elderly patients. Statistically significant differences were present in most of the study period, in 7 of 12 studies for serum albumin (Table 9) and 7 of 12 studies for serum creatinine.

Table 2. Mean serum albumin (g/L).

	Total		<65 years		≥65 years	
	Patients n±SD	Serum albumin	Patients n±SD	Serum albumin	Patients n±SD	Serum albumin
2017	75±4	40.01±4.13	41±3	41.19±4.27	35±2	38.65±3.55
2018	77±2	40.24±3.65	40±1	41.26±3.31	37±1	39.14±3.68
2019	75±7	40.06±4.09	41±4	40.02±3.65	34±3	39.16±4.32
2020	82±4	40.14±3.43	43±1	41.25±3.30	39±2	38.90±3.15
2021	72±1	41.72±3.68	40±1	42.10±3.70	32±1	41.22±3.61

Table 3. Serum creatinine (μmol/L).

	Total		<65 years		≥65 years	
	Patients n±SD	Serum creatinin	Patients n±SD	Serum creatinin	Patients n±SD	Serum creatinin
2017	75±4	756±209	40±3	782±184	35±2	731±234
2018	77±2	821±234	40±1	873±235	37±1	763±223
2019	75±7	833±245	41±4	875±234	34±3	784±252
2020	82±4	810±209	43±1	869±213	39±2	744±187
2021	72±1	831±240	40±1	906±237	32±1	736±208

Vascular access. Cases of vascular access with arteriovenous anastomoses and those with tunneled catheters in the whole patient population were almost equivalently represented (Fig. 7). Arteriovenous anastomosis was the more common vascular access option for HD in young patients. In contrast, tunneled catheters marginally dominated in older patients. The differences were nonsignificant.

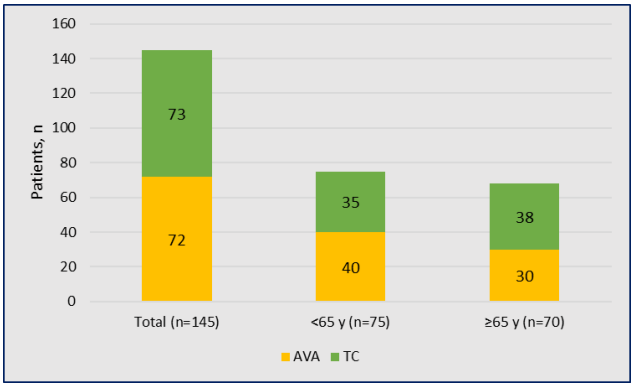

Figure 7. Patient distribution by vascular access. AVA – arterio-venous anastomosis, TC – tunneled catheter.

Examination of physical independence showed that the young age group of patients had significantly greater physical independence ($p<0.001$), Fig. 8.

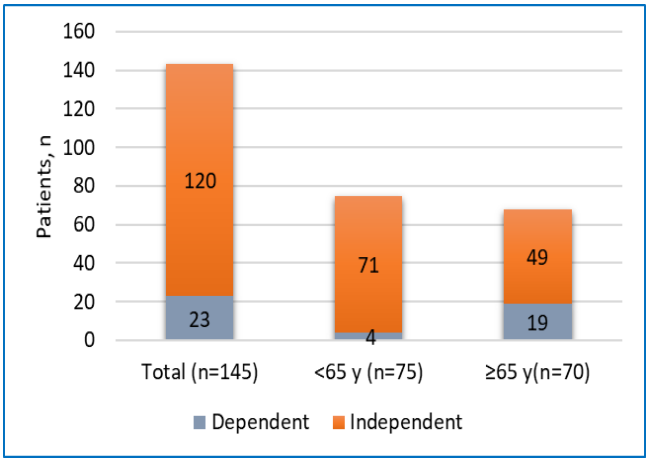

Figure 8. Distribution of patients according to physical independence.

The mean duration of the observation period was 32.8±19.7 months in the total group. The mean observation period in the young patients was 34.6±21.0 months, whereas the elderly patients were observed for a mean of 31±18.6 months (Fig. 9). No statistically significant differences were found.

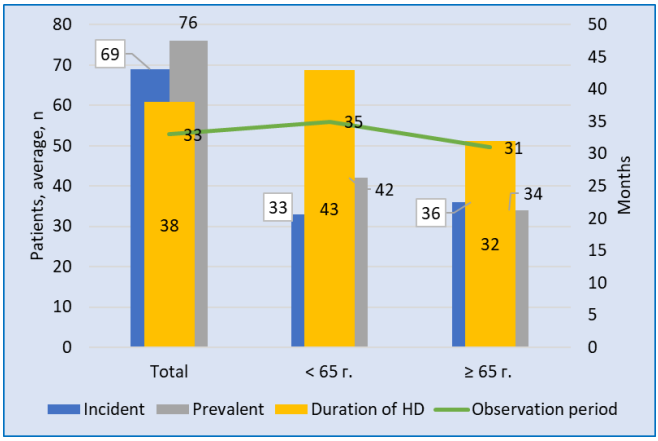

Figure 9. Distribution of patients according to duration of hemodialysis treatment and observation period. HD – hemodialysis.

2. Characteristics of disorders of mineral and bone metabolism in CKD stage 5D. Biochemical markers.

2.1. Serum calcium

The laboratory data from the studies of serum total calcium levels are summarized in Table 4.

Table 4. Mean total serum calcium (mmol/L).

	Total		<65 years		≥65 years	
Year	Patients n±SD	Mean Calcium	Patients n±SD	Mean Calcium	Patients n±SD	Mean Calcium
2017	77±3	**2.22±0.21**	42±2	**2.22±0.20**	35±2	**2.23±0.21**
2018	77±1	**2.24±0.18**	40±1	**2.24±0.19**	37±1	**2.24±0.16**
2019	78±2	**2.21±0.18**	41±2	**2.21±0.20**	37±1	**2.22±0.15**
2020	82±3	**2.15±0.21**	44±2	**2.14±0.22**	38±2	**2.15±0.19**
2021	71±1	**2.16±0.24**	40±2	**2.16±0.25**	32±2	**2.17±0.21**

During the study period, the mean total serum calcium ranged from 2.15±0.21 to 2.24±0.18 mmol/L in the whole group of observed patients. The age group <65 years had mean total calcium of 2.14-2.22 mmol/L, and patients aged ≥65 years had mean total calcium ranging from 2.15 to 2.24 mmol/L. For the entire study period, comparative analysis of the index showed no significant age-determined differences.

2.2. Serum phosphorus

During the observation period, serum phosphate levels in the general population had mean values ranging from 1.94±0.63 to 2.1±0.72 mmol/L. During all periods, the younger age group of patients had higher mean serum phosphorus levels (ranging from 2.15±0.65 to 2.35±0.71 mmol/L) compared with the older age group (ranging from 1.7±0.49 to 1.85±0.61 mmol/L), and the differences were significant ($p<0.05$) (Fig. 10).

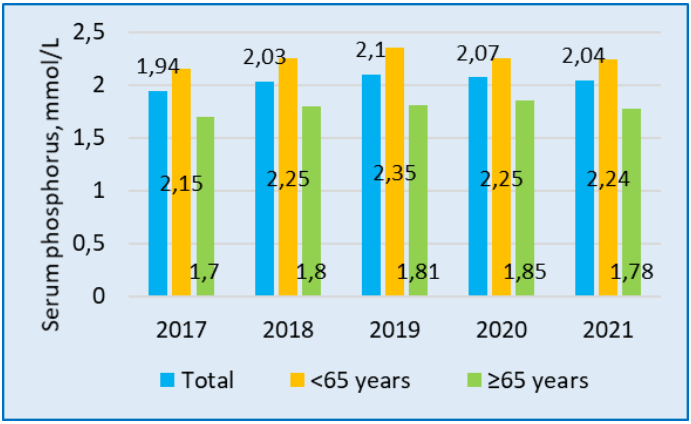

Figure 10. Patients distribution by mean serum phosphorus (mmol/L).

2.3. Calcium-phosphate product

A statistically significant difference in the two age groups under and over 65 years was also found in the value of the calcium-phosphate product - a higher product was reported more often in young patients compared with older ones (4.8±1.6 to 5.2±1.6 mmol2/L2, compared with 3.8±1.2 to 4.0±1.4 mmol2/L2, respectively). All product values were within the target range in older patients (Table 5).

Table 5. Calcium-phosphate product (Ca x P, mmol2/L2).

	Total		<65 years		≥65 years	
Year	Patients n±SD	Mean Ca x P mmol2/L2	Patients n±SD	Mean Ca x P mmol2/L2	Patients n±SD	Mean Ca x P mmol2/L2
2017	77±3	**4.3±1.5**	42±2	**4.8±1.6**	35±2	**3.8±1.2**
2018	77±1	**4.6±1.5**	40±1	**5.0±1.6**	37±1	**4.0±1.2**
2019	78±2	**4.6±1.6**	41±2	**5.2±1.6**	37±1	**4.0±1.4**
2020	82±3	**4.4±1.5**	44±2	**4.9±1.4**	38±2	**3.9±1.3**
2021	71±1	**4.4±1.5**	40±2	**4.8±1.6**	32±2	**3.9±1.2**

2.4. Parathyroid hormone

The mean serum intact PTH (i-PTH) values in the whole study population ranged from 558±481 to 467±431 pg/ml by year during the study period. Higher mean i-PTH values were again reported more frequently in younger patients compared with

those aged ≥65 years (Fig. 11), although no significant difference was found over the entire study period.

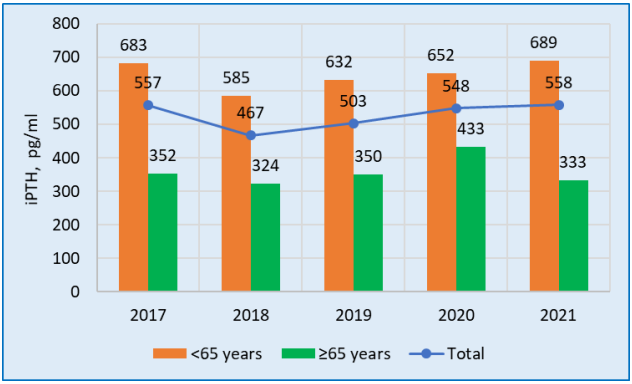

Figure 11. Patients distribution by mean intact i-PTH.

In a total of 60 months of follow-up, tests for the significance of the difference in serum i-PTH levels according to age were positive ($p<0.05$) in only 10 of them. The lack of a significant difference in i-PTH levels according to age in the remaining months may be explained by the fact that the indicator study was conducted at different time intervals from 1 to 6 months for individual patients and did not cover all patients at the same time every month. Older patients with low i-PTH, especially those with PTH below 100 pg/ml, were studied less frequently as no significant short-term dynamics were expected. Thus, data from patients with high PTH were more frequently included in the comparative analysis.

Comparative analysis of the mean serum phosphate and i-PTH values of the two age groups <65 and ≥65 years over the entire period showed a significant difference of 21.1% and 81.2% for the two parameters, respectively (Fig. 12).

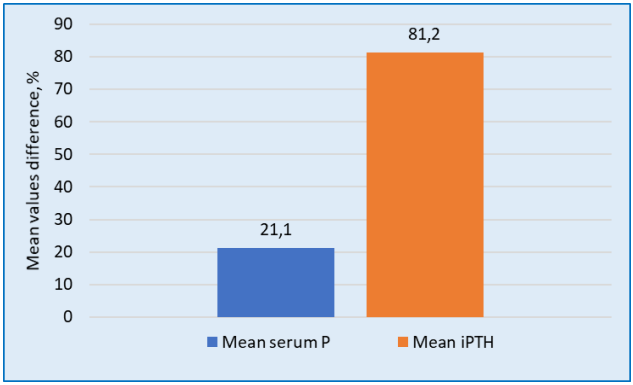

Figure 12. Difference (%) in mean values of serum phosphorus (P) and i-PTH over the entire study period between age groups <65 and ≥65 years.

2.5. Total alkaline phosphatase

The mean total alkaline phosphatase (t-ALP) in patients aged <65 years ranged from 112-123 IU/L, and in patients aged ≥65 years ranged from 106-113 IU/L. Comparative analysis revealed that the differences in the index in the two age groups were insignificant (Table 6).

Table 6. Serum total alkaline phosphatase levels (IU/L).

	Total		**<65 years**		**≥65 years**	
Year	Patients n±SD	t-ALP n±SD	Patients n±SD	t-ALP n±SD	Patients n±SD	t-ALP n±SD
2017	75±4	**118±55**	40±3	**123±60**	35±2	**113±47**
2018	74±2	**110±44**	38±2	**113±48**	37±1	**107±40**
2019	77±3	**111±49**	40±2	**112±53**	36±2	**110±46**
2020	77±4	**109±46**	39±2	**112±53**	38±2	**106±39**
2021	69±1	**114±48**	38±2	**118±52**	31±2	**110±44**

2.6. 25(OH)Vitamin D

The serum 25(OH)D level was studied once in October 2020 in 90 patients undergoing hemodialysis treatment at the time of the study and indicated the vitamin

D status at the end of the summer season. The median 25(OH)D level was 26.3 (4.30-92.5) ng/ml. The distribution of patients according to Vit. D showed that 55.0% had a suboptimal 25(OH)D level (<30 ng/mL), of whom 23.6% had an insufficient level (20-29 ng/mL) and 31.4% were vitamin deficient (<20 ng/mL). A severe Vit. D deficiency (<10 ng/mL) was found in 8.8% of the subjects. A sufficient level of 25(OH)D - above 30 ng/mL was found in 45% of patients on dialysis treatment, 23.5% of whom had Vit. D levels above 40 ng/mL. Older patients had lower 25(OH)D levels of 23 (Q1-3: 15-34) compared to 30 (Q1-3: 20-51) in the younger age group, p=0.012 (Fig. 13).

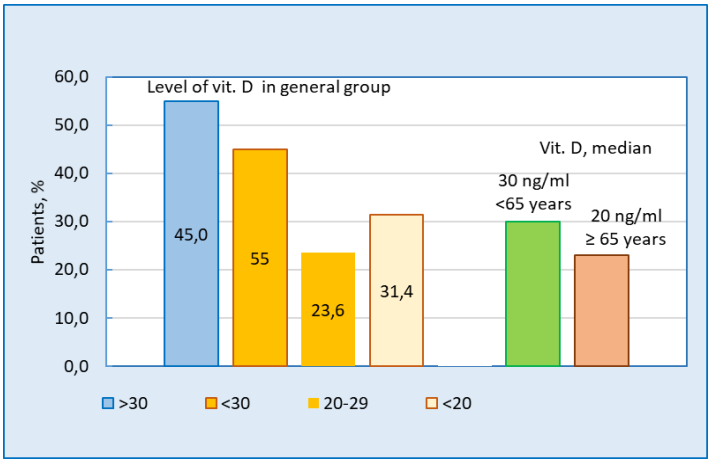

Figure 13. Vitamine D level in the general and both age studied groups.

Patients in this subgroup also showed significant age differences in the indicators presented in Table 7.

Table 7. Indicators with significant age differences in the study of vitamin D status.

	Total	< 65 years	≥ 65 years	p
Patients, n (%)	90 (100.0%)	44 (48.9%)	46 (51.1%)	-
Diabetes	24 (26.7%)	7 (15.9%)	17 (37.0%)	0.041
Phosphates, mmol/L	1.97 (0.83-3.94)	2.14 (1.26-3.94)	1.78 (0.83-3.37)	0.008
Ca x P, mmol2/L2)	4.27 (1.76-8.33)	4.53 (2.50-8.33)	4.02 (1.76-7.58)	0.006
Serum albumin, g/L	40.80±2.851	41.59±3.040	40.04±2.460	0.009
i-PTH (15-65 pg/ml)	307 (15-2895)	408 (35-2895)	199(15-1637)	0.000
25(OH)D, ng/ml	26.2 (4.3-92.5)	29.9 (10.6-88.4)	22.9 (4.3-92.5)	0.012

A significant negative correlation of vitamin D level with age was found ($p<0.01$) (Fig. 14).

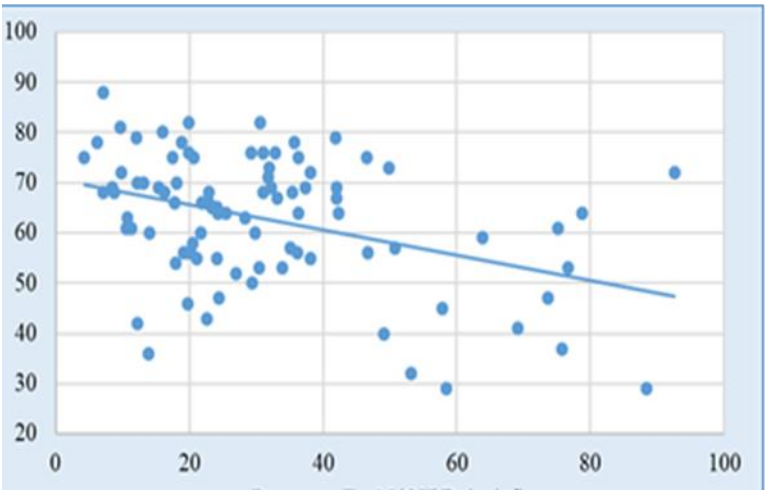

Figure 14. Correlation of vitamin D level with age.
On the x-axis – 25(OH)D level in ng/ml, on the y-axis – age in years.

2.7. Serum magnesium

Serum magnesium levels were tested on a single occasion in November 2021 in 70 patients undergoing hemodialysis (Table 8).

Table 8. Mean serum magnesium (Mg), mmol/L.

Total		< 65 years		≥ 65 years		p
Patients, n	Mg $\overline{X} \pm SD$	Patients, n	Mg $\overline{X} \pm SD$	Patients, n	Mg $\overline{X} \pm SD$	
70	1,32±0,24	41	1,33±0,26	29	1,30±0,23	0,235

All patients were dialysed at a magnesium concentration in dialysis solution of 1.5 mmol/L, therefore, the mean serum magnesium in the general population in both groups <65 and ≥65 years was high (normal 0.66-1.07 mmol/L) and without significant age differences. One patient had evidence of hypomagnesemia, 9 patients (12.9%) had normal serum magnesium levels, and all the remaining 60 (85.7%) had mild hypermagnesemia up to 1.75 mmol/L.

The **correlation analysis** performed for the relationship between age and laboratory parameters of MBD did not demonstrate a statistically significant correlation between age and serum calcium level, BMI (-0.021, $p<0.01$), and serum magnesium (-0.183, $p<0.01$). A weak inverse correlation was present between age and serum total ALP level. Age correlated negatively with vitamin D level and moderately in strength with serum phosphate level in almost all observation periods. Intact PTH correlated negatively with age in a few periods studied, with correlations varying in strength from moderate to strong.

2. Bone disease

Bone fractures are one of the significant clinical manifestations of CKD-BMD and, in turn, a risk factor for increased morbidity and mortality in the dialysis patient population. Twenty-five (17.3%) of the patients studied had experienced one or two fractures before enrollment in the clinical study, 12 (16.0%) of them were aged <65 years and 13 (18.6%) were aged ≥65 years (Table 9).

The most common fractures were of the lower limb (11.0% overall, 9.3% and 12.9% in the two age groups) and upper limb (4.1%, 4.0%, and 4.3%, respectively), with no significant differences by age. Vertebral fractures were registered in only two of the older patients.

Table 9. Bone fractures and other bone disease.

	Total		< 65 y.		≥ 65 y.		p
	n	%	n	%	n	%	
Fractures at study entry							
1 fracture	22	15.2	11	14.7	11	15.7	0.861
2 fractures	3	2.1	1	1.3	2	2.9	
Fractures type							
Upper limb	6	4.1	3	4.0	3	4.3	0.928
Vertebrae	2	1.4	0	0	2	2.9	0.139
Lower limb	16	11.0	7	9.3	9	12.9	0.491
Others	4	2.8	3	4.0	1	1.4	0.340
New fracture	15	10.4	7	9.3	8	11.4	0.887
Fracture type							0.974
Upper limb	6	4.1	3	4.0	3	4.3	
Vertebrae	1	0.7	0	0.0	1	1.4	
Lower limb	6	4.1	3	4.0	3	4.3	
Others	2	1.4	1	1.3	1	1.4	
Fracture - total	40	27.6	19	25.4	21	30	0.706
Osteoporosis - total	16	11.0	5	6.7	11	15.7	0.112

During the study period, a total of 15 (10.4%) new cases of fractures were recorded over a 5-year period, 7 (9.3%) in the young age group and 8 (11.4%) in the elderly patients over 65 years of age. Upper and lower limb fractures were again the most common, with similar incidence, in the general population and in both age groups. A total of 40 (27.6%) of the patients had a bone fracture in the overall cohort, 19 (25.4%) of the patients under 65 years of age, and 21 (30%) of the patients over 65 years of age, with no statistically significant differences (Figs. 15, 16, and 17).

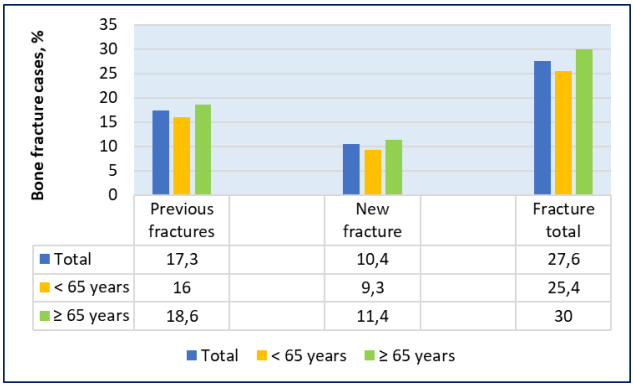

Figure 15. Total number of bone fracture cases.

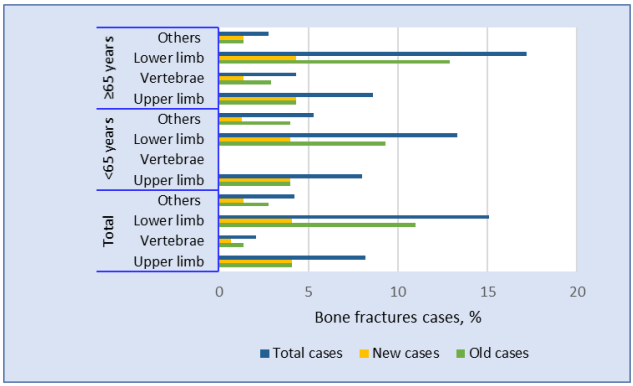

Figure 16. Distribution of cases with bone fractures by age and location.

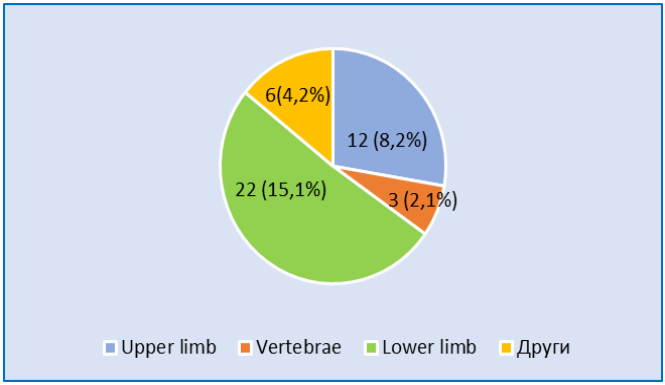

Figure 17. Distribution of the total number of bone fracture cases by location.

Specialized bone density testing (DXA) was performed on a small number of patients. Data on changes in bone structure were also taken from the bone radiographs performed. Hence, there was information about osteoporosis in only 16 (11.0%) of all the patients, 5 (6.7%) patients in the young age group and 11 (15.7%) patients aged ≥65 years.

4. Extraosseous calcifications

On inclusion in the clinical study, 23 (15.8%) of all patients had calcifications detected on radiography: there were 9 (6.2%) cases of such calcifications in the abdominal aorta, 6 (4.1%) in the iliac arteries, 6 (4.1%) in the femoral arteries, and 2 (1.4%) in the radial arteries. In the age groups <65 years and ≥65 years, the affected patients were 12 (16%) and 11 (15.8%) overall, respectively, with no significant difference associated with age. Multiple localization of vascular calcification was present in some cases.

During the study period, new cases of vascular calcification were found in a total of 39 (26.9.7%) patients: in the abdominal aorta in 19 (13.1%) of the patients, in the iliac arteries 10 (6.9%), in the femoral arteries 12 (8.3%) and radial arteries 2(1.4%). Newly diagnosed calcifications in the vascular wall of large blood vessels amounted to 20 (26.6%) in patients younger than 65 years and 23 (32.9%) in patients older than 65 years, with no statistically significant difference. There were no significant differences in calcification localization according to age.

Echocardiograms of 26 patients were available when they were included in the clinical follow-up. Ten of the echocardiograms showed no valvular calcinosis, and 16 (61.5%) of all echocardiograms (11% of patients in the study) showed calcifications

of the heart valves, 8 cases of mitral and 8 cases of aortic valvular calcinosis involving the valve annulus and leaflets, respectively; according to age, there were 4 cases of aortic valve calcinosis and 3 cases of mitral valve calcinosis in the young age group of patients, and 4 and 5 cases, respectively, in patients aged 65 years and older. Echocardiography was performed on 20 patients during the follow-up period. Six of the examinations were without evidence of valvular calcinosis, and 17 (85% of cases with echography, 11.7% of all patients studied) were positive: 6 cases of Ao valve calcinosis and 11 cases of M valve calcinosis; 4 Ao and 5 M valves in patients younger than 65 years of age and 2 Ao and 6 M valves in older patients. Mitral valve involvement was more frequent than aortic valve involvement, with 19 cases (13.1% of all patients studied) and 14 cases (9.6% of patients), respectively. There was no statistically significant difference according to age.

There was no evidence of coronary calcinosis detected by coronary angiography on study inclusion. During the study period, a total of 6 patients (3 under 65 and 3 over 65) underwent coronary angiography with evidence of LCx and RCA calcinosis. In one patient under 65 years, the coronary angiography did not reveal calcinosis.

When summarizing the data on the presence of extraosseous calcifications, regardless of their localization, we found an equal involvement of patients without significant age dependence (Fig. 18).

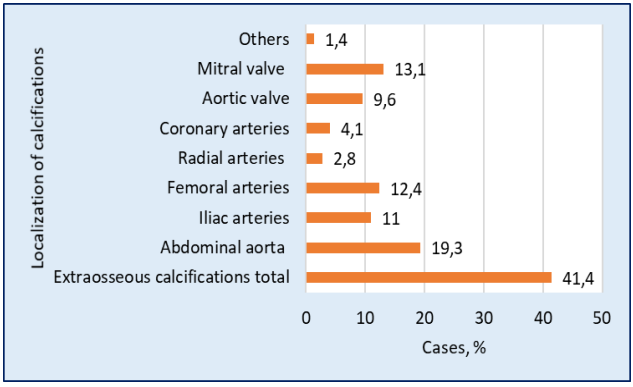

Figure 18. Localization of registered soft tissue calcifications. (The graph represents the incidence of all cases of extraosseous calcifications, at study entry and in the observation period).

5. Analysis of the conducted therapy

5.1. Therapy with vitamin D receptor activators (calcitriol, paricalcitol).

Two medications were used to treat secondary hyperparathyroidism (SHPT) - calcitriol in oral form and paricalcitol in oral and intravenous form. For the purpose of comparative analysis, vitamin D receptor activators (VDRAs) were grouped and presented in dose equivalent to those of the more commonly used preparation calcitriol (0.25 μg calcitriol = 1 μg paricalcitol).

The relative proportion of patients in the whole group treated with VDRAs during the study period ranged from 33.8±4.1 to 42.7±2.9% (Fig. 19). VDRAs were used in the treatment of impaired CKD-BMD more frequently in the elderly patients (from 43.3±4.5 to 59.9±7.6% during the period) than in the younger age group (from 28.9±5.8 to 37.9±3.8%). Statistically significant differences in the frequency of VDRA use according to age were found in only 4 of the 60 months of the study.

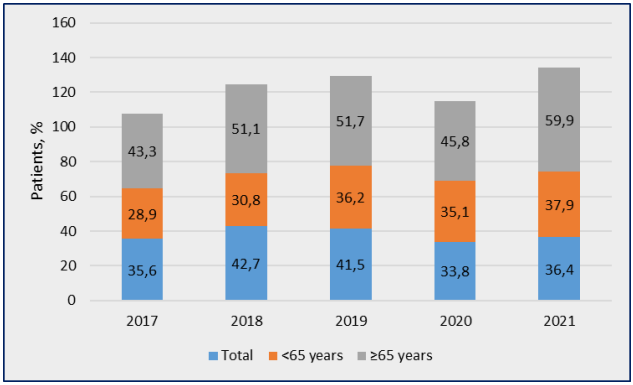

Figure 19. Treatment of patients with vitamin D receptor activators.

In some of the patients aged <65 years, the presence of hyperphosphatemia was a limitation to prescribing VDRA despite the presence of SHPT. The calcium-phosphate profile of patients aged ≥65 years was significantly better than younger patients, allowing therapy with VDRA for appropriate indications. In the age group ≥65, a VDRA (calcitriol) was given in low doses to correct concomitant hypocalcemia.

The mean weekly dose of VDRA used in the general patient group ranged from 1.19 to 1.45 μg/week. It was significantly higher in patients <65 years of age

(1.27-1.48 µg/week, p<0.05) compared with patients >65 years of age (1.03-1.44 µg/week) in 3 of the 5 years of the study (Fig. 20).

Figure 20. Treatment with vitamin D receptor activators - average weekly dose.

Low weekly doses of VDPA 0.75-0.875 mcg were used more frequently (in 55.3%) and longer (51-60 months) compared with higher doses of 2.5-3.75 mcg/week in 15.6% of patients for 22-34 months of therapy in the overall cohort (Fig. 21). Of patients aged 65 years and older, 61.1% received doses of 0.75-0.85 mcg/week compared with 49.2% of patients aged less than 65 years. At high doses (2.5-3.75 mcg/week), the incidence was slightly higher in young patients (15.5%) compared to 12.2% in older patients. The groups analyzed were small, and tests for statistical significance remained negative.

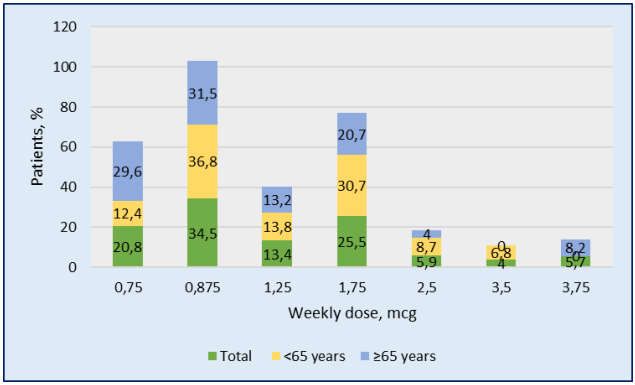

Figure 21. Distribution of patients by weekly dose of VDRAs.

5.2 Phosphate-lowering therapy. Calcium carbonate.

Calcium carbonate is most commonly used as a phosphate binder in the complex treatment of CKD-MBD disorders. As a source of elemental calcium, its use may also be mandated in treating hypocalcemic states. Calcium carbonate therapy accounted for a significant proportion of the patient population studied, ranging from 80.0±1.5 to 95.2±2.1%. Younger patients received calcium carbonate more frequently than older patients, but no significant differences were found between the two age groups (Fig. 22).

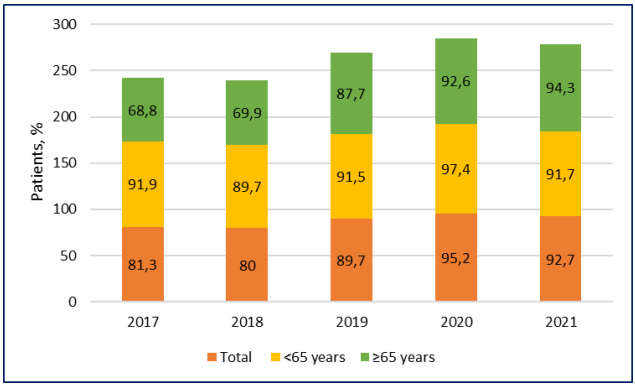

Figure 22. Treatment with calcium carbonate.

Analysis of the mean daily dose of calcium carbonate showed that it was significantly higher in younger patients <65 years of age (ranging from 1120 to 1378 mg daily) compared with the older (906-1266 mg daily) (Fig. 23).

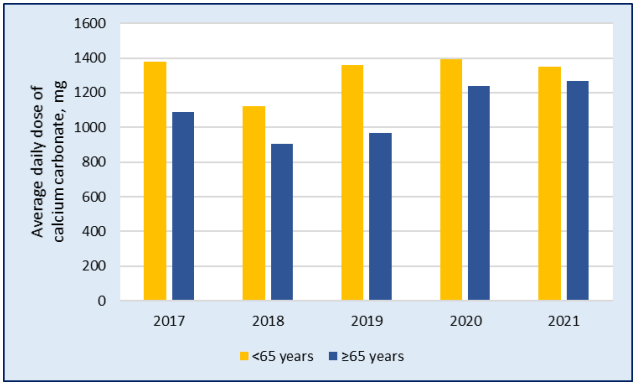

Figure 23. Average daily dose of calcium carbonate by age group.

The most commonly used doses of calcium carbonate were 600, 1200, and 1800 mg daily (Fig. 24). The largest proportion of patients were prescribed a daily dose of 1200 mg - 41.6%, 41.9% and 40.9% in the general population and the two age subgroups under and over 65 years, respectively. Higher doses of the medication were again more common in younger patients. Statistically significant differences were found in 31 of the 60 months of therapy studied over the five-year period.

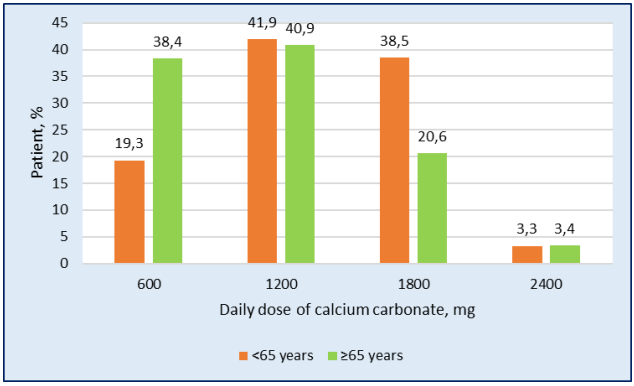

Figure 24. Distribution of patients by age and daily dose of calcium carbonate. Results are presented as a percentage (%) of all patients receiving calcium carbonate.

Treatment of CKD-MBD disorders with calcium carbonate alone (for hypocalcemia and/or hyperphosphatemia) was administered to an average of 25 patients in the whole group: 13 patients over 65 and 12 patients under 65 during the study period (Fig. 25).

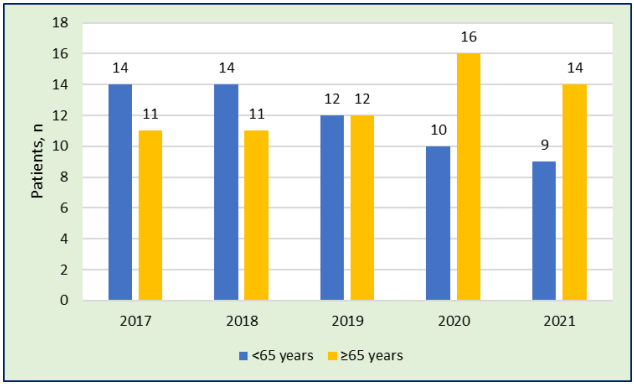

Figure 25. Calcium carbonate monotherapy by age group.

5.3 Sevelamer hydrochloride/carbonate treatment.

All patients were treated with sevelamer between 17.5% by 2017 and 37.2% by 2021. In the study period, the use of the drug increased in the young age group (from 14.5 to 52.9%) and decreased in the over-65 age group (from 23.3 to 17.4%), which can be explained by a lower incidence of hyperphosphatemia and its better control in the older patients (Fig. 26).

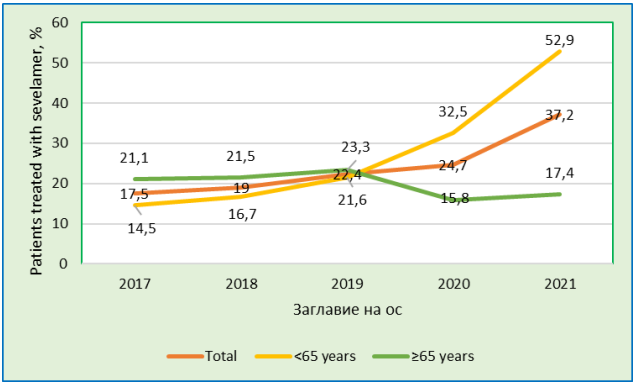

Figure 26. Treatment with sevelamer.

In only two of the 60 months studied, there was a significant difference in mean and most frequently used daily doses according to age. This difference was due to using relatively low doses because of side effects, irregular drug intake by some patients, and combination therapy with calcium carbonate (Fig. 27).

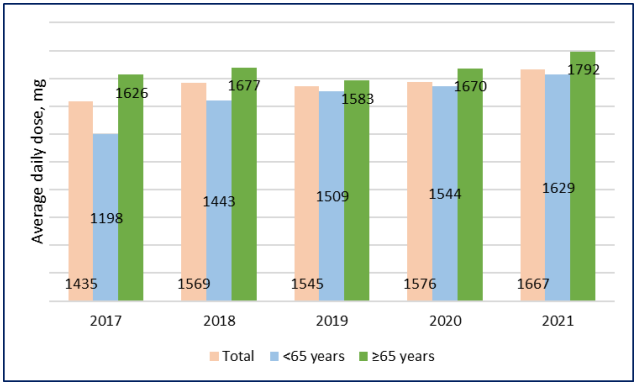

Figure 27. Treatment with sevelamer - distribution of patients according to mean daily dose (mg) and age.

The most common daily doses of sevelamer were 800, 1600, and 2400 mg (Fig. 28).

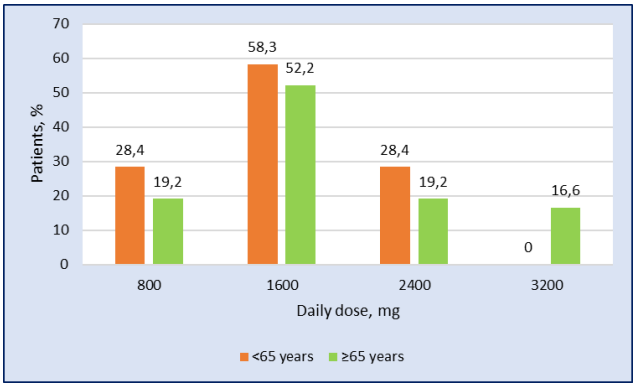

Figure 28. Treatment with sevelamer - distribution of patients under and over 65 years according to daily dose.

Combination therapy with sevelamer and calcium carbonate was applied in a small number of patients, 14 (%) in the total group, due to combined calcium and phosphate abnormalities, unsatisfactory effect of phosphate binding monotherapy, or side effects requiring dose reduction. Figure 29 presents the distribution of cases according to age.

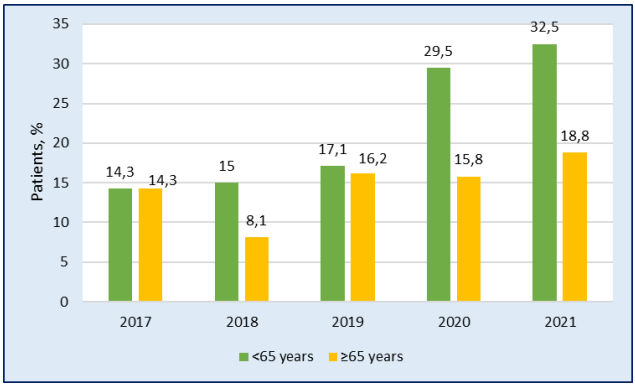

Figure 29. Combined therapy with phosphate binders (sevelamer and calcium carbonate).

5.4 Cinacalcet treatment

Between 17% and 20% of the whole patient population had a severe SHPT (i-PTH > 800 pg/ml) or biochemical profile that necessitated the inclusion of cinacalcet

in the therapy. The relative proportion of young patients <65 years on cinacalcet therapy was higher than that of elderly patients (between 23.2 and 32.9% compared with 5.5 and 11.1% in the older). The group of patients aged 65 years and older was statistically under-representative, so statistical analysis could not be performed (Fig. 30).

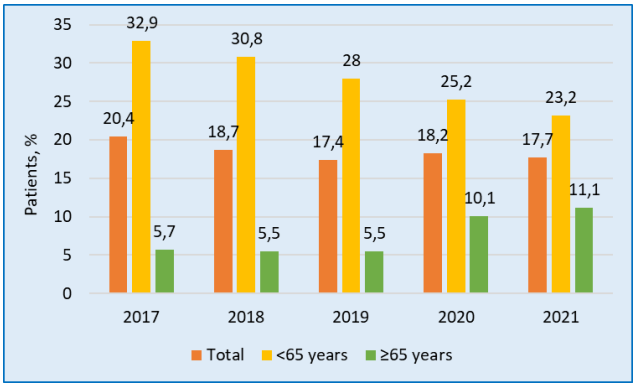

Figure 30. Treatment with cinacalcet in the general and both age groups.

Mean daily doses of cinacalcet were comparable in the two age groups (Fig. 31).

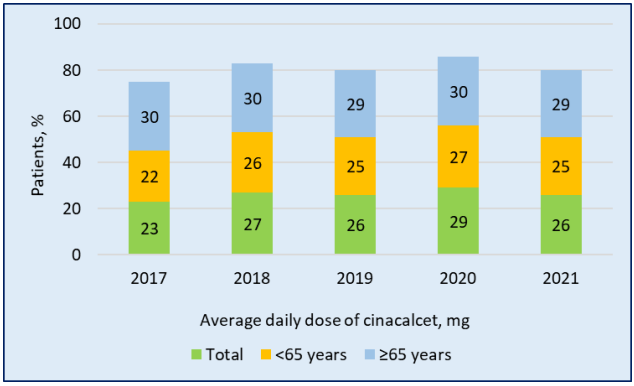

Figure 31. Comparative analysis by mean daily dose of cinacalcet.

The most commonly used daily doses were 30 mg/48 h, 30 mg/day, and 30 mg on 5 days of the week (Fig. 32).

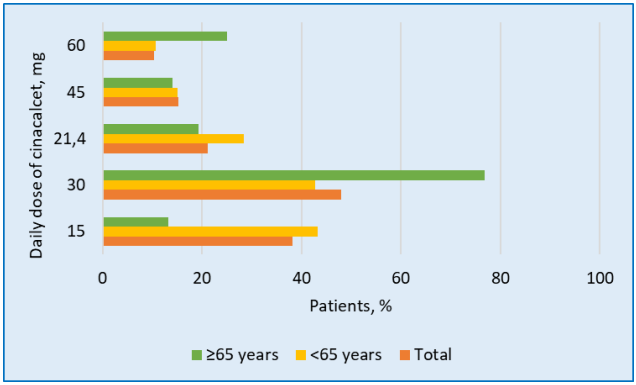

Figure 32. Comparative analysis by daily dose of cinacalcet. The patients with the indicated dose are given as a proportion of the total number of patients treated with cinacalcet in the respective group.

5.5. Patients without therapy

Patients not on medication therapy for CKD-MBD disorders were few, both in the total group (1 to 6 patients) and in the two age subgroups (1 to 2 in the young age group and 1 to 8 in the older patients in the monthly periods studied) (Fig. 33).

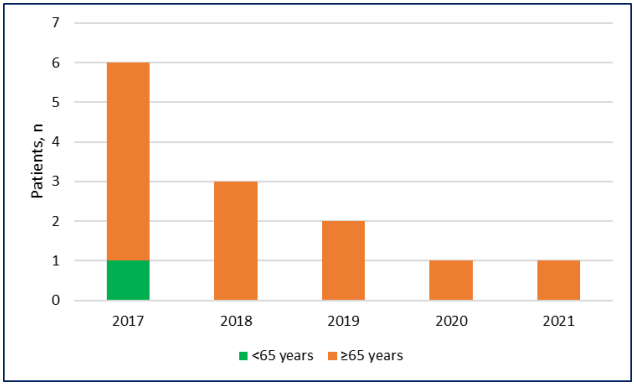

Figure 33. Patients without drug therapy for CKD-MBD.

Treatment with sintrom was recorded in 6 (9.3%) of patients younger than 65 years and in 1 (1.4%) of those aged 65 years and older.

The comparative analysis of the patients' collaboration, based on data on the use of medications provided by the Dialysis Unit (Fig. 34), showed that: 1. A

significantly lower proportion of the older patients received medication to treat CKD-MBD disorders ($p<0.05$). 2. There was no significant difference between the two age groups in the regular use of the free medications provided - VDRAs, sevelamer and cinacalcet.

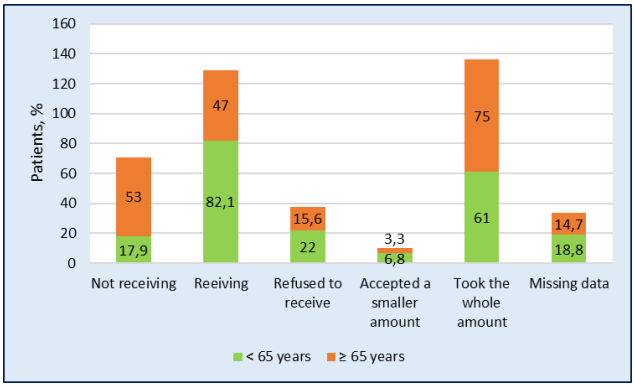

Figure 34. Patient collaboration.

6. Therapeutic outcomes

6.1. Therapeutic outcomes. Serum calcium.

We found no significant difference in the two age groups according to serum calcium levels <2.1 mmol/L, 2.1-2.55 mmol/L and >2.55 mmol/L. There was no clear trend of correlation between age and this biochemical parameter. For all periods studied, in the whole group and in the two age subgroups, serum calcium levels in the reference range of 2.1-2.55 mmol/L dominated (Fig. 35), followed by the frequency of patients with hypocalcemia below 2.1 mmol/L presented in Fig. 35 by age group. Single patients with hypercalcemia above 2.55 mmol/L were also studied.

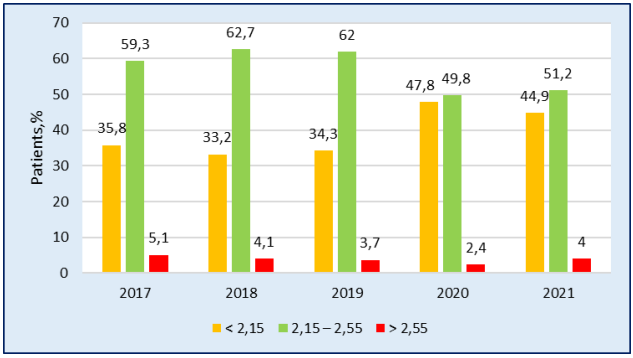

Figure 35. Distribution of patients in the overall group according to serum calcium level <2.1 mmol/l, 2.1-2.55 mmol/l and ≥2.55 mmol/l.

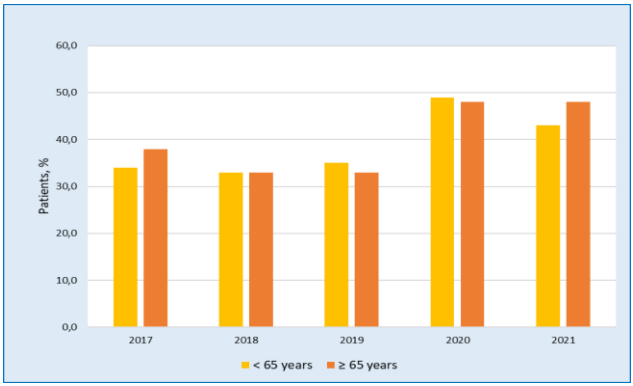

Figure 36. Distribution of patients with hypocalcemia <2.1 mmol/L by age group.

There was a negative trend of increasing the relative proportion of patients with hypocalcemia (Figure 36).

6.2. Therapeutic outcomes. Serum phosphorus.

Figure 37 shows the distribution of patients in the total group according to serum phosphorus level. There was a trend for a slight increase of cases with hypophosphatemia <0.85 mmol/L, a retention and subsequent rise in cases with phosphatemia in the range of 0.85-1.9 mmol/L, and a decrease in cases of hyperphosphatemia above 1.9 mmol/L.

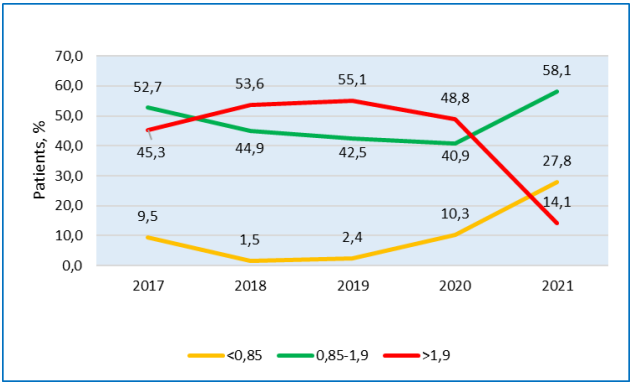

Figure 37. Distribution of patients in the total group according to the level of achieved serum phosphorus < 0.85 mmol/l, 0.85-1.9 mmol/l and ≥1.9 mmol/l.

We found a similar distribution and trend in both age groups: under and over 65 years.

Patients in the younger age group had significantly higher serum phosphate levels more often than patients older than 65, who were more likely to have serum phosphorus values within the desired range ($p<0.05$). Hyperphosphatemia remains a challenging problem in the treatment and control of CKD-MBD disorders.

1.3 Alkaline phosphatase.

We found no significant association between different levels of total alkaline phosphatase and age. All groups were dominated by patients with serum t-ALP levels of 80 to 120 IU/L, which were within the reference range (Fig. 38).

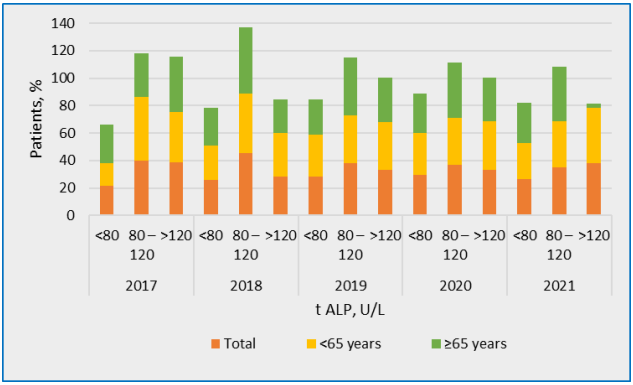

Figure 38. Comparative analysis of patients by t-ALP levels.

6.4. Parathyroid hormone.

Figures 39, 40 and 41 present the distribution of patients according to serum i-PTH levels in the total and both age groups. In all periods studied, patients with i-PTH values of 300-600 pg/ml and 150-300 pg/ml, i.e. in the desirable range, dominated, followed by patients with values <150 pg/ml, 600-800 pg/ml and those with above 800 pg/ml.

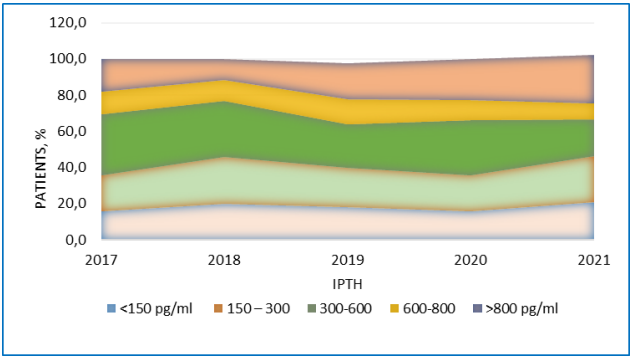

Figure 39. Distribution of patients in the total cochort according to i-PTH levels.

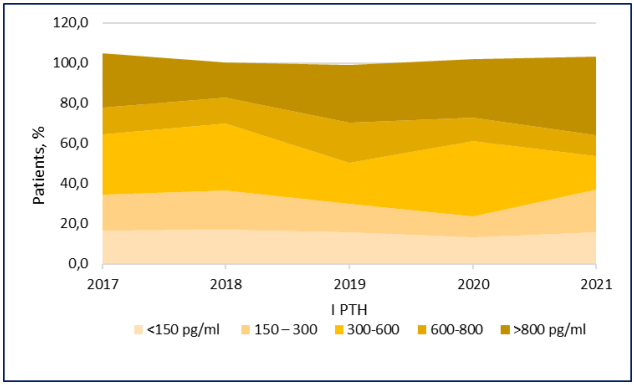

Figure 40. Distribution of patients <65 years according to i-PTH levels.

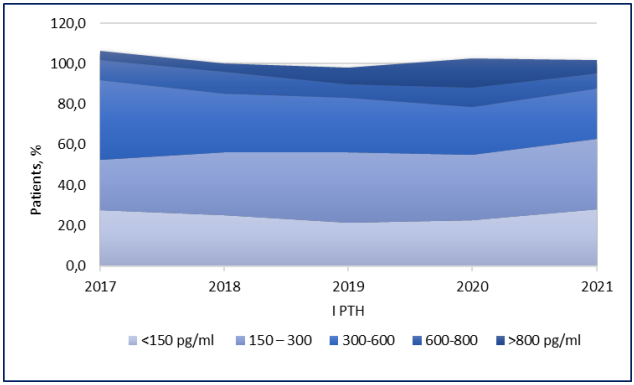

Figure 41. Distribution of patients ≥65 years according to i-PTH levels.

The dynamics of mean i-PTH levels in the two age groups showed positive trends of the increasing number of patients with PTH 150-300 pg/ml and the decreasing number of cases with PTX 600-800 and 800 pg/ml. The statistical analysis of parathyroid hormone levels achieved according to age was complicated because of the different frequency of testing by month, in different years, in the two age groups. Significant differences with heterogeneous patterns in i-PTH levels according to age were found in 5 of the 60 months studied. In a study of vitamin D levels in October 2020 (presented above in the vitamin D analysis), the i-PTH level was examined in all patients simultaneously. It showed that older patients had lower i-PTH levels, 199 (Q1-

3: 96-421), compared to 435 (Q1-3: 240-772) in patients younger than 65 years, at p=0.000.

6.5. Analysis of achieved target serum calcium values (2.15-2.55 mmol/L).

Between 49.8 and 62.7% of all patients achieved target serum calcium values (Fig. 42). In younger patients, this proportion was insignificantly lower than in patients aged 65 years and older, 49.0% and 61.9%, compared with 50.7 and 64.9%, respectively. There was no statistically significant age-dependent difference throughout the study period.

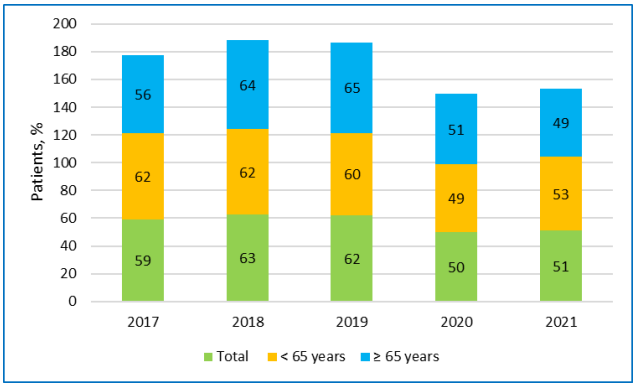

Figure 42. Distribution of patients with achieved target serum calcium level (2.1-2.55 mmol/L). The patients are given as a proportion of the total number of patients in the given year.

6.6. Analysis of achieved target values for serum phosphate (0.85-1.9 mmol/L).

We found that for the whole sample, the relative proportion of patients achieving these values ranged between 41.9 and 59.0% (Fig. 43).

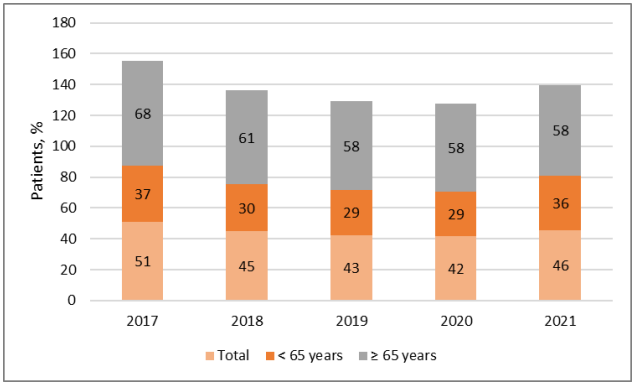

Figure 43. Comparative analysis of patients achieved target serum phosphorus (0.85-1.9 mmol/L).

Adequate control of phosphatemia was achieved less frequently in younger patients than in older patients, between 29.1% and 36.6%, compared with 57.5% and 67.7%, respectively. The difference between the two age groups was significant. There was a slow trend towards improvement in the indicator.

6.7. Analysis of achieved target values for i-PTH (150-600 pg/ml).

Achievement of i-PTH values in the range of 150-600 pg/ml was more frequent in patients aged 65 years and older. In the total group, 31.3% to 76.9% of patients had i-PTH in the indicated range (Fig. 44).

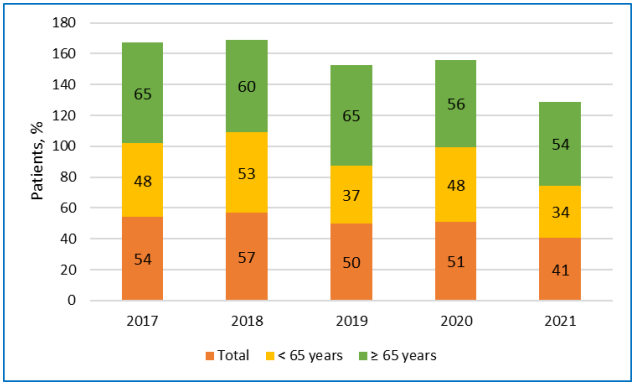

Figure 44. Distribution of patients with i-PTH in a target range of 150-600 pg/ml.

There were slight fluctuations over the five-year period studied, with no significant change in the overall trend.

6.8. Analysis of achieved target values simultaneously for serum Ca (2.15-2.55 mmol/L) and i-PTH (150-600 pg/ml).

Concurrent target values for serum calcium and i-PTH levels were achieved by 18.2 and 32.6% of patients in the overall group, 12.7 and 25.3% in the age group <65 years, and 26.7 and 47.5% in the patients ≥65 years (Fig. 45).

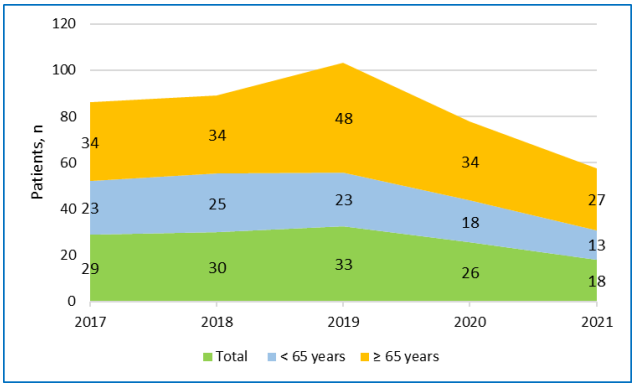

Figure 45. Distribution of patients with i-PTH 150-600 pg/ml and calcium 2.1-2.55 mmol/L.

6.9. Analysis of target values achieved simultaneously for three parameters - serum Ca (2.15-2.55 mmol/L), P (0.85-1.9 mmol/L) and i-PTH (150-600 pg/ml).

The three major biochemical parameters of CKD-MBD were achieved simultaneously in a small relative proportion of patients, ranging from 9.4-21.1% in the total group, between 6.3 and 15.7% in younger patients, and between 13.5 and 29.4% in the over 65 age group (Fig. 46).

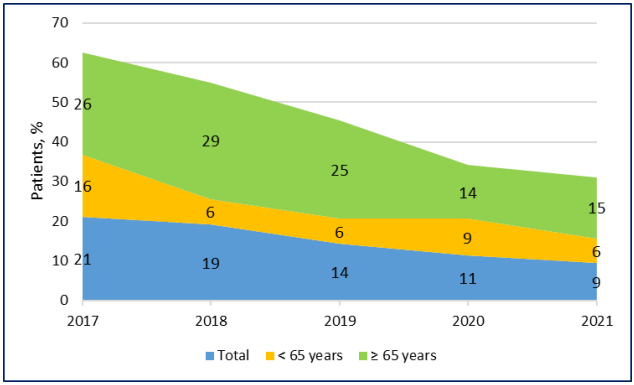

Figure 46. Distribution of patients with achieved three targets - Ca (2.1-2.55 mmol/l), P (0.85-1.9 mmol/l) and i-PTH (150-600 pg/ml).

7. Study outcome.

At the end of the study period, 72 (49.7%) patients continued hemodialysis treatment, 71 (49.0%) died, and two patients moved to another dialysis center (Table 11). In absolute and relative terms, death was more common in patients over 65 years of age, but no statistically significant difference was found between age groups.

Table 11: Distribution of patients according to study outcome.

Study outcome	Total		< 65 years		≥ 65 years	
	n	%	n	%	n	%
Alive	72	**49.7**	43	**57.3**	29	**41.4**
Died	71	**49.0**	31	**41.3**	40	**57.1**
Other outcome	2	**1.4**	1	**1.3**	1	**1.4**

We found no significant difference in the causes of death according to age. The leading cause of death during the study period was associated with infectious complications - 23 (32.4%) in the general population, 9 (29.0%) and 14 (35.0%) in the two age groups, respectively. Infections were related to vascular access (11 patients, 47.8%) and Sars-Cov2 infection (12 patients, 52.2%). Cardiovascular disease was the second leading cause of death, with 15 (21.1%), 6 (19.4%) and 9 (22,4%) in the whole group, in patients <65 and ≥65, respectively (Fig. 47).

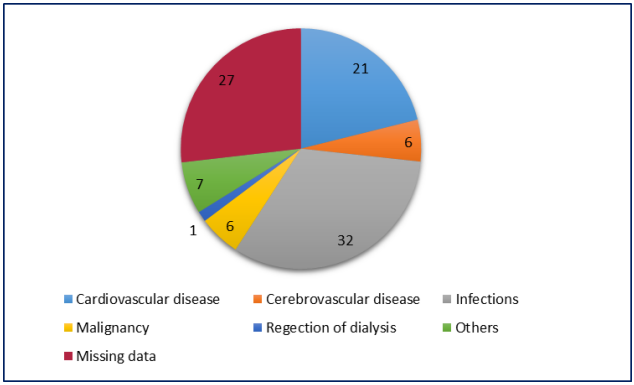

Figure 47. Distribution of the total cases of deaths by cause (%).

Discussion

Despite significant scientific advances worldwide, the diagnosis and treatment of CKD-MBD disorders associated with chronic kidney disease remain challenging in actual clinical practice. This single-centre ambispective study presents an analysis of the clinico-laboratory characteristics of abnormal CKD-MBD and the potential for correcting some of these abnormalities in patients with stage 5D CKD on hemodialysis treatment.

Demographic characteristics

Our study cohort of 145 patients had a mean age of 60.9±13.1 years, which is consistent with the mean age of the American (63.0±14.9 years) and general European (62.1 years) dialysis populations, younger by 4.5 years than the Japanese population (65.5±12.3), 10 years than the French population, and about 6 years younger than the Western European dialysis population.

The age profile of the study group is consistent with global trends in the age structure of the dialysis population, with 48.3% of patients aged ≥65 years and 51.7% in the <65 years age group. According to data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Practice Monitor - Hemodialysis, as of February 2021, the age of 51.2% of hemodialysis patients in the United States is 55-74 years, and 22.0% are aged ≥75 years. In Europe, as of December 2020, 45% of patients starting renal replacement therapy (RRT) are ≥65, and according to data from the Renal Epidemiology Information Network (REIN) registry (France), as of December 2017, there is an increasing trend in the absolute number of patients undergoing kidney replacement therapy, which is particularly pronounced in the group of patients aged 65-74, 75-84, and over 84 years.

Males dominated the dialysis population we studied, accounting for 62.8%. This percentage is entirely consistent with the gender distribution of patients in the general European (60% males) and American (59.1% males) dialysis populations. We found no significant difference by sex according to the age group of the patients. In the study population, males dominated the age group 50-59 years, and females dominated the 70-79 group.

In contrast to data for other dialysis populations, diabetic nephropathy was not the leading nephropathy in our study cohort of patients. It ranked third as a cause of chronic renal failure (CRF) in the whole group with a proportion of 16.0%, after chronic glomerulonephritis at 41.3% and hypertensive nephropathy at 18.7%. In comparison, diabetic nephropathy had a prevalence of 21% of ESRD cases in European countries, followed by hypertensive nephropathy (14%), "miscellaneous"

(14%), and chronic glomerulonephritis (11%). The prevalence of diabetic nephropathy is also significantly higher in the USA, accounting for 49.6% of CKD stage 5D cases as of February 2021. We also found differences in terms of underlying kidney disease according to age. Patients under and over 65 retained diabetes mellitus as the leading cause of ESRD in 22%, and in Europe, these amounted to 24%, respectively. However, in the patients we studied, the CKD ranking first in both age groups was hypertensive nephrosclerosis in 32.9% and 24.1%, respectively.

We found no significant age-related differences in BMI, unlike other authors finding lower BMI in patients over 65. According to M. Lassalle et al., obesity (BMI ≥30.0 kg/m2) was significantly more common in patients younger than 70 years in the French dialysis population. The BMI for the entire cohort we studied had a mean value of 25.0±5.40 kg/m2, a result similar to data from other European studies and DOPPS phase 5, but different from data for dialysis cohorts in China (21.8±3.7 kg/m2) and Japan (21.5±3.5 kg/m2). We found no data (BMI, serum albumin, and serum creatinine level before HD session) on which to argue that elderly patients suffer from malnutrition.

Worldwide, patients with CKD stage 5D have significant comorbidity. The majority of the 134 patients we studied (92.4%) had 1 to 3 comorbidities. The most common were hypertensive disease (96.6%), diabetes mellitus (28.3%), and various forms of ischemic heart disease (IHD) (37.2%-48.3%). Similar data have been reported for other dialysis populations, although there are also differences. According to data from the 2017 Annual Report Digest of REIN registry in the French dialysis population, the prevalence of diabetes mellitus and cardiovascular disease in incident dialysis patients was higher, estimated at 47 and 57%, respectively. In a subanalysis of 6307 patients from a multicentre European study of CKD-BMD disorders in haemodialysis patients (COSMOS), a significantly higher incidence of cardiovascular disease was found in 72.1% of cases. In a cohort of 5132 patients aged less than 70 years and 5874 aged more than 70 years, M. Lassalle et al. found a significant difference in the incidence of diabetes mellitus, ischaemic heart disease (IHD), chronic cardiac insufficiency, rhythm disturbances, peripheral vascular disease, cerebrovascular accidents and age-related carcinomas. We found significant age-related differences in the incidence of IHD (chronic IHD) and rhythm disorders recorded at inclusion in the study but did not find these for other comorbidities and new cases during the study.

The mean duration of dialysis treatment in patients undergoing dialysis treatment before inclusion in the study was 37.8 ± 63.0 months (median 57.6 months (IQR 20.1-89.2)). The duration of dialysis treatment of our study patients was similar to that of patients in Eastern Europe and was lower than that in Western European countries covered in COSMOS and Japan, as reported in DOPPS phase 5. Patients in

the younger age group underwent more extended dialysis treatment than patients aged 65 years and older (mean 43 months compared with 32 months), but we found no significant difference by age.

As assessed by URR%, dialysis therapy was effective in both age groups, and there were no significant differences.

The use of a tunnelled catheter as vascular access for hemodialysis was significantly higher than commonly reported in other dialysis populations and was similar in both age groups. This difference may be explained by a significant relative proportion of elderly patients and age-associated concomitant vascular pathology, diabetes mellitus, patient reluctance to construct an arterio-venous anastomosis, etc.

Features of mineral metabolism and bone metabolism. Biochemical profile

Biochemical profile. Serum calcium

The accumulation of multiple lines of evidence for an existing association between body calcium load, hypercalcemia, and vascular calcification with increased cardiovascular morbidity and mortality in patients with CKD has made nephrologists worldwide more careful in monitoring serum calcium. According to DOPPS phase 5 data, mean serum calcium concentrations ranged from 2.31 mmol/L in Europe and the United States to 2.27 mmol/L and 2.28 mmol/L in samples for Japan and China. For 2016-2020, the DOPPS Monitoring report communicated a continued downward trend in serum calcium. In our study cohort of dialysis patients, the mean serum total calcium concentration ranged from 2.15 to 2.24 mmol/L during the monitoring period. We report similar data in both age groups of patients. There was no age-determined difference in the level of calcemia throughout the study period, as was pointed out by S. Pelletier et al. in their results. We found no correlation between age and serum calcium level (-0.021 at $p<0.01$).

The use of different concentrations of calcium in the dialysis solution (1.25, 1.5 and 1.75 mmol/L) was based on the evidence that its increase leads to increased calcium import into the patients and reduces PTH synthesis and secretion. Conversely, lowering it results in a slight decrease in the serum calcium level but stimulates PTH secretion and increases bone turnover. In our study, the dialysate calcium concentration used was 1.5 mmol/L according to KDIGO recommendations. The lower concentration of 1.25 mmol/L and the higher concentration of 1.75 mmol/L were used for a short period (in 2017) of the study in cases of adynamic bone disease and hypocalcemia.

Biochemical profile. Serum phosphorus

Control of serum phosphorus has been identified as a cornerstone in managing CKD-MBD in patients with CKD stage 3-5, ensuing from its importance as a contributor to impaired mineral metabolism, development of renal bone disease, bone fractures, vascular calcification, and increased cardiovascular mortality. Achieving target values is difficult in clinical practice in hemodialysis patients, so current guidelines recommend that therapy aim to achieve near-normal laboratory values.

Hyperphosphatemia is an almost universal abnormality in patients with ESRD, and regardless of available therapeutic options, its incidence remains high. From 2016 to 2020, according to DOPPS data, serum phosphorus levels continue to rise in the US dialysis population, and the relative proportion of patients with phosphorus >2.26 mmol/L has increased from 13% to 18%. By August 2021, the mean serum level in the same population was 1.8±0.01 mmol/L. In the dialysis cohort we studied, mean serum phosphorus ranged from 1.94±0.63 to 2.1±0.72 mmol/L, highlighting hyperphosphatemia as a current problem. Similar results were reported in China (1.94±0.66 mmol/L) and significantly better ones in Western Europe (1.57±0.49 mmol/L) and Japan (1.75±0.43 mmol/L). The reasons for these differences are probably multiple: a higher proportion of elderly patients ≥65 years maintaining lower levels and easier control of phosphatemia in different populations, a higher prevalence of diabetes mellitus as an underlying or concomitant pathology, more frequent conventional dialysis with high flux dialysers, more effective use of patient education programs, financial and marketing differences, different local rules in clinical practice, and last but not least different patient cooperation.

Serum phosphate levels depend on a patient's age. Our results confirm the findings of S. Pelletier and I. Kiss: patients aged ≥65 years have significantly lower serum phosphate levels than younger patients. The lower serum phosphate level in the elderly patients studied is most likely due to reduced dietary phosphate intake and low bone turnover. Age correlated negatively and moderately in strength with serum phosphate level at almost all periods in our observation.

Biochemical profile. Calcium-phosphate product

Because of the minor variations in serum calcium level and the possibility of marked ones in serum phosphorus values, the value of the calcium-phosphate product is more determined by the latter. The data in the present study showed a statistically significant ($p<0.05$) difference in the calcium-phosphate product between the two age groups. Adults had lower calcium-phosphate product values, ranging from 3.8±1.2 to 4.0±1.4 mmol2/L2, all at target levels, compared with 4.8±1.6 to 5.2±1.6 $mmol^2/L^2$ in patients in the age group younger than 65 years. It is now recommended that nephrologists' efforts be directed towards maximizing serum calcium and phosphorus levels, which will also result in a target value for the calcium-phosphate product.

Biochemical profile. Parathyroid hormone

The optimal PTH level in hemodialysis patients remains an unspecified parameter. According to the KDIGO 2017 recommendations, it could be 2 to 9 times above the upper standard value of PTH for the respective laboratory, which is 65 pg/ml, including the Central Clinical Laboratory of the University Hospital - Pleven. Thus, the optimal value of i-PTH for patients with CKD 5D is defined in the range 130(150)-600 pg/ml. The mean value of i-PTH in the general population in our study had a minimum value of 467±431 pg/ml and a maximum value of 558±481 pg/ml. Its level was higher in patients under the age of 65 years, 689±511 pg/ml and 585±500 pg/ml, compared with the level in patients over 65 years, the maximum mean value of 433±357 pg/ml, the minimum mean value of 324±220 pg/ml. As of August 2020, the mean PTH in the US was 491.1±10.7 pg/ml. In Europe, the DOPPS phase 5 data indicated a mean i-PTH of 333±320 pg/ml and in Japan - 149±130 pg/ml. When interpreting and evaluating these results, one should bear in mind that populations vary and that there are different rules at a national level to which the relevant specialists adhere. Based on an analysis of data from the national registry of Japanese dialysis patients, with mortality as the "endpoint", the Japanese Society for Dialysis Therapy recommends the following levels as the most appropriate for the Japanese dialysis population: i-PTH 60-240 pg/ml, serum calcium 2.1-2.5 mmol/L, serum phosphorus 1.13-1.938 mmol/L. In an analysis of data on 4500 patients on maintenance hemodialysis treatment from 20 European countries, J. Fernandes-Martin et al. found a minimum relative risk of death (all-cause) at i-PTH levels of 398 pg/ml and a safe interval (the interval with the lowest relative risk of death) between 168 and 674 pg/ml for the European dialysis population. The authors suggest these values could be an addition or alternative to the KDIGO recommendations.

A significant difference in hormone levels according to age was found in 10 of the 60 months of follow-up. We relate the results to the way i-PTH was followed up and investigated in the present study and believe that our data are consistent with the results of other authors. In a sub-study of vitamin D levels in the dialysis patients we studied, we found that older dialysis patients had significantly lower parathyroid hormone (PTH) levels, 199 (Q1-3: 96-421) compared to 435 (Q1-3: 240-772) at p=0.000. There was a moderate to strong negative correlation between age and i-PTH level.

Biochemical profile. Total Alkaline phosphatase

Despite the higher mean value of total ALP in the younger age group studied, we did not find a statistically significant difference compared with the patients over 65. The mean alkaline phosphatase in the total group of patients had a value of 109-118 IU/L in the periods studied, which, in combination with the level of i-PTH,

indicated a trend toward high bone turnover states. The present study found a low statistically significant inverse correlation between age and serum total ALP level.

Biochemical profile. 25(OH)D

A study of vitamin D status revealed insufficient levels in 55% of the patients. The lower result compared to the data of J. Guillaume et al. can be explained by the characteristics of our study patient population - an essential risk factor for Vit. D deficiency and states like diabetes mellitus, obesity and malnutrition were less prevalent. Besides, the study coincided with the COVID-19 pandemic, during which increased intake of the vitamin as a dietary supplement was recommended. There was a significant negative correlation between age and 25(OH)D level in agreement with the results of the authors cited above. The available evidence that 25(OH)D exerts an effective paracrine effect on the parathyroid glands and leads to a significant reduction in i-PTH levels in patients with CKD stage 3-4 make us believe that optimisation of therapy and maintenance of a level of >50 ng/ml of native Vit. D in our study population would have a positive effect. For effective control, regular testing of its serum level is necessary and should be formally regulated.

Biochemical profile. Serum magnesium

Recent observational studies have found that higher serum magnesium levels in hemodialysis patients are associated with lower cardiovascular and total mortality, probably because the phosphatemia-cardiovascular mortality association is "neutralised" at higher magnesium levels. M. Vervloet, therefore, identified magnesium as a modifier of phosphate toxicity. Using a dialysis solution with a magnesium concentration of 1.5 mmol/L, we report a mean serum magnesium value of 1.32±0.24 mmol/L in the general population in both age groups, i.e. the presence of mild hypermagnesemia. We found no significant age differences or evidence that age correlated with serum magnesium level (-0.183, $p < 0.01$) in our study patients.

Bone disease. Bone metabolism

Accurate assessment of bone disorders in CKD stage 5D, for a given patient, in a given period can only be done with bone biopsy and histomorphological examination. Bone biopsy is not a routine method, and the most commonly used in clinical interpretation of bone turnover is PTH. According to S. M. Sprague et al., PTH has a fair but not suboptimal ability to assess bone turnover. To avoid excessive suppression of bone turnover and treat patients with high serum PTH but histologically low bone turnover, KDIGO extends the target values for PTH mentioned above. S. M. Sprague et al. found that PTH levels 2 to 9 times the upper average value had high specificity (85.8%) but low sensitivity (37%) in distinguishing high from non-high bone turnover. In distinguishing low from non-low bone turnover, PTH had equivalent

sensitivity and specificity, 65% and 67%, respectively. The authors found the predictive diagnostic accuracy of PTH to be analogous to bone specific ALP. Combining PTH and bone specific ALP may improve low and high bone turnover differentiation. In light of these statements, in the population examined in our study, those with PTH <150 pg/ml and possibly low bone turnover were between 16% and 20% of the patients in the general group, 13% and 16% in the group younger than 65 years, and between 22% and 28% in patients older than 65. The incidence we found was lower than that reported by H. Malluche et al., who found evidence of low bone turnover in 62% of 630 bone biopsies in Caucasian dialysis patients. Those with high bone turnover (i-PTH>600 pg/ml) were 23-36% of the general patient population, 31-49% of patients younger than 65, and 14-24% of patients older than 65. These results were higher than those reported in DOPPS phase 5.

Bone fractures

The evolution of CKD has been associated with an increasing incidence of bone fractures, peaking in patients on dialysis treatment. In a study by F. Tentori et al., the overall incidence of new bone fractures (femoral and all bone fractures) in dialysis patients was higher than in the general population. According to data from a number of studies, 10 to 52% of hemodialysis patients have experienced bone fractures. Besides traditional risk factors - advanced age, low BMI, gender, familial history of osteoporotic fractures, prior fracture, smoking, low serum albumin, low Vit. D, and falls, in patients with CRF have the additional effects of uremic toxins, high serum phosphate, SHPT, adynamic bone disease (ABD), low vit. K levels, the influence of medications, reduction of bone mass with disturbed microarchitectonics, disturbed bone remodelling process, defective mineralisation, etc. We found a total of 40 (in 27.6% of patients in the total group) cases of fractures in the study population, which included 25 (17.3%) fractures experienced before inclusion in the study and 15 (10.4%) new cases of fractures recorded during the study. There was no statistically significant difference by age in total number of fractures, time of occurrence, or location. Lower extremity fractures were the most common cases. A number of studies have suggested that patients on dialysis treatment have a 4-fold higher risk of femoral fracture compared with individuals of the same age, sex and ethnicity. We found lower limb fractures in 22 (15.1%) of the total cohort of patients, and 14 of these (63.6% of patients with lower limb fractures) had a femoral fracture. The femoral fracture was present in 9.7% of all patients examined in the study (n=145).

In contrast to our results, in the DOPPS phase 2 study of 12782 dialysis patients from 12 countries, M. Jadoul et al. found that older age and female sex had predictive value for new femoral fractures as well as for new fractures of any type. The authors also reported that 2.6% of dialysis patients had a history of a previous femoral fracture and that this incidence varied between countries (from 1.4% for Germany to

3.9% for France). During the follow-up period of the study mentioned above, 174 cases of new femoral fracture and 489 new cases of fracture of any type were reported. The high incidence of bone fractures in dialysis patients, the possibility of disability, the risk of a new fracture, rehospitalisation, and the increased risk of death in the postfracture period highlight the need to detect patients at risk for bone fracture early and to implement the appropriate therapeutic measures in each case.

Calcifications in extraosseous structures

Analysing data from multiple observational studies among dialysis patients, B. Caplin et al. summarised that, regardless of the heterogeneity of the populations studied and the study method, vascular calcifications are highly prevalent among patients with CKD. Between 60 and 90% of dialysis patients have vascular calcifications, most commonly associated with advanced age and duration of dialysis treatment. Other risk factors include calcium load, serum calcium (coronary calcification) and phosphates levels, calcium-phosphate product, PTH (aortic calcification), C-reactive protein, concomitant arterial hypertension, and others. The authors also conclude that assessing the severity of arterial calcification is a helpful tool for predicting associated bone pathology and long-term prognosis in patients. Evidence that early detection of vascular calcification will lead to real patient benefits is still lacking, as is evidence that vascular calcification is reversible or attempting to slow progression and improve patient outcomes. Routine testing for vascular calcifications is, therefore, not recommended by the available guidelines. In clinical practice, lateral abdominal radiography and echocardiography may be alternatives to CT-based methods of investigating vascular calcifications. Our data showed that in clinical practice, imaging studies with other indications revealed a total of 60 (41.4%) cases of vascular calcification, with the majority of these cases having multiple calcifications.

The most commonly affected arteries were the abdominal aorta in 19.3% of patients and the arteries of the lower extremities (femoral arteries, 12.4%, and iliac arteries in 11% of the patients). M. Kraus et al. studied 275 haemodialysis patients and found that 77% of patients had evidence of calcification in the abdominal aortic wall, and its incidence and severity were significantly more severe in elderly patients. We found no age-dependent significant difference in the incidence of vascular calcification, in contrast to the data of I. Kiss et al. We believe that the lower reported incidence of vascular calcification in our study does not indicate that the pathology is less frequent in the study population. The lower incidence was most likely since a large proportion of imaging studies were not performed with the indication of detecting vascular calcifications. The lack of a statistically significant difference in vascular wall calcification by age is due to the above reasons. It also corresponds to some features

of vascular calcification in CKD - its development starts earlier, in younger individuals, especially those with CKD stage 5D and has a progressive course.

Calcification of the heart valves is also common - in 20% to 47% of patients on dialysis treatment. We found positive results for cardiac valve calcinosis in 33 patients (22.7%). The mitral valve was affected more frequently (19 patients - 13.1%) than the aortic valve - 14 cases (9.6%). No significant difference was confirmed to depend on the age of the patient, which differs from the data of M. Kraus et al. Interpretation of the results of coronarographies is difficult due to their small number.

Although frequently identified in clinical practice as a concomitant pathology, vascular calcifications are not always assessed in the focus of CKD-MBD disorders and cardiovascular risk. They should be used as an additional important target in the therapeutic plan of impaired CKD-MBD to improve the long-term prognosis of patients on dialysis.

Analysis of ongoing therapy

Cholecalciferol

Due to the self-provision of 25(OH)Vit D by patients, reliable data is necessary on the frequency of use and doses administered. It is reasonable to include regular testing of serum levels in national standards for dialysis treatment. Substitution should provide a 25(OH)D level >30 ng/ml, even >50 ng/ml, to avoid increased PTH synthesis, a decrease in bone mineral density and increased fracture risk, and for the autocrine and paracrine effects of the vitamin to take place.

Treatment with vitamin D receptor activators

In the absence of contraindications and with an appropriate biochemical profile of the patient, treatment with active vitamin D and other VDRAs is part of the therapy of SPTH in dialysis patients. The frequency of use of VDRAs and the type of VDRAs used varies worldwide. In the United States, for the period 2017-2021, approximately 80% of dialysis patients received active Vit. D (of any type), 36% were treated with calcitriol, 9.7% with paricalcitol, and 51% with doxercalciferol. Slightly more than 50% of the patients received intravenous therapy with the same medications. A high prevalence of VDRAs use was reported in Europe and Japan in 79 and 75% of patients. In our study cohort, only two types of VDRAs, calcitriol and paricalcitol, were used; oral intake dominated, with a total of 33.8 to 42.7% of patients having active vit. D in the study period. In contrast to S. Pelletier et al. we found no significant difference in the frequency of VDRA therapy according to age in a substantial part of the study period. Older patients received more frequent VDPA, but more frequently at lower doses (0.75 and 0.875 mcg/week). Although the mean i-PTH was higher in younger patients, they had significantly more frequent hyperphosphatemia, which

limited therapy with active Vit. D. The biochemical profile of patients over 65 was with better serum phosphate values, and, in some of them, an indication for treatment with Vit. D was also a state of hypocalcemia. There was a significant difference in the mean weekly dose of VDPAs in the two age groups - it was higher in the younger age group, which is consistent with data from other investigators.

Phosphate-lowering medications

The importance of hyperphosphatemia in the complex of disorders of mineral and bone metabolism and fracture risk, vascular calcification and increased risk of cardiovascular and total mortality justifies the need for its correction. It makes phosphate binders a necessary part of the therapeutic plan. T. Isakova et al. reported that using phosphate binders (of any type) is independently associated with improved survival in patients starting hemodialysis therapy. According to DOPPS Monitor data, between 78% and 87% of dialysis patients in the United States took a phosphate-lowering drug between 2015 and 2021. By early 2021, 44% of patients were on calcium carbonate therapy, 23% on sevelamer, and combination therapy (calcium-containing binder and sevelamer) was registered in 11% of patients. Iron-containing binders (as of May 2015), which have not been introduced in clinical practice in our country, were used in 14% of patients. For Europe, the DOPPS phase 5 data are similar, with 79% of patients on phosphate trap therapy, 23% on calcium-containing phosphate trap monotherapy, 21% on sevelamer monotherapy, and 12% on combination therapy of calcium-containing phosphate trap and sevelamer. Data analysis on calcium carbonate therapy in the present study showed that between 80 and 95% of patients take calcium carbonate. Some of the subjects take the preparation because of hypocalcemia. Patients in the younger age group received calcium carbonate more frequently at the beginning of the study period. At the end of the study, however, the older patients received calcium carbonate therapy more often. There was no significant age difference in the frequency of phosphate binders use, in contrast to the results reported by I. Kiss et al., who found this in a national study in Hungary: older dialysis patients more frequently used calcium-containing phosphate binders. We note that the older patients needed a significantly lower dose of calcium carbonate, as was also reported by S. Pelletier et al. The low-dose regimens most commonly used were 600-1800 mg/day calcium carbonate, corresponding to 240 to 720 mg elemental calcium daily. Therefore, despite the frequent use of calcium carbonate, we assume patients are not at high risk for calcium loading.

Treatment with sevelamer (hydrochloride or carbonate) was carried out in between 17.5 and 37.2% of the patients we studied, a result comparable to that of other investigators cited above. Because of the significant differences in phosphatemia in the two age groups, younger patients under 65 were more often indicated for therapy with this phosphate trap. The mean and most frequently used doses did not show significant

differences in the two age groups due to the need to limit the maximum amount of the drug in case of side effects, lack of good patient compliance, combination therapy with calcium carbonate, etc.

The phosphate-binding capacity of phosphate scavengers is commensurate, but due to accumulating evidence of increased calcium load with the use of calcium-containing phosphate binders, progression of vascular calcification, and the possibility of suppression of bone metabolism, as well as the additional benefits of using sevelamer (lowering LDL-cholesterol level, delayed progression of vascular calcification, lower levels of calcium-protein particle 1 (CPP-1) and interleukin 8), we agree with the opinion of many authors on the need to limit calcium-containing phosphate trap therapy, which is not well defined in terms of indication and dose regimen. However, the need for its complete negation is unproven and, therefore, unjustified.

Calcimimetics. Cinacalcet

Initially used in patients with severe, resistant to conventional therapy SHPT, cinacalcet may now be used as the first agent of choice in SHPT therapy as well as in vitamin D receptor activator combination therapy. Multiple extensive studies confirm that cinacalcet effectively improves biochemical control of SHPT in patients with CKD stage 5. Cinacalcet therapy resulted in a 54% reduction in bone fractures and a 93% reduction in the need for parathyroidectomy. In a randomised controlled trial, P. Raggi et al. found that treatment of moderate to severe SHPT for 20 weeks with cinacalcet in combination with low-dose calcitriol or paricalcitol reduced the rate of calcification in the aorta, aortic and mitral valves (significant only for the aortic valve). The drug has been used to treat SHPT in 13-34% of the European dialysis population, 24% - of Japanese dialysis practice, and up to 30% for 2017-2021 in the US. Our study shows a similar incidence of using cinacalcet to treat SHPT. Between 23% and 33% of patients younger than 65 and 5 and 11% of patients older than 65 were on calcimimetic therapy. The difference is statistically significant, as reported by other authors.

In contrast, we did not find lower mean doses in patients older than 65. The most commonly used daily doses were below 60 mg (15, 30 and 45 mg), as P. Urena et al. reported in a comparative study of the use and effectiveness of SHPT therapy with cinacalcet in 12 European countries. Several main reasons can be discussed regarding the dose of the calcimimetic: clinical and laboratory side effects, reasons for dose reduction or temporary discontinuation of the drug, nonadherence to therapy in some patients, the severity of SHPT, temporary difficulties with the supply of the medication, etc. Reserves for improving the effectiveness of treatment are seen in improving the effectiveness of hemodialysis treatment and patient compliance, as well

as increasing the use of parenteral preparations (e.g. etelcalcetide), which allow greater objective control of therapy.

Patients without therapy for CKD-MBD disorders

The total number of patients without therapy for CKD-MBD was small. More patients aged 65 years and older in absolute numbers did not need treatment compared with the younger age group.

Concomitant therapy with effect on CKD-MBD

We identified the use of three main drug groups that have proven effects on bone disease, vascular calcification, etc. These are anticonvulsants, indirect anticoagulants and corticosteroids. Vitamin K antagonists have been "incriminated" in accelerating the process of vascular calcification, most likely by exacerbating functional vit. K deficiency. Seven of the patients we studied received sintrome (acenocoumarol) for cardiovascular disease, and use was more commonly in the younger age group.

Collaboration of the patients

The question of a patient's role and involvement in therapy is always relevant. Therapy adherence is an essential factor that can influence the effectiveness of any treatment, including that for impaired CKD-MBD. Analysing data from 44 studies on the issue, S. Ghimire et al. indicated a significant variation, from 13 to 99%, of nonadherence to oral therapy in hemodialysis patients. The reasons for non-compliance with the prescribed treatment are multiple factors related to the characteristics of the patient, the disease and the characteristics of the therapy itself - young age, smoking status, living alone, duration of haemodialysis treatment, comorbidities such as hypertension and diabetes mellitus, frequent hospitalisations, depressive state, number of medications taken per day, total number of tablets taken per day, number of phosphate trap tablets, complex drug regimen, etc. Amidst the numerous instructions, restrictions and requirements concerning the complex treatment of dialysis patients, many find it difficult to comply with the prescribed therapy without understanding its significance, discussing only changes in laboratory parameters and related complications that seem abstract and indefinitely distant in time. Our analysis of the studied patients' collaboration revealed no significant age-dependent difference. The lack of a significant correlation with age, sex, or duration of dialysis treatment, the number of medications taken, and the number of tablets taken daily were also reported by M. Arenas et al. in a study of adherence to phosphate binding therapy among 165 hemodialysis patients - 21% of whom did not adhere to the treatment assigned. Further analysis of the reasons for lacking collaboration in the

population we studied could positively affect the final results from treating disorders of CKD-MBD.

Therapeutic outcomes

In an analysis of data on the achievement of target levels of several parameters (serum calcium and phosphorus, PTH, 25(OH)D, haemoglobin, blood pressure, serum LD-cholesterol and serum bicarbonate) in the EURODOPPS phase 4 trial (2009-2011) involving 7 European countries (Belgium, France, Germany, Italy, Spain, Sweden and the United Kingdom), S. Liabeuf et al. reported a low overall level of achievement of recommended values, with significant differences between European countries. Similar results were reported by J. Wang et al. in a comparative study of CKD-BMD in China, Japan, North America and Europe, using data from DOPPS Phase 5 (2012-2015).

Therapeutic outcomes. Serum calcium

Control of serum calcium levels in patients with CKD on dialysis treatment should be aimed at maintaining it in the normal range of 2.1-2.55 mmol/L. Hypercalcaemia >2.6 mmol/L should be avoided because of its proven importance for vascular calcification and increased risk of death from cardiovascular and general causes in dialysis populations in multiple studies. The importance of hypocalcemia (<2.1 mmol/L) in increasing the risk of total mortality in dialysis patients has not been demonstrated in all relevant studies. The need for its adjustment is pertinent because of its influence on CKD-BMD, hemodynamic instability during HD sessions, risk for rhythm disturbances, and clinical symptoms. In our study, the whole cohort and the two age groups were dominated by patients with achieved target values for serum calcium in the range of 2.1-2.55 mmol/L, an average of 57% in the three groups studied. The proportion of patients with calcium <2.1 mmol/L remained high, between 33 and 48%, but hypercalcaemia was rare, occurring in 4 to 5% of patients. These results differed from those reported in other dialysis populations, with an average of 82% of dialysis patients in Europe: 77% in the USA, and 71% in those in Japan having target values, at the expense of a lower proportion of patients with hypocalcemia. Unlike I. Kiss et al., we found no significant difference in this indicator depending on the age of the patients. The increase in the relative proportion of patients with hypocalcemia is most likely related to insufficient dietary calcium intake, low calcium doses in substitution therapy, problems with the patients' collaboration, concomitant treatment with cinacalcet, etc. The problem requires further analysis for more precise correction.

Calcium concentration in dialysate solution

As of 2018, all patients were dialyzed with a dialysate solution calcium concentration of 1.5 mmol/L. Using a dialysate calcium concentration of 1.5 mmol/L is the most common practice in other European countries - Italy (65%) and France (91%).

Therapeutic results. Serum phosphate

As recommended by KDIGO, serum phosphate control in patients with stage 5D CKD aims to achieve normal or near-normal serum levels. Multiple observational studies have demonstrated a J-type curve of phosphatemia - cardiovascular and all-couse mortality relationships in dialysis patients. Both high and low phosphate values increase the relative risk of death. The adverse effect of hyperphosphatemia has been demonstrated in single measured high values, in the mean values of serial studies, and in studies of the duration of the period in which it persisted as an outlier. There are three main options to control phosphatemia: a low-phosphate diet, optimal dialysis and use of phosphate binders. However, the complex pathogenesis of CKD-BMD and the interdependence of its many factors lead to the fact that the treatment of one deviation may affect another. The latter, in turn, requires new therapy changes, making it complex and difficult. Many authors have reported significant variations in therapeutic outcomes. We chose an upper limit of phosphate levels of 1.9 mmol/L because the results of multiple observational studies suggest that the risk for adverse events increases significantly with phosphatemia values above this level. We found a significant age-related difference in the achievement of target serum phosphate values. Patients older than 65 achieved a phosphate level of 0.85-1.9 mmol/L significantly more often (50-64%) than younger patients (29-60%). Possible reasons for this are the considerably lower serum phosphorus values in the over 65 age group due to lower dietary intake, low bone turnover, better dialysis dose, and better treatment compliance from some of them. Similar differences between the two groups were observed in the incidence of hypophosphatemia <0.85 mmol/L (2% to 36% in older patients, compared with 2% to 21% in younger ones) and hyperphosphatemia >1.9 mmol/L, which was more common in patients younger than 65 years. For a number of European countries and Canada, a similar or lower incidence of hyperphosphatemia >1.8 mmol/L was reported as follows: France - 26%, United Kingdom - 36%, Spain - 28%, Belgium - 29%, Italy -33%, and Canada - 38%. We can note the positive trend over the study period, a decrease in the incidence of cases with phosphate >1.9 mmol/L and an increase in the incidence of cases with a desirable level of phosphatemia in both age groups of patients.

Serum total alkaline phosphatase is a biochemical marker for diagnosing and monitoring bone turnover. Studying a cohort of 739 600 dialysis patients in the national database of hemodialysis patients at DaVita dialysis clinics in the United States, Deborah Regidor et al. demonstrated that regardless of serum calcium, phosphorus,

and PTH levels, t-ALP >120 IU/L was associated with an increased risk of death in dialysis patients (HR 1.25 (95% CI 1.21-1.29). Higher t-ALP in the authors' cohort was associated with higher PTH levels, younger age, female sex, and diabetes mellitus. Patients with i-PTH >600 pg/mL were more likely to have t-ALP >120 IU/L. No age-specific differences in enzyme levels were found in our study population. Patients with t-ALP dominated the entire group and the two age groups in the 80-120 IU/L range. However, over 30% of patients fell into the high-risk group because of t-ALP levels above 120 IU/L.

Therapeutic outcomes - parathyroid hormone

Given the risk for overall and cardiovascular mortality in dialysis patients and its association with bone turnover and pathology, the desirable serum PTH level for patients with CKD stage 5D should be in the range of 2 to 9 times the upper normal limit, which is usually 65 pg/mL. On average, 50.4% of patients in the total group, 44% of patients aged below 65 years, and 60.3% of patients aged ≥65 years achieved target i-PTH levels. As described above, 18%, 16%, and 28% of patients in the overall cohort and under- and over-65 age groups, respectively, had probable low bone turnover on average. Patients with evidence of SHPT and high bone turnover, respectively, were more common in the younger age group, averaging 42% over the entire period, compared with 17% in the over-65 group. In the overall group, this proportion averaged 32% of patients. As with other biochemical markers of CKD-BMD, there were significant differences in PTH control across countries and regions of the world. The PTH level in prevalent hemodialysis patients depends on the duration of HD and in those newly started on dialysis treatment on its level before starting HD therapy. There are different targets in treatement, and while PTH up to 300 pg/ml is within the desirable range in European countries and the USA, in Japan, the target range is 60-240 pg/ml. Compared with data from other European countries (DOPPS phase 5), in the total patient group, we report a higher proportion of patients with high bone turnover and a lower incidence of ABD, which correlates with problematic control of hyperphosphatemia.

In a nationwide study of the Hungarian dialysis population, I. Kiss et al. reported a significant difference in the incidence of achieving the targets of all CKD-BMD parameters, which was higher in patients older than 65 years. The lack of a substantial difference between the two age groups in our study regarding PTH can be explained by the difference in the frequency of PTH testing in clinical practice, so our data does not contradict the results of other authors.

Analyzing data from DOPPS phases 1 (1996-2001) and 2 (2002-2004) involving seven countries - France, Germany, Italy, Spain, the United Kingdom, Japan, and the United States, E. Young et al. reported that only 6% of dialysis patients

achieved all four parameters (Ca, P, CaxP and PTH) of the mineral and bone metabolism according to the K/DOQI criteria. Achievement of target values for two or three parameters (Ca, P, PTH) has been monitored in the US dialysis population. For the period 2017-2020, an average of 30% of patients achieved simultaneous serum calcium levels of 2.1-2.55 mmol/L, serum phosphorus levels of 1.13-1.78 mmol/L, and PTH levels of 150-600 pg/ml. On average, 40% of the study population (10,770 dialysis patients) had two indicators achieved, 24% of patients had achieved only one indicator, and 5% of all patients had all three indicators outside the desired range. Concurrent target values for serum calcium, and i-PTH were achieved by 18.2 and 32.6% of patients in the whole group, 12.7 and 25.3% in the age group <65 years, and 26.7 and 47.5% in the older patients ≥65 years in our study. The three major biochemical parameters of mineral and bone metabolism were achieved simultaneously in a small proportion of patients, 9.4-21.1% in the total group, 6.3 - 15.7% in younger patients, and 13.5. -29.4% in the age group ≥65 years. According to I. Kiss et al., in a study of mineral metabolism in the Hungarian dialysis population of 5008 patients, 15.8% and 19.8% in the age groups under 65 and over 65 years, respectively, achieved the target goals of all three significant biochemical markers of CKD-MBD, and the reported difference was statistically significant. Our results were similar, but the small number of patients in the analyzed groups did not allow the determination of statistical significance.

Study outcome

During the study, 72 patients died. The two most common causes of death were: 1. Infections; 2. Cardiovascular disease. We found no age differences in the incidence and cause of death between patients in the two age groups. We believe the reason why mortality from infectious causes dominates over cardiovascular causes is the COVID-19 pandemic.

Chronic kidney disease is a significant public health problem. Its high incidence against the background of a global trend of the ageing of the human population and the multiple complexes, interrelated and progressive homeostatic disorders associated with disrupting renal function determine their importance as a risk factor for increased cardiovascular morbidity, bone disease and fractures, and increased risk of premature death due to cardiovascular and other causes. Disorders of mineral and bone metabolism associated with chronic kidney disease are significant causes of multiple adverse events and outcomes in patients with chronic renal failure.

The continuous drive in medicine to improve patient outcomes and prolong their life expectancy at the best possible quality has led to extraordinary advances in understanding the pathogenesis of CKD-MBD and their therapy. However, impaired

mineral and bone metabolism in CKD are still a challenge in clinical practice, and many of the desired goals recommended in specialty guidelines still need to be improved in all dialysis patients. There are differences, sometimes significant, between countries, regions and dialysis units. The reasons for such differences are multiple. The specific gene pool and the influence of social, economic, cultural, health, and other environmental factors render particular characteristics of individual dialysis populations, which in turn influence the manifestation of mineral and bone disorders. National, local, and other rules modify the possibilities of therapeutically influencing existing abnormalities in mineral and bone metabolism. And as a result of all the factors mentioned above, the final results are different.

Continuous monitoring, analysis and evaluation at local and national levels of mineral and bone metabolism are critical for identifying current problems, assessing available options and their effective use, the results achieved and present failures. This will make it possible to identify the most appropriate targets in therapy for a given population, as well as reserves for further improvement.

Conclusions

1. Different dialysis populations, according to age, underlying kidney disease, concomitant pathology, dialysis treatment and other factors, have specific characteristics influencing the presentation and therapy of MBD in stage 5D CKD.

2. Disorders of mineral and bone metabolism are age dependent. The levels of serum phosphate, PTH, serum albumin and creatinine, and Vit. D were significantly lower in dialysis patients older than 65 than in patients younger than 65. Age over 65 correlated negatively with serum phosphate, PTH and 25(OH)D levels.

3. Therapy of MBD disorders is age dependent. Patients older than 65 years require lower doses of active vitamin D, phosphate binders, and calcimimetics, achieving better results in controlling biochemical indicators of MBD.

4. Compared with other authors, we report good results in monitoring MBD associated with CKD stage 5 D, in a comprehensive approach to therapy, in increasing the use of calcium-free phosphate binders and calcimimetics, but also more pronounced problems in controlling hyperphosphatemia and high turnover bone disease.

5. We find possible reserves to improve the control of CKD-MBD in monitoring and therapy of 25(OH)D deficiency, optimization of complex treatment of high serum phosphate levels, improvement of patients' collaboration, diagnosis and treatment of bone disease in the direction of fracture risk reduction, including with antiresorptive drugs, utilization of treatment options with vit. K1 - to improve bone health and cardiovascular risk.

We believe it is necessary to establish a registry of dialysis patients in Bulgaria to track multiple indicators, especially those proven to be associated with morbidity and mortality. Their periodic analysis would help control them better and improve dialysis patients' survival in our country.

Contributions

Original:

1. A longitudinal study was conducted to follow up on mineral and bone disorders in a Bulgarian dialysis patient population in one dialysis unit for a period of five years.

2. A comprehensive analysis and evaluation of the available disturbances in biochemical parameters of calcium-phosphate metabolism, bone metabolism and vascular calcification in hemodialysis patients in the aspect of the characteristics of the studied population, the influence of the age of the patients, the possibilities for correction of the disturbances, the achieved results and the existing reservations in therapeutic terms is presented.

3. The study reflects the clinical practice compared to global trends and achievements.

Affirmative:

1. The characteristics of individual dialysis populations show differences between countries. These differences in demographics, dialysis therapy, and local policies and practices, among others, influence the clinical and biochemical characteristics of mineral and bone disorders, therapeutic management, and outcomes.

2. The biochemical profile and some of the clinical manifestations of CKD-MBD in hemodialysis patients are age dependent. Serum phosphate level, PTH, calcium-phosphate product, and vitamin D status depend on the patient's age. The latter correlated negatively with these indices.

3. It was confirmed that although the same agents were used, the therapeutic goals and the way to achieve them differ in patients under and over 65 years of age. Older patients require less frequent treatment with vitamin D receptor activators, phosphorus-lowering drugs, and lower dose regimens.

4. It was confirmed that achieving the values recommended by the world guidelines for the main parameters of mineral and bone metabolism in patients with chronic kidney disease in stage 5D shows a dependence on the age of patients and is easier to achieve in patients over 65 years.

PUBLICATIONS ON THE DISSERTATION TOPIC

Scientific papers printed in journals:

1. M. Yankova, V.Todorov. Spontaneous fracture due to brown tumor of the femur. Nephrology, dialysis and transplantation, 2012, 17, 3-4.

2. M. Yankova, V. Todorov, A. Ruseva, G. Todorova, I. Gencheva. Vitamin D status and risk factors for 25(OH)D deficiency in patients with chronic kidney disease. Nephrology, dialysis and transplantation, 2021, year 27, issue 2, 26-31.

3. M. Yankova, G. V. Todorova, V. V. Todorov. Age-related mineral and bone metabolism characteristics in patients with CKD 5D on hemodialysis. Journal of Biomedical and Clinical Research, vol.15, no.2, 2022, pp.165-170.

Scientific papers presented at conferences:

Age-related mineral and bone metabolism features in CKD 5D patients on hemodialysis, M. Jankova, G. M. Todorov, NEPHROLOGY ACADEMY, 12-15 May 2022, Sofia. Plovdiv, poster.

Participation in scientific projects on the topic:

1. Investigation of the frequency of vitamin D deficiency in patients with chronic kidney disease.

2. Possibilities of correcting some abnormalities of calcium-phosphate metabolism and bone metabolism in patients with chronic renal failure over 65.