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Comparative study of two immunoassays used for the determination of serum vitamin D

Raquel Lahoz [a,*], Juan Pelegrín Sánchez [a], Silvia Górriz [a,b], Pilar Calmarza [a]

[a] Servicio de Bioquímica Clínica, Hospital Universitario Miguel Servet, Zaragoza, Spain
[b] Servicio de Bioquímica Clínica, Hospital de Alcañiz, Teruel, Spain

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ABSTRACT

Objectives: The best indicator of vitamin D level is the determination of serum 25-hydroxyvitamin D (25(OH)D). Due to the lack of standardization of the available methods, there are problems of precision and reproducibility in its measurement. The objective of this study was to compare the results of 25(OH)D concentration determined by two different immunoassay methods.
Design: and Methods: 25(OH)D was analyzed in 184 serum samples in an IDS-iSYS Multi-Discipline Automated System Analyzer (Vitro) and in an Alinity i automated Analyzer (Abbott). Then, results were compared. Three groups were considered: group of total patients, group with vitamin D supplements and group without treatment.
Results: Of the total patients, 52.7% received vitamin D supplements. The correlation coefficient of agreement for general group, supplementation group and group without supplementation was 0.92, 0.91 and 0.89, respectively. In all of them, a kappa index>0.75 was obtained.
Conclusions: The assays evaluated are not comparable to each other. Despite this, they show an excellent concordance in the evaluation of the vitamin D status.

1. Introduction

In the last years, there has been a lot of progress in the knowledge of vitamin D, proving that it can have both bone [1] and non-bone effects. It has been observed that this vitamin could be involved in cardiovascular diseases, metabolic syndrome, diabetes, thyroid diseases, cancer, immune and neurological diseases among others [2–7]. Most cells express vitamin D receptors and about 3% of the human genome is directly or indirectly regulated by the endocrine system of vitamin D [8].

Vitamin D is a fat-soluble steroid prohormone that is generated mainly in the skin by photochemical conversion of 7-dehydrocholesterol. The term "vitamin D" defines two relevant biological forms, both precursors of its active form, 1,25-dihydroxyvitamin D (calcitriol), which are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). For its activation two hydroxylation occur, generating an intermediate metabolite that is 25-hydroxyvitamin D (25(OH)D).

25(OH)D is its main storage form and is present in blood at very high concentration with respect to the active form 1,25-dihydroxyvitamin D. It has a half-life of 2–3 weeks compared to the 4 h of 1,25-dihydroxyvitamin D. For this reason, 25(OH)D is the analyte chosen to know the state of the organism in relation to the concentration of vitamin D [1,9], being the form measured in the clinical laboratory.

* Corresponding author. Servicio de Bioquímica Clínica, Hospital Universitario Miguel Servet, C/ Padre Arrupe, s/n, Consultas Externas, Planta 3a, 50009, Zaragoza, Spain.
E-mail address: rlahoz@salud.aragon.es (R. Lahoz).

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Epidemiological studies have shown a high prevalence of both insufficiency and vitamin D deficiency [10], so measurements of vitamin D status represent an opportunity to intervene preventively and therapeutically [11,12].

Tai et al. [13] in 2010 developed a tandem liquid chromatography method with mass spectrometry (LC-MS/MS), which has been recognized as a reference method by the Joint Committee for Traceability in Laboratory Medicine for the determination of 25(OH)D. But it is an impractical method in the routine laboratory, given its complexity and the need for specialized personnel to use it. Therefore, automated immunoassays have been implemented in laboratories for its determination in recent years, which have allowed us to assume the increase in the demand for this parameter, but they present a series of difficulties.

The difficulties in the determination of 25(OH)D are related to its fat soluble nature, as well as its strong binding to the vitamin D binding protein (VDBP) and to serum albumin. On the other hand, although it would be appropriate for the different immunoassays to detect vitamin D2 and D3 in equimolar form, this is not usually fulfilled.

Besides all this, it is necessary to consider the presence in serum of multiple metabolites of vitamin D: 24,25-dihydroxyvitamin D, c3 epimers of vitamin D2 and D3 and other interference in the assay as the presence of heterophile antibodies. The study conducted by Farrel et al. [14] showed that around 40% of individuals have the epimer c3 vitamin D and therefore this epimer represents a source of analytical variation across the available immunoassays.

Likewise, all the immunoassays used for the determination of vitamin D show cross reactions with 24,25(OH)2D, that may be present in serum at concentrations of 10–12 nmol/L [15].

Another important aspect in the determination of 25(OH)D is its separation from its binding protein. LC-MS/MS separates it by solvent extraction but in the immunoassays solvent extraction has been replaced by the use of different blocking agents that displace vitamin D from its binding protein, with more or less efficacy. Elsenberg et al. [16] found that the deviation in the determination of vitamin D by different methods was greater when the concentration of VDBP was higher.

The main objective of this study is to compare the concentrations of 25(OH)D carry out through two different automated immunoassays and secondarily to verify the scope of the classification of patients according to high, normal or insufficient concentrations.

2. Material and methods

2.1. Study design

It was a transversal descriptive observational study carried out in the Chromatography Section of the Clinical Biochemistry Department of the University Hospital Miguel Servet in Zaragoza (Reference Hospital of the Autonomous Community of Aragon) during March 2018.

2.2. Subjects

The study group consisted of a total of 184 individuals from Aragon that includes patients belonging to the Health Sectors I and II of our autonomous community. These areas give service to a population of 980,000 inhabitants, which, with respect to the total population of the Aragonese community (1,315,000 inhabitants), represents more than 75% of it. The samples came from both healthy patients and patients with various pathologies as bone, kidney, thyroid, cardiovascular, immune, neurological diseases and cancer.

2.3. Methods

The samples were distributed into three study groups. The first group included the total number of patients (n = 184). Then, it was divided into two groups according to the vitamin D supplementation: a supplemented group, consisting of 97 samples from patients who received vitamin D3 supplements (cholecalciferol and calcifediol), and a second group consisting of 87 samples from non-supplemented patients. Some assays are not able to discern the exogenous form of vitamin D (administered as a supplement or with diet) [17], hence the distribution in these groups. None of the patients was supplemented with vitamin D2.

The concentration of 25(OH)D was measured, as routine health analysis, by the automated IDS-iSYS 25VitD assay in an IDS-iSYS Multi-Discipline Automated System Analyzer (Vitro). Then, the same samples were processed by the Alinity i 25-OH Vitamin D assay in an Alinity i automated Analyzer (Abbott). Both of them were performed according to the manufacturer's recommendations. Finally, the results obtained were compared.

IDS-iSYS 25 VitD analyzer (Immunodiagnostic Systems Holdings PLC, Vitro, Boldon, United Kingdom) uses a chemiluminescence technology, which is fully automated. According to manufacturer, the functional sensitivity of the assay is 10.8 nmol/L (4.3 ng/ml) and the linearity through the measurement interval is between 10.8 nmol/L and 350 nmol/L (4.3 ng/ml to 140 ng/ml). The analytical specificity reflected through the percentage of cross-reactivity with other metabolites is 101% for 25-hydroxyvitamin D3 (25(OH)D3), 105% for 25-hydroxyvitamin D2 (25(OH)D2) and 37% for 24,25-dihydroxyvitamin D2 (24.25(OH)2D2) [18]. The cross-reactivity with the C3 epimers of 25-hydroxyvitamin-D2 (3-*epi*-25(OH)D2) does not exceed 1%.

In the case of Alinity i 25-OH Vitamin D (Abbott Laboratories, Illinois, United States), the assay used for the quantitative determination of 25(OH)D is a one-step delayed-action immunoassay using chemiluminescent microparticles immunoassay (CMIA) technology. According to the manufacturer, the functional sensitivity of the assay is 8.8 nmol/L (3.5 ng/ml) and the linearity through the measurement range is between 8.8 nmol/L and 385.5 nmol/L (3.5 ng/ml to 154.2 ng/ml). The analytical specificity expressed through percentage of cross-reactivity with other metabolites is 98.6%–101.1% for 25(OH)D3, from 80.5% to 82.4% for 25(OH)D2, from

101.9% to 189.2% for 24.25(OH)2D3 and from 71.4% to 114.2% for 24.25(OH)2D2. The cross-reactivity with the C3 epimer of 25 (OH)D3 is 1.3% and 25(OH)D2 0.8%.

Also, the intra- and interday imprecision (expressed as coefficients of variation) of both assays were evaluated following the CLSI EP15-A3 protocol (Clinical and Laboratory Standards Institute). In the case of iSYS, the imprecision of the method was only calculated using the level of control determinant of vitamin D insufficiency, given its proper functioning to date.

Finally, using as a guide the Position Paper on the needs and optimal levels of vitamin D prepared by the Spanish Society of Bone Research and Mineral Metabolism (SEIOMM) and related Societies [19], the concordance in the evaluation of the vitamin D status was also evaluated. The serum concentration of vitamin D was considered as sufficient concentrations when it was above 75 nmol/L (>30 ng/ml), insufficient 50–75 nmol/L (20–30 ng/ml) and deficient below 50 nmol/L (<20 ng/ml).

2.4. Statistical analysis

A descriptive study of the data was carried out to know the demographic characteristics and the statistical parameters of the samples. For each qualitative variable the frequency distribution of the percentages of each category was calculated. The Kolgomorov-Smirnov test was accomplished for the quantitative variables of the study and indicators of central tendency (mean or median) as well as dispersion tendency (standard deviation or percentiles) were calculated as proceeded.

For the comparison of both assays, the results were studied by the Bland-Altman analysis, the Passing-Bablok regression and the concordance correlation coefficient (CCC). The kappa index (k) was also performed to the assessment of concordance in the evaluation of the status of vitamin D. The concentration of 25(OH)D was expressed in nmol/L (1 nmol/L equals 0.4 ng/ml). The statistical analysis was performed using Medcalc software version 11.4.2.0. Values of $p < 0.05$ were considered statistically significant.

3. Results

The median age of the total of patients was 61 years old, with a minimum of 1 year and a maximum of 92. There were 31.5% men and 68.5% women.

The intraday and interday imprecision of each assay of 3 daily replications during 5 days of 3 control levels for Abbott and 1 control level for Vitro are summarized in Table 1.

The results of 25(OH)D concentration measured by IDS-iSYS 25 VitD (Vitro) and Alinity i 25-OH Vitamin D (Abbott) are shown in Table 2.

The concordance between the results obtained by the IDS-iSYS 25 VitD (Vitro) and Alinity i 25-OH Vitamin D (Abbott) assays by Bland-Altman analysis is shown in Fig. 1. The mean value of the bias was higher for the supplemented group (−20.4 nmol/L) than for the group without treatment (−4.9 nmol/L). Graphically, a greater concordance between both assays was observed when patients receiving vitamin D supplements were excluded. The dispersion of the results obtained (negative trend in all groups) indicated that the degree of agreement decreases the higher the value of 25(OH)D measured.

The results of the Passing-Bablok regression as well as the CCC analysis are shown in Table 3.

Table 1
Imprecision for both 25(OH)D assays, the IDS-iSYS 25 VitD (Immunodiagnosticsystems) (Vitro) and the Alinity i 25-OH Vitamin D (Abbott).

Samples	Day	Intraday			Interday		
		Mean (nmol/L)	SD	% CV	Mean (nmol/L)	SD	% CV
ABBOTT							
Low control	1	50.2	0.5	0.9	48.7	2.1	4.4
	2	47.4	1.7	3.6			
	3	49.5	4.0	8.1			
	4	47.3	1.1	2.3			
	5	48.8	1.4	2.8			
Medium control	1	108.2	5.4	5.0	103.4	3.7	3.6
	2	100.9	2.9	2.8			
	3	102.4	2.8	2.8			
	4	102.1	0.8	0.8			
	5	103.3	2.2	2.1			
High control	1	201.2	8.6	4.3	189.6	10.9	5.7
	2	196.0	12.3	6.3			
	3	179.3	7.1	4.0			
	4	182.4	3.0	1.6			
	5	189.2	6.7	3.5			
VITRO							
Low control	1	54.0	3.9	7.2	52.4	5.0	9.5
	2	51.9	7.6	14.7			
	3	51.7	1.5	2.9			
	4	56.8	3.6	6.3			
	5	47.6	4.2	8.8			

SD: standard deviation; CV: variation coefficient.

Table 2
Statistical parameters of 25(OH)D concentration of the patients studied by the IDS-iSYS 25 VitD (Immunodiagnosticsystems) (Vitro) and the Alinity i 25-OH Vitamin D (Abbott).

	General Group		Supplemented Group		Unsupplemented group	
	Vitro	Abbott	Vitro	Abbott	Vitro	Abbott
Kolgomorov-Smirnov test	P < 0.0001	P < 0.0001	P = 0.0004	P = 0.0003	P = 0.0441	P = 0.0005
Median (Q1-Q3)	80.7 (46.9–122.2)	76.4 (41.3–136.5)	105.1 (68.5–147.2)	113.9 (61.4–174.1)	59.9 (27.0–89.7)	54.3 (24.8–82.9)
Mínimum	10.8	8.9	10.8	8.9	10.8	10.1
Maximum	653.7	770.6	653.7	770.6	338.0	546.7
Interquartile range	75.3	95,2	78.7	112.7	62.7	58.1

Q1: first quarter; Q3: third quarter.

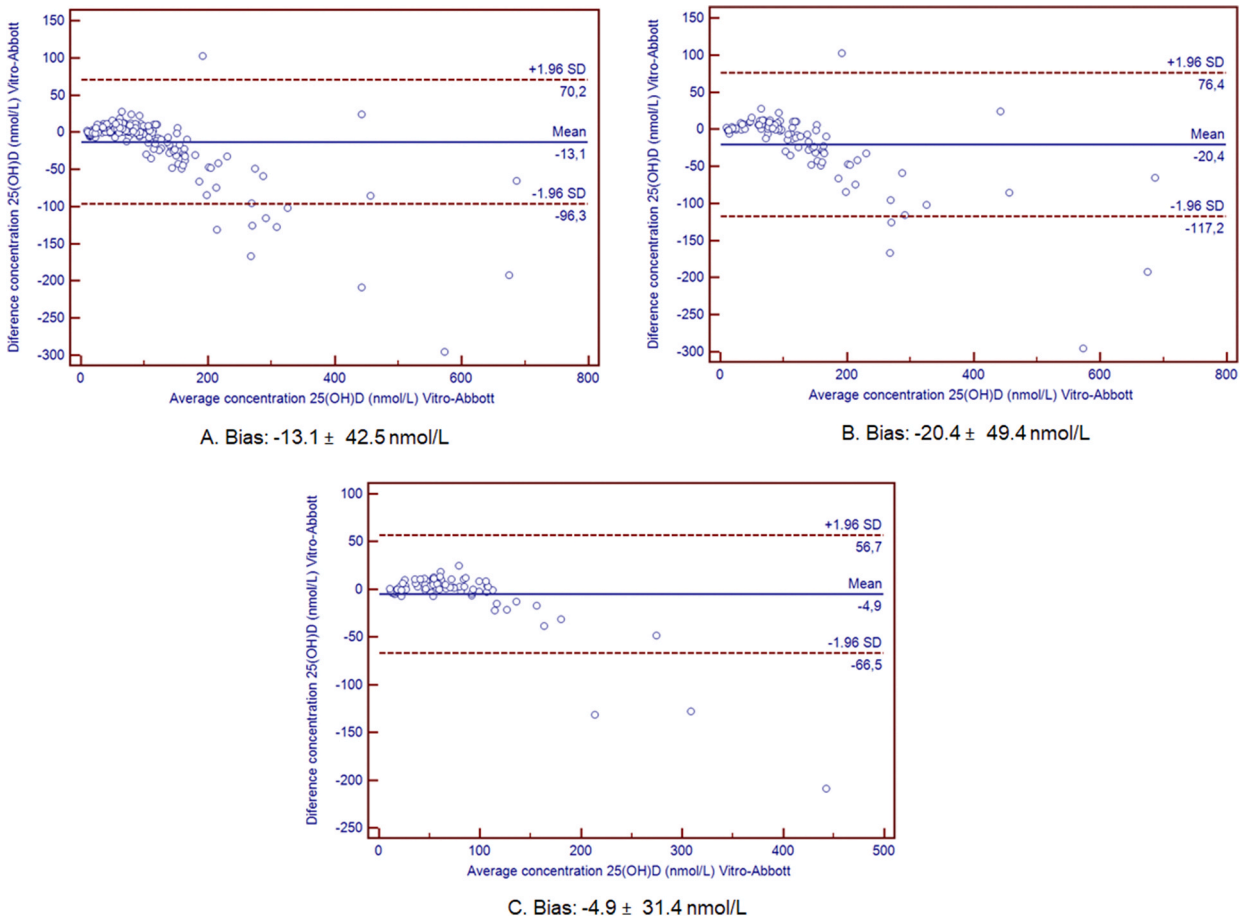

Fig. 1. Methods comparison using Bland-Altman plots. The graphs show the bias in nmol/L (1 nmol/L is equal to 0.4 ng/ml). A. All patients group; B. Supplemented group; C. Group without supplementation.

Table 3
Passing-Bablok analysis and correlation agreement between the IDS-iSYS 25 VitD (Immunodiagnosticsystems) (Vitro) and the Alinity i 25-OH Vitamin D (Abbott) assay in all patient groups.

Group	Passing-Bablok Analysis					Concordance correlation Analysis		
	A (CI 95%)	B (CI 95%)	RSD	±1,96 RSD	Cusum test	CCC (CI 95%)	r	Cb
General	−13.14 (−16.8–8.55)	1.21 (1.15–1.26)	34.06	−66.76–66.76	p < 0.01	0.92 (0.90–0.93)	0.96	0.95
Supplemented	−22.18 (−31.80–15.56)	1.33 (1.25–1.41)	40.09	−78.58–78.58	p < 0.01	0.91 (0.88–0.93)	0.96	0.95
Unsupplemented	−2.53 (−8.01–0.63)	1.03 (0.97–1.11)	31.01	−59.85–59.85	p < 0.01	0.89 (0.87–0.92)	0.96	0.93

A: y-intercept; B: slope; Cb: bias correction factor; CCC: concordance correlation coefficient; CI: confidence interval; r: Pearson's correlation coefficient; RSD: residual standard deviation.

There were constant systematic differences (95% of the confidence interval of A did not contain the value 0) and proportional systematic differences (95% of the confidence interval of B did not contain the value 1) in the general and supplemented group between the two methods. These differences were not found in the unsupplemented group. The CCC indicated a substantial concordance for the two assays in the general and supplemented groups (CCC was between 0.99 and 0.95) and moderate concordance for the group without treatment (CCC was between 0.90 and 0.94).

Based on the serum concentrations obtained with both methods, we have also compared the proportion of samples that were classified in the different states of vitamin D concentration proposed by the SEIOMM. Taking into account the different serum concentrations in each group of patients (sufficient concentrations: above 75 nmol/L (>30 ng/ml); insufficient 50–75 nmol/L (20–30 ng/ml); and deficient below 50 nmol/L (<20 ng/ml), we obtained similar k values in the three groups studied (k = 0.90, 0.89, and 0.89, for general, supplemented and without supplementation groups, respectively). K values higher than 0.75 indicate excellent concordance in the evaluation of the vitamin D status.

4. Discussion

In this study, we observed lower concentrations of 25(OH)D in the IDS-iSYS 25 VitD assay (Vitro) when compared to the results obtained with the Alinity i 25-OH Vitamin D assay (Abbott).

Regarding the imprecision of the measurements with both analyzers, the coefficient of variation does not exceed 10% in any of the levels, both intraday and interday, except for an isolated day for the Vitro technique, so we can affirm that they have good repeatability and reproducibility. Other studies have similar results to ours. Saleh et al. found an intraassay CV < 6,6% an interassay CV < 9,2% [20] and in the study carried out by Kocak FE et al. [21] the intraassay CVs were 4.2% for low, 2.7% for medium and 2.2% for high level). The intra-assay CV obtained by Iglesias Álvarez et al. was <3.7% [22] and as in our study, and M. K. Kaoivula et al. found a coefficient of variation greater for Vitro than for Abbott (CV 6.2–16.9% and 3.8–4.6% for low control and CV 5.4–8.9% and 2.8% for high control, respectively [23]).

First of all, we must consider that when we compare our results with the results obtained in other studies, although we have used the Alinity i Abbott equipment, after reviewing the bibliography we have not found any comparative study with this analyzer. Therefore, we will refer to the studies made with the Abbott Architect analyzer that uses the same assay and reagents as the Alinity i.

When comparing the two methods studied we have found statistically significant differences in all the statistical tests performed. In the Bland-Altman analysis, we observed a greater bias or systematic error in the group of patients supplemented (Fig. 1) and despite having found acceptable values of Pearson correlation coefficient (Table 3), CCC results that range between 0.90 and 0.94 in both the general and supplemented groups, indicate a moderate concordance between these methods. In the unsupplemented group there is also little agreement, since the CCC is less than 0.90. Regarding the Passing-Bablok regression, we found proportional and constant errors simultaneously in the general and the supplemented groups (Table 3).

The finding of these differences between both methods could be due to the design of the assays. The functional sensitivity of the Abbott method is 8.8 nmol/L (3.5 ng/ml) while that of Vitro is 10.8 nmol/L (4.3 ng/ml), which could explain the highest values obtained with the Alinity i instrument and if we analyze the specificity according to the manufacturer, deserves special attention the cross reactivity with the metabolite 24,25(OH)2D2. In the Vitro assay, it is 37% while this cross-reactivity for the Abbott assay ranges from 71.4 to 114.2%. There are also differences in the 25(OH)D2 detection, being in this case 105% for Vitro and between 80.5 and 82.4% for Abbott. These differences can influence the results obtained according to the assay employed.

The Abbott assay in comparison with the reference method ID-LC-MS/MS underestimates the values of 25(OH)D2, which would be irrelevant in European countries, as is our case, since patients they do not receive vitamin D2 supplementation [20].

Furthermore, both methodologies use different pretreatment systems for the release of the 25(OH)D of the vitamin D binding protein, which could generate a different amount of 25(OH)D suitable for its measurement. In general, incomplete release has been suggested as a potential source of variability between different immunoassays for the determination of 25(OH)D, being implicated also different polymorphisms of this protein [21,24].

E. Cavalier et al. in their comparison study of several automated immunoassays for the determination of this parameter, conclude that the iSYS method seems less influenced by the concentration of the vitamin D binding protein [25]. Likewise, in other studies it seems that the inaccuracy in the measurement of 25(OH)D using the Architect's Abbott assay is highly dependent on the concentration of this protein [20].

On the other hand, Vitamin D deficiency is a common problem in many populations around the world [26], constituting a public health problem. In Europe it was estimated, regardless of the age group, the ethnic mix and the latitude of the populations studied, that 13.0% of the 55,844 individuals studied had serum concentrations of 25(OH)D < 30 nmol/L [27]. This state of generalized hypovitaminosis can be influenced to a large extent by the analysis methodology used in the determination of the concentration of 25(OH)D.

Regarding the classification of the patients, we found an excellent agreement between the methods when applying the values proposed by the SEIOMM (k > 0.75). The prevalence of vitamin D insufficiency is very similar among the methods studied. We have found that it is slightly higher for Abbott in all groups of patients (49.5, 30.9 and 70.1%, general group, with supplement and without treatment, respectively) than for Vitro (47.8, 29.9 and 67.8%, respectively). As expected, the highest percentage of patients with insufficient levels of vitamin D is in the group without treatment, finding only a difference of 2.3% between both assays. This means that patients, regardless of the method used, would receive pharmacological treatment if needed.

Our study had some limitations. One of them is that we have not compared the measurement of the samples with the reference method. This is due to the lack of availability of an LC-MS/MS equipment to perform these measurements. The determination of

vitamin D by this last method is not possible in most laboratories, both because of the increased demand for this determination and because of the complexity of the method; this is why this limitation is common to most published studies.

However, this comparison has been carried out in some studies, observing a great variability of results, which shows the need for standardization of the immunoassays used for the measurement of 25(OH)D.

In this sense, M. J. W. Janssen et al. found variable systematic differences when comparing the Abbott method with ID-LC-MS/MS, but not for the iSYS equipment [28], and in the study carried out by M. K. Koivula et al. although none of the results obtained by the different immunoassays, including Architect and iSYS, were equivalent to those obtained with PerkinElmer LC-MS/MS, iSYS was more generally in accordance with the reference method [23]. Saleh et al. also observed that the proportion of patients who were classified as having vitamin D insufficiency was significantly overestimated by the automated tests (including Abbott) compared to the LC-MS/MS method [20]. F.E. Kocak et al. found a greater concentration-dependent deviation when using the Architect equipment compared to the measurements using LC-MS/MS [21].

Another important issue that may affect the performance of 25(OH)D automated immunoassays is the differences in the serum matrix between the study populations (eg. healthy subjects, patients with chronic kidney disease, dialysis patients, pregnant women, different ethnic groups or patients in intensive care with fluid changes) [26]. In our study, we grouped the patients according to the supplementation received without taking into account their basic characteristics. However, it is true that this matrix effect was greater in those techniques based on binding proteins than in the current immunoassays that use antibodies for the determination of 25(OH)D, as is the case of the assays compared in this study [23].

5. Conclusions

The assays evaluated were not comparable to each other. Despite this, they showed an excellent concordance in the evaluation of the vitamin D status. This was the only comparative study in which 25(OH)D has been measured in the Alinity i analyzer (Abbott).

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Declaration of competing interest

None of the authors have any conflicts of interest to disclose.

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