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CLINICAL BIOCHEMISTRY DATABASE ANALYSIS: A RESTRICTED DIALYSIS COHORT

ANALIZA KLINIČKO-BIOHEMIJSKE BAZE PODATAKA: OGRANIČENA GRUPA BOLESNIKA NA DIJALIZI

Martina Bellini, Paolo Paparella

San Carlo Clinic, Paderno Dugnano, Milan, Italy

Summary

Background: A retrospective study was undertaken to investigate the biochemistry data of a restricted cohort of dialysis-related arthropathy patients. The aim of our study was to characterize this specific cohort of dialysis patients using a clinical chemistry database analysis.
Methods: An elaboration of more than 160,000 items of biochemical data, collected from 2001 to 2011, was made of 50 patients, 25 with dialysis-related arthropathy and 25 patients asymptomatic for arthropathy. A Student's t-test was applied, considering a P-value less than 0.05 as statistically significant.
Results: Significant and relevant unexpected biochemical differences were found between the two groups of patients. The serum level of β2-microglobulin was similar, while ferritin values were significantly higher in symptomatic patients. We excluded the possibility that the ferritin difference between the two groups was due to different iron storage and to an inflammatory profile.
Conclusions: The correct use of a biochemical database could permit to identify significant values which must be correlated with clinical data, but which could be the first step to a wider research.

Keywords: arthropathy, ferritin, β2-microglobulin, biochemical panel, dialysis, iron storage

Kratak sadržaj

Uvod: Ova retrospektivna studija sprovedena je kako bi se istražili biohemijski podaci ograničene grupe pacijenata sa artropatijom usled dijalize. Cilj studije je da se pomoću analize kliničko-hemijske baze podataka napravi karakterizacija ove specifične grupe pacijenata na dijalizi.
Metode: Više od 160000 stavki obrađeno je u skupu biohemijskih podataka sakupljenih između 2001. i 2011. od 50 pacijenata, 25 sa artropatijom usled dijalize i 25 pacijenata bez simptoma artropatije. Primenjen je Studentov t-test, a statistički značajnim smatrane su vrednosti P manje od 0,05.
Rezultati: Između ove dve grupe pacijenata neočekivano su pronađene značajne i relevantne biohemijske razlike. Serumski nivo β2-mikroglobulina bio je sličan, dok su vrednosti feritina kod pacijenata sa ovom bolešću bile značajno više. Isključena je mogućnost da razlika u feritinu između dve grupe potiče od različitih zaliha gvožđa ili inflamatornog profila.
Zaključak: Pravilna upotreba biohemijske baze podataka mogla bi omogućiti identifikaciju značajnih vrednosti koje moraju korelisati sa kliničkim podacima, što bi ipak moglo biti prvi korak u jednom opsežnom istraživanju.

Ključne reči: artropatija, feritin, β2-mikroglobulin, biohemijski panel, dijaliza, zalihe gvožđa

Address for correspondence:

Martina Bellini MS
San Carlo Clinic, Paderno Dugnano, Milan, Italy
e-mail: bellini_martina@libero.it

Introduction

An objective interpretation of the dialysis clinical biochemistry database is useful for the correct management of patients, especially complex cases such as those in a dialysis unit. An in-depth analysis of a large quantity of data could be helpful to find unexpected characteristics.

Dialysis-related arthropathy affects patients on maintenance hemodialysis treatment. Osteoarticular disease called renal osteodystrophy, which includes signs of secondary hyperparathyroidism, osteoporosis, osteosclerosis and osteomalacia (1, 2), is found in 80% of patients after ten years of dialysis. These patients show stiffness in the large joints, 64% show a restriction in movement, and 43% show carpal tunnel syndrome (3, 4) and, less frequently, cubital tunnel syndrome (5).

Caused by insufficient elimination during the therapy (6), beta-2-microglobulin deposits in the joints of patients, in the form of fibrils in the synovium, are known to be the main cause of this condition. Amyloid deposits were first found in the articular cartilage and were later found in the synovial membrane, joint capsule, and subchondral bone with a pathogenesis that was probably multifactorial, and related to the duration of renal failure, patient's age, age at the beginning of hemodialysis and duration of hemodialysis (7).

Our intention was to screen a large database of a restricted cohort of dialysis patients in order to study the behavior of biochemical analytes.

Subjects and Methods

A large computerized hospital database containing extensive clinical, laboratory and pathological information has been consulted.

A retrospective study was undertaken on 50 patients who had received hemodialysis for more than 10 years (range 10–30 years) and who had been monitored at the San Carlo Clinic of Paderno Dugnano (Milan) throughout their courses of hemodialysis. Twenty-five were symptomatic patients with surgical or instrumental diagnostic (radiological, sonographic and magnetic resonance) evidence of joint amyloidosis (group B), and twenty-five were included in a control group of asymptomatic patients (group A). Unfortunately, we could not demonstrate iron deposits through articular biopsy. The patients in the two different groups were standardized for age and gender: median age was 73 (±16) and each group included 15 women and 10 men.

Clinical characteristics are described in *Table I*. All patients were Caucasian.

Table I Clinical characteristics of both groups of patients.

		Group A (Symptomatic)	Group B (Asymptomatic)
Causes of chronic renal insufficiency	Kidney malformations	8%	8%
	Chronic interstitial nephropathy	12%	4%
	Bilateral polycystic disease	20%	20%
	Vascular nephropathy	16%	12%
	Renal calculosis	4%	4%
	IgA nephropathy	12%	4%
	Diabetic nephropathy	16%	44%
	Partial nephrectomy	12%	4%
Therapy	Endovenous iron	68%	72%
	Erythropoietin	76%	76%
	Phosphorus binder	80%	84%
	Vitamin D	76%	72%
	Cinacalcet	52%	44%
	Paricalcitol	44%	48%
Kidney transplantations		24%	28%

Diabetes, arterial hypertension, ischemic heart disease and cerebrovascular disease were present in both groups. Rheumatic diseases were not present.

A central venous catheter was used with patients from both groups.

Among the symptomatic patients, who underwent a more specific diagnostic investigation, we found acute monoarthritis or polyarthritis due to periarticular calcification, ruptured tendons from gout or pseudogout, and carpal tunnel syndrome. Carpal tunnel syndrome is treated by surgical release of the medial nerve. Fractures were present, namely: distal radius fracture, bilateral lesions of the rotator cuff, femoral fracture, and ischiopubic fracture.

The plasma level of analytes was recorded periodically from 2001 to 2011 as a routine procedure, for a total of 160,000 determinations. This represented our standard dialysis panel.

Particular attention was focused on: uremic toxicity parameters (urea, creatinine, uric acid), the parameters for bone and mineral pathophysiology (calcium, phosphate, vitamin D, PTH–parathyroid

Table II Standard dialysis panel adopted at San Carlo Clinic for CKD (chronic kidney disease).

Blood Test	Normal Values	Expected Values for CKD
Albumin, g/L	35–50	Goal: >4.0 BCG (preferred); Lab normal BCP
Bicarbonate [CO_2], mmol/L	21–30	>22
Blood Urea Nitrogen [BUN], mmol/L	1.1655–3.4965 mmol/L, Expected ratio of BUN: Creatinine ~ 10	<100; depends on protein intake
Ca X PO_4 Product	NA	<55
Chloride [Cl], mmol/L	95–108	Same
Creatinine, µmol/L	44.2–123.76	1060.8–1768: varies with muscle mass
Ferritin, pmol/L	26.9–674, male 22.47–337, female	224.7–1123.5 CKD 1–4; <1123.5 evaluate
Hematocrit [Hct], %	45–52, male 37–46, female	30–36% if on erythropoiesis stimulating agent (ESA)
Hemoglobin, g/L	132–162, male 120–152, female	100–120 if on erythropoiesis stimulating agent (ESA)
Magnesium [Mg], mmol/L	0.65–0.98	Same
Mean corpuscular volume [MCV]	82–102, male 78–101, female	Same
Parathyroid Hormone Level [PTH], ng/mL	10–65	Stage 3: 35–70; Stage 4: 70–110; Stage 5: 150–300
Phosphorus [PO_4], mmol/L	0.96–1.61	Goal: 1.12 – 1.77 mmol/L
Platelet count, × 10^9/L	140–450	Same
Potassium [K], mmol/L	3.6–5	Same
Red Blood Cells [RBC], 10^{12}/L	4.3–6.2, male 3.8–5.5, female	Same
Reticulocyte count, %	0.5–1.5	Same
Sodium [Na], mmol/L	133–145	Same
Transferrin saturation [TSAT], %	15–50%	Goal: >20%
White Blood Cell Count [WBC], × 10^6/L	4.8–10.8	Same

hormone), inflammatory parameters (WBC – white blood cells, lymphocytes, neutrophils, platelets, ESR – erythrocyte sedimentation rate, CRP – C-reactive protein), anemia parameters (RBC – red blood cells, Hb – hemoglobin, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, CHr – reticulocyte hemoglobin content, iron), ferritin as a marker of iron storage, beta-2-microglobulin as an indicator of dialysis-related amyloid deposits.

Analytes were measured before the dialysis procedure, and relevant analytes are shown in *Table II*.

Particular attention was focused on β2-microglobulin and on inflammatory, mineral concentration, iron storage and uremic toxicity parameters.

The significance of difference between groups was determined by unpaired Student's t-test. A P-value of <0.05 was considered statistically significant. Mean ±SD is given for quantitative variables.

Results

All symptomatic patients showed radiological, sonographic and magnetic resonance signs of periarticular small erosions and subcortical periarticular bone cysts, rotator cuff thickness and rotator cuff hyper-hypoechogenic deposit.

Uremic toxicity parameters (urea, creatinine, uric acid) and the parameters for bone and mineral pathophysiology (calcium, phosphate, vitamin D, PTH – parathyroid hormone) were similar in both patient groups ($p>0.5$) as shown in *Tables III* and *IV* respectively.

Uremic toxicity was evaluated by measurements of serum concentrations of small molecules (urea, creatinine, uric acid, phosphate) and it was excluded in both groups. Secondary hyperparathyroidism affected the totality of the cohort, and it was treated equally in the two groups.

Table III Urea, creatinine and uric acid values in our cohort of patients.

Group	Urea, mmol/L		Creatinine, μmol/L		Uric acid, μmol/L	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
B (n=25)	47.5 ± 20	1525	480 ± 220	1597	380.67 ± 89.22	443
A (n=25)	44.2 ± 19.32	1925	548.6 ± 236.4	1929	368.77 ± 83.27	436
P value						
B vs A	>0.05	>0.05	>0.05			

Table IV Calcium, phosphate, vitamin D and PTH values in our cohort of patients.

Group	Calcium, mmol/L		Phosphate, mmol/L		Vitamin D, pmol/L		PTH, ng/L	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
B (n=25)	2.27 ± 0.175	1371	1.5 ± 0.38	1308	38.74 ± 14	62	263 ± 91	78
A (n=25)	2.27 ± 0.19	2038	1.6 ± 0.41	1973	40.56 ± 14.5	42	275.15 ± 94	59
P value								
B vs A	>0.05		>0.05		>0.05		>0.05	

Table V Ferritin and beta-2-microglobulin values in our cohort of patients.

Group	Ferritin, pmol/L				Beta-2-microglobulin, g/L	
	Women		Men			
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
B (n=25)	553.28 ± 120	219	660 ± 176	314	0.313 ± 0.0107	60
A (n=25)	1030.17 ± 123.3	252	900 ± 161.2	349	0.3324 ± 0.08	54
P value						
Asymptomatic vs Symptomatic	<0.01		<0.01		>0.05	>0.05

Table VI Iron panel, except ferritin values.

Group	MCHC		Chr		MCH		Hemoglobin, g/L		Iron, g/L		MCV, μmol/L	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
B (n=25)	32.17±1.46	2320	32.44±2.6	967	29.89±2.44	2320	113±13.5	2320	8.77±3.9	353	92.9±6.25	2320
A (n=25)	32.29±1.49	2497	32.17±3.24	3097	29.67±3.28	3097	112.8±14	3097	9.2±5	1905	91.75±8.27	2040
P value												
B vs A	>0.05		>0.05		>0.05		>0.05		>0.05		>0.05	

Table VII Inflammatory panel, except ferritin values.

Group	White cells $\times 10^9$/L		Lymphocytes $\times 10^9$/L		Neutrophils $\times 10^9$/L		Platelet $\times 10^9$/L		ESR		PCR	
	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD	N
B (n=25)	6.88±2.4	2320	24.42±8.2	1975	64.8±9.34	1975	224.84±77	2320	81±30	1535	2.08±4	1780
A (n=25)	7.42±2.25	2497	22.09±5.81	2092	68±7	2092	239.38±78	2797	71.17±28.5	1905	2.02±3.74	2040
P value												
B VS A	>0.05		>0.05		>0.05		>0.05		>0.05		>0.05	

The β2-microglobulin level was normal in both patient groups ($p>0.5$). We observed a statistical difference in serum ferritin mean values ($p<0.01$) as shown in *Table V.*

Due to these results, we decided to investigate the different roles of serum ferritin, within an inflammatory and an iron panel. We obtained more accurate results through the elaboration of compared data in a temporal window of seven days.

The iron panel included analytes that are known to be iron status indicators such as MCHC, Chr, MCH, hemoglobin, iron itself, MCV and ferritin. The inflammatory panel included white cells, lymphocytes, neutrophils, platelets, ESR and CRP. Neither of the panels showed any differences in ferritin behavior. The values are reported in *Tables VI* and *VII*.

Discussion

The discussion of laboratory values related to dialysis is basic to the management of the pathology aspect. Examination of the complex relationship between calcium, phosphorus, vitamin D and PTH allows to review bone and mineral pathophysiology, and to determine the treatment of bone and mineral abnormalities with medications and surgery. During the progression of renal failure, the kidney's excretion of phosphorus decreases, causing serum phosphorus to increase. The kidney does not reabsorb calcium and vitamin D is not activated, causing decreased serum calcium levels and parathyroid gland hypertrophy and hyperplasia. Decreased serum calcium and increased serum phosphorus levels cause increased secretion of PTH. So, first of all, a thorough analysis of the biochemical database permits a better definition of the renal failure stage and biochemical parameters, which are helpful for therapy.

When interpreting the results, RDW (red cell distribution width), MCV (mean corpuscular volume) and MCHCH (mean corpuscular hemoglobin concentration) are extremely important to anemia management.

When examining the iron study results, focusing on iron saturation and ferritin is indispensable, too. A high ferritin level does not necessarily indicate adequate iron stores; many factors can increase ferritin levels (recent iron infusion, infection, inflammation, autoimmune disorders, malignancy, blood transfusions).

Moreover, personalized medicine aims to provide information that allows the right treatment option to be given to the right patient. The first step in this approach is to find relevant subtypes of patients for which a different treatment strategy would clearly be beneficial. In our study, we were able to define two different groups of dialysis patients, differentiated by clinical and biochemical aspects.

Unexpectedly, we found a different behavior of ferritin in respect of β2-microglobulin values. Even though recent literature has focused on β2-microglobulin deposits, in 1986 Cary et al. (8) included the hypothesis of iron involvement. They found synovial hemosiderin deposits in stromal macrophages and connective tissue, with smaller amounts in lining cells. These iron deposits may also cause arthropathy. Subsequently, scientists tried to define the role of iron status indicators (9, 10), but they did not consider a complete biochemical panel.

Different reports regarding the connection between the level of β2-microglobulin and joint symptoms have been published: Chattopadhyay et al. (11) found the β2-microglobulin level was raised in all patients; Nagi et al. (12) found no connection between the plasmatic level of β2-microglobulin and detachment of the capsule bone (joint effusion), which is one of the most important parameters of painful shoulder in dialyzed patients; Baldrati et al. (13) found no connection between the plasmatic level of β2-microglobulin in patients with dialysis-related amyloidosis and dialyzed patients without it; Sethi et al. (14) reported that the plasmatic level of β2-microglobulin was higher in patients with arthropathy than in dialyzed patients without it. Serum β2-microglobulin seems to be an inconstant indicator of dialy-

sis-related arthropathy. In our study, this common arthropathy dialysis-related indicator, β2-microglobulin (15, 16), was similar in the two groups.

There are only a few reports concerning ferritin levels in chronic hemodialysis patients; we found them statistically different in our two groups.

Brown et al. (10) measured isolated ferritin levels, showing that the four patients in their study with the most severe dialysis arthropathy had higher values. However, Hurst et al. (9) showed that there was a wide scatter in serum ferritin levels in patients with large joint chronic synovitis, and these levels were not different to those in patients without synovitis. Ferritin levels seem to be inconstant too, but it is important to underline that previous researchers have not improved the complete iron storage biochemical panel and no one has standardized patients from the clinical point of view.

To correctly study biochemical analytes, first it is necessary to standardize the dialysis patients in terms of: gender, age, years of dialysis, therapy panel, kidney transplantation, comorbidity (diabetes mellitus, arterial hypertension, ischemic heart disease, cerebrovascular disease, peripheral vascular disease), and then secondary hyperparathyroidism in order to guarantee the best available homogeneity in the two groups. These parameters were similar in both our groups.

The serum level of inorganic phosphate, which is related to bone metabolism and to abnormalities in bone mineral density and which is a known disorder in dialysis patients (17, 20), was similar in the two groups. Due to the groups' homogeneity, we can exclude the role of the central venous catheter (which is known to be a potential source of inflammation) as a cause of differences in ferritin mean values in the two groups (15).

Even if our research has some limitations, such as the small number of patients, we are the first to describe a complete panel to investigate the biochemical and clinical characteristics of these patients from a different point of view using a wide database. This leads us to speculate that the different ferritin behavior in our symptomatic patients is independent of iron storage and inflammatory aspects. The result opens a new area of research for future investigation, and database analysis could be helpful in this context.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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