INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2021.86548
Int J Med Biochem 2021;4(2):00-00

Technical Report

Data to assist in the determination of biochemistry test ranges to assess hemodialysis efficacy in patients with chronic renal failure

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Abstract

Objectives: Reference intervals are usually defined based on blood samples from healthy subjects and specific reference ranges for patients on hemodialysis (HD) are not currently available. The aim of the study was to establish expected ranges of biochemical analytes before and after HD for patients with chronic renal failure (CRF).

Methods: The findings of the 4 most recent quarterly check-ups of 684 patients (233 women and 451 men; age 18-95 years) treated with HD in several dialysis units attached to a single laboratory were studied. Biochemical analytes were measured using fully automated Roche Cobas C 501 or C 701 analyzers (Roche Diagnostics, Basel, Switzerland). Expected ranges were set according to International Federation of Clinical Chemistry and Clinical and Laboratory Standards Institute guidelines using the nonparametric method.

Results: Compared with pre-HD values, beta-2 microglobulin (β2m), chloride, creatinine, phosphate, potassium, and urea concentrations were lower post-HD ($p<0.001$), while bicarbonate, calcium, protein, and sodium concentrations were higher ($p<0.001$). Comparison with healthy subjects revealed that the levels of β2m, creatinine, and urea were higher before and after HD. Other analyte ranges were either lower, higher, or equivalent to healthy subjects in pre- and post-dialysis measurements. Differences between sexes were not significant, with the exception of creatinine, as well as a significant difference (>10%) in the creatinine level between individuals under and over 60 years of age ($p<0.0001$).

Conclusion: The establishment of specific ranges for dialysis patients could contribute to finding specific thresholds to monitor the effectiveness of HD.

Keywords: Biochemical, blood serum, hemodialysis, reference intervals, renal failure

The concept of a reference interval (RI) in human medicine was developed in the late 1960s by Grasbeck and Saris [1]. An RI is usually defined based on blood samples from healthy men and women, but similar studies of patients could contribute to finding specific values that provide a better ability to discriminate between states of health and disease.

Chronic renal failure (CRF) is characterized by a progressive loss of renal function [2]. CRF is associated with increased and decreased levels of some biochemical measurands. RIs based on healthy subjects are not optimal in this clinical context.

The objective of this study was to establish expected ranges for biochemical analytes commonly used in the monitoring before and after hemodialysis (HD) in a single laboratory.

Materials and Methods

Patients and sample collection

The subjects included in the study were male or female patients of a minimum 18 years of age with diagnosed CRF. A total of 684 patients (aged 18-95 years; 63% male, 37% female)

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Submitted Date: December 04, 2020 **Accepted Date:** February 02, 2021 **Available Online Date:** March 17, 2021

with signs and symptoms of renal failure identified by expert nephrologists and confirmed by an estimated glomerular filtration rate (GFR) of <15 mL/min/1.73 sqm. The study period was January 2016 to December 2019. The samples analyzed were from the 4 most recent check-ups (2736 samples) and the separated serum was analyzed using routine methods.

Estimation of biochemical analytes

The biochemical analytes were measured using a fully automated Roche Cobas C 501 or C 701 analyzer (Roche Diagnostics, Basel, Switzerland). The methods and results are described in Table 1.

Statistical and data analysis

Data collection

Results were retrieved from the laboratory information system and statistical analysis was performed using XLSTAT software version 2018.1.1 (Addinsoft SARL, Paris, France). Retrospective data were used in accordance with the ethical standards of EU regulation 2016/676 on the protection of natural persons and the processing of personal data and the free movement of such data. The procedures used by our institution have been approved by French National Commission on Informatics and Liberty. All of the biochemical investigations were ordered by the treating physicians. The Labosud database is registered with the French National Commission on Informat-ics and Liberty, record no. 2073511v0.

Detecting and eliminating outliers

Dixon's range test [3], recommended by the CLSI [4] for statistical analysis in reference interval studies, was used to detect and eliminate extreme values as outliers. This test identifies the single most extreme value at the upper or lower limit as an outlier: after sorting the data into ascending order (smallest to largest), the ratio (Q_{exp}) was defined as the difference of the suspect value from its nearest one (D) divided by the range of the values (R). The obtained Q_{exp} value was compared to a critical Q-value (Q_{crit}) found in tables. This critical value should correspond to the confidence level (CL) we have decided to run the test (usually: CL=95%). The extreme value must be eliminated if Q_{exp} value was greater than Q_{crit}. XLSTAT software repeat application of criteria until no further observations are rejected.

Establishment of expected values

The expected values corresponded to the central 95th percentile based on nonparametric estimates defined by the 2.5th and 97.5th percentiles as the lower and upper reference limits, respectively. Reference limits with a confidence interval of 95% (95% CI) were estimated according to the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) recommendation [5]. The results were partitioned by age group and gender when the range exceeded the 95% CI.

Statistical analysis

The Mann-Whitney test was used to establish differences and the results were considered significant at $p<0.05$.

Results

The demographic profiles of the study patients are presented in Table 2. Expected values before and after hemodialysis were examined in all patients, by gender, and in 2 age groups (18-60 years, 61-93 years), and are summarized in Table 3. There was a significant difference in the serum creatinine and urea values between men and women ($p<0.0001$) and a significant difference (>10%) between patients who were older or younger than 60 years of age ($p<0.0001$). The pre-dialysis values of beta-2 microglobulin (β2m), creatinine, phosphate, potassium, and urea were significantly higher than the post-dialysis values and significantly lower for bicarbonate, calcium, chloride, total protein, and sodium ($p<0.0001$).

Table 1. Analytical and methodological characteristics of analytes with CVa data

Parameters measured in serum	Methods used	Reference standard	Imprecision (CVa %)
Bicarbonate	Enzymatic method using phosphoenolpyruvate carboxylase	Primary reference material	4.0
Beta-2 microglobulin	Immunoturbidimetric method	WHO reference	4.5
Calcium	Colorimetric method using 5 nitro 5' méthyl BAPTA	SRM 956 c level 2	1.6
Chloride	Indirect ion-selective electrode method	PC gravimetrically prepared	2.1
Creatinine	Enzymatic method	ID-MS	3.5
Phosphate	Ultraviolet phosphomolybdate method	Primary reference material	2.5
Potassium	Indirect ion-selective electrode method	PC gravimetrically prepared	1.2
Sodium	Indirect ion-selective electrode method	PC gravimetrically prepared	1.4
Total protein	Colorimetric biuret method	SRM 927	2.0
Urea	Kinetic test using urease and glutamate dehydrogenase	ID-MS	3.7

CVa: Analytical variation; ID-MS: Isotope dilution-mass spectrometry; PC: Primary calibrator; SRM: Standard reference material; WHO: World Health Organization.

Table 2. The age and gender distribution of the chronic renal failure patients

Age (years)	All patients		Female		Male	
	Number of patients	Number of results/analytes	Number of patients	Number of results/analytes	Number of patients	Number of results/analytes
18 - 30	15	60	5	20	10	40
31 - 40	25	100	7	28	18	72
41 - 50	29	116	11	44	18	72
51 - 60	70	280	23	92	47	188
61 - 70	189	756	62	248	127	508
71 - 80	188	752	63	252	125	500
81 - 90	156	624	56	224	100	400
>90	12	48	6	24	6	24
Total	684	2736	233	932	451	1804
Total %	100%		37%		63%	

Table 3. The upper (97.5th percentile) and lower (2.5th percentile) expected values before and after hemodialysis

Parameters	Age and gender	Before hemodialysis		After hemodialysis		Healthy subjects [14]
		Lower limit - 2.5th (95% CI)	Upper limit - 97.5th (95% CI)	Lower limit - 2.5th (95% CI)	Upper limit - 97.5th (95% CI)	
Beta-2 microglobulin (mmol/L)	Total patients	1234 (1195-1255)	3375 (3348-3414)	270 (249-288)	1528 (1492-1544)	68-186
	Female (F)	1302 (1248-1354)	3410 (3355-3461)	250 (220-274)	1405 (1373-1430)	
	Male (M)	1179 (1133-1212)	3552 (3314-3387)	286 (249-308)	1606 (1574-1633)	
	F :18-60 years	1099 (846-1087)	3614 (3501-3741)	250 (228-287)	1616 (1512-1746)	
	F: >60 years	1401 (1282-500)	3667 (3569-3835)	267 (222-323)	1519 (1445-1572)	
	M: 18-60 years	1084 (993-1137)	3741 (3643-3896)	280 (223-320)	1546 (1514-1643)	
	M: >60 years	1370 (1288-1459)	3512 (3429-3643)	268 (231-335)	1670 (1580-1723)	
Bicarbonate (mmol/L)	Total patients	17 (17-18)	28 (27-28)	22 (22-23)	31 (30-31)	22-29
Calcium (mmol/L)	Total patients	1.91 (1.90-1.92)	2.52 (2.51-2.53)	2.26 (2.26-2.27)	2.79 (2.78-2.80)	2.15-2.55
	Female (F)	1.93 (1.89-1.95)	2.53 (2.50-2.54)	2.26 (2.25-2.27)	2.78 (2.77-2.79)	
	Male (M)	1.89 (1.87-1.90)	2.52(2.51-2.54)	2.26 (2.26-2.27)	2.80 (2.79-2.81)	
	F: 18-60 years	2.00 (1.99-2.03)	2.52 (2.51-2.54)	2.25(2.24-2.26)	2.74 (2.73-2.75)	
	F: >60 years	1.78 (1.73-1.81)	2.55 (2.52-2.59)	2.31 (2.30-2.34)	2.83 (2.80-2.85)	
	M: 18-60 years	1.94 (1.92-1.96)	2.52 (2.51-2.54)	2.25 (2.24-2.26)	2.79 (2.78-2.80)	
	M: >60 years	1.81 (1.79-1.84)	2.49 (2.45-2.52)	2.26 (2.25-2.29)	2.88 (2 .86-2.90)	
Chloride (mmol/L)	Total patients	90 (90-91)	105 (104-105)	94 (94-95)	103 (102-103)	98-107
Creatinine (µmol/L)	Total patients	298 (291-302)	1071 (1063-1080)	71 (69-73)	368 (364-373)	<84
	Female (F)	251 (243-261)	970 (957-985)	60 (58-62)	297 (291-304)	
	Male (M)	325 (319-334)	1115 (1104-1125)	90 (88-92)	394 (389-400)	
	F: 18-60 years	238 (230-248)	885 (863-897)	58 (56-60)	275 (264-285)	
	F: >60 years	376 (355-400)	1144 (1111-1164)	82 (76-91)	376 (362-390)	
	M: 18-60 years	323 (316-332)	1024 (1011-1035)	88 (86-90)	392 (367-389)	
	M: >60 years	368 (355-390)	1316 (1289-1336)	101 (95-111)	509 (495-524)	
Phosphate (mmol/L)	Total patients	0.57 (0.56-0.58)	2.53 (2.51-2.56)	0.23 (0.22-0.24)	0.93 (0.92-0.95)	0.87-1.45
	Female (F)	0.57 (0.55-0.59)	2.49 (2.46-2.53)	0.22 (0.21-0.24)	0.83 (0.82-0.85)	
	Male (M)	0.56 (0.55-0.58)	2.54 (2.51-2.57)	0.25 (0.24-0.27)	0.99 (0.97-1.01)	
	F: 18-60 years	0.59 (0.57-0.63)	2.42 (2.36-2.48)	0.21 (0.20-0.23)	0.84 (0.81-0.86)	
	F: >60 years	0.50 (0.56-0.58)	2.93 (2.85-3.01)	0.28 (0.25-0.32)	0.88 (0.84-0.93)	
	M: 18-60 years	0.56 (0.55-0.58)	2.47 (2.42-2.52)	0.25 (0.24-0.27)	0.94 (0.91-0.97)	
	M: >60 years	0.61 (0.56-0.66)	3.09 (3.01-3.16)	0.27 (0.23-0.30)	1.25 (1.20-1.30)	
Potassium (mmol/L)	Total patients	3.5 (3.5-3.6)	6.5 (6.5-6.6)	2.8 (2.7-2.8)	4.5 (4.4-4.5)	3.5-5.1
	F: 18-60 years	3.5 (3.6-3.7)	6.5 (6.4-6.6)	2.8 (2.7-2.8)	4.4 (4.3-4.4)	

Table 3. Cont.

Parameters	Age and gender	Before hemodialysis		After hemodialysis		Healthy subjects [14]
		Lower limit - 2.5th (95% CI)	Upper limit - 97.5th (95% CI)	Lower limit - 2.5th (95% CI)	Upper limit - 97.5th (95% CI)	
	F: >60 years	3.8 (3.7-3.9)	6.7 (6.5-6.8)	2.7 (2.7-2.8)	4.5 (4.3-4.7)	
	M: 18-60 years	3.5 (3.4-3.5)	6.4 (6.4-6.5)	2.8 (2.8-2.9)	4.5 (4.5-4.6)	
	M: >60 years	3.6 (3.6-3.8)	6.7 (6.6-6.8)	2.8 (2.8-3.0)	4.6 (4.3-4.7)	
Protein total (g/L)	Total patients	56 (56-57)	79 (79-80)	59 (59-60)	89 (89-90)	64-83
	F: 18-60 years	56 (55-56)	77 (77-78)	58 (58-59)	86 (85-87)	
	F: >60 years	56 (56-57)	81 (79-82)	62 (61-63)	93 (92-94)	
	M: 18-60 years	55 (55-56)	79 (79-80)	59(59-60)	89 (89-90)	
	M: >60 years	57 (56-58)	80 (79-80)	62 (62-64)	92 (90-94)	
Sodium (mmol/L)	Total patients	131 (130-131)	144 (143-144)	135 (135-135)	144 (144-144)	132-146
	F: 18-60 years	131 (131-132)	144 (143-144)	135 (135-135)	144 (144-144)	
	F: >60 years	131 (131-132)	144 (144-145)	136 (134-136)	145 (145-146)	
	M: 18-60 years	132 (132-133)	144 (144-145)	135 (134-135)	144 (144-145)	
	M: >60 years	129 (128-130)	144 (144-145)	135 (134-135)	144 (144-145)	
Urea (mmol/L)	Total patients	8.3 (8.2-8.5)	32.8 (32.4-33.2)	1.3 (1.3-1.4)	10.2 (9.9-10.4)	3.2-8.1
	Female (F)	7.8 (7.6-8.1)	31.8 (31.6-32.4)	1.1 (1.0-1.1)	8.3 (7.7-8.7)	
	Male (M)	8.6 (8.5-8.8)	33.5 (32.9-33.9)	1.5 (1.4-1.6)	10.9 (10.5-11.2)	
	F: 18-60 years	7.7 (7.5-7.9)	30.5 (29.8-34.2)	1.1 (1.0-1.1)	7.1 (6.7-7.4)	
	F: >60 years	9.2 (8.3-10.7)	34.8 (32.9-35.9)	1.3 (1.2-1.5)	10.2 (9.7-12.8)	
	M: 18-60 years	8.5 (8.4-8.7)	32.8(32.0-33.3)	1.5 (1.4-1.5)	10.3 (9.9-10.7)	
	M: >60 years	8.9 (8.5-9.5)	35.3 (34.1-35.9)	1.5 (1.4-1.7)	12.6 (11.4-13.5)	

Parameters that differ by gender or age before or after HD are shown in bold and underlined. CI: Confidence interval; HD: Hemodialysis.

Discussion

Our results were consistent with those of numerous studies demonstrating biological disorders in the blood induced by continuous decrease in renal clearance or GFR [6-12]. Despite the effectiveness of dialysis in filtering and purifying the blood, we found that some analytes remained at very high levels and an interpretation of the result using the existing RIs was meaningless. In cases of renal failure, our results showed that the β2m level was about 18 times higher than the RI before dialysis and 8 times higher after dialysis. The serum level of β2m remained significantly elevated despite dialysis treatment. It would seem to be important for a laboratory to define expected values according to the practices of dialysis departments. The multiplicity of dialysis protocols could lead to greater variability of post-dialysis reference values. For example, the characteristics of the dialysis membrane or the duration of hemodialysis may be important factors influencing the level of β2m after dialysis [13]. The decrease in creatinine during dialysis is used to measure the effectiveness of the treatment. Although dialysis significantly reduces the serum creatinine level, it remains higher overall than that of healthy subjects (about 4 times higher). Defining post-dialysis reference values would be valuable as a means to alert the healthcare team to any changes, particularly if an increase is observed. Normal blood contains 3.2–8.1 mmol/L of urea [14]. Our results indicated that the minimum urea limit was lower after dialysis (~1.3 mmol/L). Excess urea was likely eliminated to prevent accumulation between dialysis sessions. When compared with healthy subjects, the post-dialysis results for calcium were higher, while phosphate was lower. According to some authors, hypocalcemia could precipitate adverse cardiac outcomes, such as cardiomyopathy, congestive cardiac failure, ventricular tachycardia, and other arrhythmias [15]. Furthermore, a high serum phosphate level could increase risk of mortality [16]. In this context, RIs appropriate for dialysis patients would be very useful to avoid the potential side effects of biochemical disturbances. As expected, we found that dialysis sessions led to a significant decrease in the plasma potassium concentration. According to some authors, an increase in dialysate potassium was associated with a smaller decrease in plasma potassium concentration, and accordingly, with a much lower prevalence of post-dialysis hypokalemia [17]. Our clinical data support the need to establish alert thresholds to prevent severe post-dialysis hypokalemia.

Conclusion

Ranges created specifically for dialysis patients could contribute to more efficient monitoring of HD treatment. These results could be adopted by laboratories using the same equipment with similar analytical performance and a patient population like the group evaluated in this study. For laboratories with dif-

ferent equipment or different patient demographic characteristics, transference of results published in this study should be performed following the protocol established by the CLSI [4].

Conflict of Interest: The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Committee Approval: The procedures used by our institution have been approved by French National Commission on Informatics and Liberty.

Financial Disclosure: No funding.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – C.I., Y.E., O.C., J.B.; Design – C.I.; Supervision – O.C., J.B.; Funding – C.I.; Materials – C.I., Y.E.; Data collection &/or processing – C.I., Y.E.; Analysis and/or interpretation – C.I.; Literature search – C.I.; Writing – C.I.; Critical review – O.C., J.B.

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