Routine Folic Acid and Vitamin B12 Supplementation in Hemodialysis Patients: A Cross-Sectional Analysis of Achieved Serum Levels

Ana Rita Ramos[1], Marta Margarido[1], Filipa Trigo[1], Rita Valério Alves[1], Patrícia Barreto[1], Karina Lopes[1], Flora Sofia[1], Paulo Santos[1]*

1. Nephrology department, ULS Médio Tejo – Torres Novas, Portugal

https://doi.org/10.71749/pkj.128

Abstract

Introduction: Patients undergoing maintenance hemodialysis are routinely supplemented with folic acid and vitamin B12 to prevent deficiency related to dietary restrictions and dialytic losses. However, contemporary data on achieved serum vitamin levels under routine supplementation practices are limited.

Methods: We conducted a single-center observational study including adult patients undergoing maintenance high-flux hemodialysis or hemodiafiltration. All patients received standardized supplementation with folic acid and vitamin B complex three times per week. Serum folic acid and vitamin B12 concentrations were measured in January 2024, and associations with clinical, laboratory, and dialysis-related variables were explored.

Results: A total of 53 patients were included (mean age 72.7 ± 11.5 years; 32.1% female). Serum folic acid levels were markedly elevated, with 94.3% of patients presenting values above the upper reference limit. Elevated serum cobalamin concentrations were observed in 28.3% of patients. A higher proportion of patients treated with high-flux hemodialysis presented vitamin B12 levels above the reference range compared with those undergoing hemodiafiltration, although no significant differences in mean serum vitamin levels were observed between dialysis modalities. Serum vitamin levels were not associated with dialysis adequacy or other clinical and laboratory parameters.

Conclusion: Routine supplementation with folic acid and vitamin B12 in maintenance hemodialysis patients is associated with high achieved serum vitamin levels. The higher proportion of elevated vitamin B12 levels observed in high-flux hemodialysis patients warrants further investigation and supports the need to re-evaluate and potentially individualize supplementation strategies in contemporary hemodialysis care.

Keywords: Dietary Supplements; Folic Acid; Kidney Failure, Chronic; Vitamin B Complex

INTRODUCTION

Patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis are at increased risk of nutritional deficiencies as a result of dietary restrictions, chronic inflammation, medication use and the dialysis procedure itself. Water-soluble vitamins are particularly vulnerable to depletion, as several of these compounds may be removed during hemodialysis; folic acid, in particular, is susceptible to dialytic losses due to its low molecular weight.[1,2]

Among water-soluble vitamins, folic acid and cobalamin (vitamin B12) are of particular relevance in hemodialysis (HD) patients given their central role in DNA synthesis, cellular metabolism and homocysteine metabolism. Deficiencies in these vitamins may contribute to impaired erythropoiesis, a suboptimal response to erythropoiesis-stimulating agents and elevated homocysteine levels, potentially increasing cardiovascular risk. In addition, vitamin B12 is required for the conversion of methylmalonic acid, and its deficiency may lead to neurologic and cognitive impairment, which can be particularly challenging to recognize in dialysis populations.[1,3] Consequently, routine supplementation with folic acid and vitamin B12 has been widely adopted in hemodialysis practice.

Received: 17/12/2025 **Accepted:** 18/01/2026 **Published Online:** 25/02/2026 **Published:** 16/03/2026

* **Corresponding Author:** Ana Rita Ramos | anaritap.ramos1996@gmail.com | Nephrology department, ULS Médio Tejo, Rua Xanana Gusmão 45, 2350-754, Torres Novas, Portugal

Despite the recognized risk of vitamin depletion, supplementation with folic acid and vitamin B12 is largely empirical and implemented as a routine, standardized practice in many dialysis centers. Current clinical guidelines suggest supplementation mainly to correct documented deficiency; however, these recommendations are largely opinion-based and do not define optimal dosing strategies, serum targets or monitoring frequency. As a result, supplementation protocols vary considerably between centers with respect to dose, frequency and timing of administration.[3,4]

At the same time, persistently elevated serum levels of folic acid and vitamin B12 may not be biologically neutral. Excessive folic acid intake has been associated with impaired zinc absorption, and high serum vitamin B12 concentrations have been linked to adverse outcomes in observational studies of chronic kidney disease populations, raising concerns regarding routine, non-individualized supplementation.[4]

This study aimed to characterize serum folic acid and vitamin B12 concentrations in patients undergoing maintenance hemodialysis receiving routine supplementation. Additionally, we sought to explore whether clinical, laboratory, and dialysis-related factors were associated with achieved serum vitamin levels.

METHODS

Study Design and Population

This was a single-center observational study including adult patients undergoing maintenance HD at a hospital-based dialysis unit. The study population consisted of patients receiving chronic HD with available serum folic acid and cobalamin measurements performed in January 2024. According to the institutional protocol, all patients received routine supplementation with folic acid (5 mg) and a vitamin B complex three times per week, administered at the end of each hemodialysis session. Each tablet of the vitamin B complex contained thiamine (vitamin B1, 15 mg), riboflavin (vitamin B2, 15 mg), nicotinamide (50 mg), calcium pantothenate (25 mg), pyridoxine hydrochloride (vitamin B6, 10 mg), biotin (0.15 mg), and cyanocobalamin (vitamin B12, 0.01 mg).

Participants were eligible for inclusion if they met all of the following criteria:

- Age ≥18 years
- Treatment with high-flux HD or online post-dilution hemodiafiltration (HDF). All patients undergoing HDF achieved convective volumes ≥23 L per session.
- Enrollment in a regular hemodialysis program for at least three months and were treated three times per week with a standardized dialysis schedule of 4-hour sessions.
- Treatment according to the institutional supplementation protocol.

The following conditions led to exclusion from the study:

- Active malignancy, defined as a current diagnosis of solid or hematological cancer, regardless of disease stage or ongoing oncologic treatment, or receipt of systemic chemotherapy at the time of vitamin assessment.
- Chronic liver disease, defined as a documented diagnosis of cirrhosis, chronic hepatitis or other clinically significant liver disease, or biochemical evidence suggestive of chronic hepatic dysfunction (persistent elevation of aspartate aminotransferase (AST), and/or alanine aminotransferase (ALT) ≥2 times the upper limit of normal, a cholestatic pattern with elevated alkaline phosphatase and/or gamma-glutamyl transferase, or total bilirubin >1.5 mg/dL in the absence of an acute cause), as well as a history of clinically relevant chronic alcohol consumption.
- Conditions known to interfere with folate or vitamin B12 absorption or metabolism, including prior gastric surgery, inflammatory bowel disease, pernicious anemia, strict vegetarian diet, use of methotrexate.
- Hospitalization, major surgery, or blood transfusion within the three months preceding vitamin measurement.

Data Collection

Clinical, dialysis-related, and laboratory data were retrospectively collected from electronic medical records. Demographic variables included age and sex. Dialysis-related parameters comprised dialysis modality (high-flux HD or HDF), dialysis vintage, vascular access type, ultrafiltration volume per session, blood flow rate (Qb), single-pool Kt/V, and urea reduction ratio (URR).

Laboratory parameters were obtained from routine monthly blood tests performed as part of standard clinical care. These included hemoglobin, platelet count, total serum protein, serum albumin, calcium, phosphorus, AST and ALT.

Serum folic acid and cobalamin concentrations were measured in January 2024 using standard automated immunoassays in the hospital's central laboratory, with blood samples collected before the midweek dialysis session in accordance with routine clinical practice. Reference ranges were defined according to the local laboratory standards: 7 – 45.1 nmol/L for serum folic acid and 85 – 378 ng/L for serum cobalamin. Elevated folic acid and cobalamin levels were defined as values above the upper limit of the reference range.

Statistical Analysis

Continuous variables were assessed for normality using the Shapiro–Wilk test and are presented as mean ± standard deviation or median [interquartile range], as appropriate. Categorical variables are presented as counts and percentages. Comparisons between dialysis modalities (online HDF

vs high-flux HD) were performed using the independent samples t-test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Associations between categorical variables were evaluated using the chi-square test or Fisher's exact test when expected cell counts were <5. Correlations were assessed using Pearson's or Spearman's correlation coefficients, as appropriate. All analyses were performed using IBM SPSS Statistics version 29, and a two-sided p-value <0.05 was considered statistically significant.

Ethical Considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki. Given its retrospective design and the use of fully anonymized data, formal ethical approval and informed consent were waived in accordance with institutional policy.

RESULTS

Baseline Characteristics of the Study Population

A total of 53 patients who met the inclusion and exclusion criteria were included in the analysis. The median age of the study population was 73 years, and 17 patients (32.1%) were female. Most patients were treated with HDF (40 patients, 75.5%), while the remaining patients underwent high-flux HD. Diabetic nephropathy was the most frequent underlying etiology of chronic kidney disease (10 patients, 18.9%). The median dialysis vintage was 40 months. Baseline clinical, dialysis-related, and laboratory characteristics are summarized in Table 1.

Table 1. Baseline clinical and dialysis-related characteristics

	Overall (n=53)	HDF patients (n=40)	High-flux HD patients (n=13)	*p*-value
Age (years)	73.0 [45.0; 92.0]	72.0 [45; 92]	78.0 [64; 88]	0.136
Female sex, n(%)	17 (32.1)	10.0 (25.0)	7.0 (53.8)	0.053
Arteriovenous access, n (%)	37 (69.8)	29.0 (72.5)	8.0 (61.5)	0.455
Dialysis vintage (months)	40.0 [3; 160]	43.5 [10;160]	35.0 [3; 103]	0.243
Ultrafiltration per session (mL)	2316.5 ± 722.0	2356.0 ± 702.4	1852.0 ± 1063.0	0.006
Single-pool Kt/v	1.6 [1.0; 3.0]	1.6 [1;3]	1.6 [1; 3]	0.311
URR (%)	77.1 [61.5; 91.3]	75.2 [61.5; 89.4]	82.6 [74.8; 91.3]	0.027
Blood flow rate (Qb, mL/min)	393.6 [267.0; 481.0]	395.2 [267; 481]	349.9 [321; 431]	0.119
Hemoglobin (g/dL)	11.4 ± 1.3	11.5 ± 1.2	11.1 ± 1.5	0.327
Platelet count (×10^9/L)	190.9 ± 54.8	200.3 ± 56.4	162.2 ± 38.8	0.028
Total serum protein (g/dL)	6.6 [5.4; 7.6]	6.5 ± 0.5	6.5 ± 0.6	0.921
Serum albumin (g/dL)	3.8 [2.8; 4.5]	3.9 [2.8; 4.5]	3.6 [3.3; 4.4]	0.082
Serum calcium (mg/dL)	9.0 ± 0.7	8.0 ± 0.5	9.1 ± 0.6	0.136
Serum phosphorus (mg/dL)	4.2 ± 1.6	4.2 ± 1.7	4.2 ± 1.5	0.454
Aspartate aminotransferase (U/L)	19.0 [10.0: 50.0]	18.0 [10; 50]	24.0 [13; 40]	0.021
Alanine aminotransferase (U/L)	14.0 [3.0; 39.0]	13.0 [3; 39]	19.0 [7; 30]	0.040
Body mass index (kg/m^2)	25.5 [15.4; 51.2]	25.4 [15.4; 41.1]	25.6 [18.6; 51.2]	0.975

Values are expressed as mean ± SD or median [IQR], as appropriate. Between-group comparisons were performed using the independent samples t-test or Mann–Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

Patients undergoing high-flux HD had lower ultrafiltration volumes per session and higher URR compared with those treated with HDF. Differences were also observed in platelet count and liver enzyme levels. Other baseline clinical and dialysis-related variables were broadly comparable between groups.

Serum Folic Acid and Cobalamin Levels

Serum folic acid levels were markedly elevated in the study population, with a median value of 106.9 nmol/L [29.6; 377.7]. Overall, 50 patients (94.3%) presented serum folic acid levels above the upper reference limit, while only 3 patients (5.7%) had values within the normal range (Table 2). Mean serum cobalamin levels were 316.0 ± 118.1 ng/L. Elevated cobalamin levels were observed in 15 patients (28.3%), whereas the remaining patients presented values within the reference range.

Table 2. Serum folic acid and cobalamin levels in the study population

Variable	Value
Serum folic acid (nmol/L)	106.9 [29.6; 377.7]
Folic acid > 45.1 nmol/L, n (%)	50 (94.3)
Serum cobalamin (ng/L)	316.0 ± 118.1
Serum cobalamin > 378 ng/L, n (%)	15 (28.3)

Reference ranges: serum folic acid 7 – 45.1 nmol/L; sérum cobalamin 85 – 378 ng/L

Comparison According to Dialysis Modality

Serum folic acid levels were similar between patients undergoing HDF and those treated with high-flux HD, with mean values of 120.2 ± 70.0 nmol/L and 125.1 ± 69.3 nmol/L, respectively (t (51) =-0.240, p = 0.812), as shown in Table 3.

Table 3. Serum folic acid and cobalamin levels according to dialysis modality

	HDF (n=40)	High-Flux HD(n=13)	*p*-value
Serum folic acid (nmol/L)	120.2 ± 70.0	125.1 ± 69.3	0.812
Serum cobalamin (ng/L)	309.0 ± 126.8	337.7 ± 86.8	0.452

Mean cobalamin levels were 309.0 ± 126.8 ng/L in patients undergoing HDF and 337.7 ± 86.8 ng/L in those treated with HD hemodialysis, with no statistically significant difference between groups (t (51) = 0.758, p = 0.452). The proportion of patients with cobalamin levels above the upper reference limit was 22.5% in the hemodiafiltration group and 46.2% in the high-flux hemodialysis group (Table 4). However, this difference was not statistically significant (Fisher's exact test, p = 0.155).

Table 4. Distribution of serum cobalamin levels according to dialysis modality

	Serum cobalamin £ 378 ng/L, n (%)	Serum cobalamin > 378 ng/L, n(%)	Total
HDF	31 (77.5)	9 (22.5)	40
High-Flux HD	7 (53.8)	6 (46.2)	13

No clinical, laboratory, or dialysis-related variables, including body mass index, dry weight, plasma albumin, urea, and creatinine, were significantly associated with serum folic acid or cobalamin levels.

DISCUSSION

In this contemporary single-center cohort, routine supplementation with folic acid and vitamin B12 in maintenance hemodialysis patients was associated with very high serum folic acid concentrations and elevated vitamin B12 levels in a substantial proportion of patients. Previous studies have reported high or supratherapeutic serum vitamin levels in hemodialysis populations receiving supplementation.[6,7] Our findings are consistent with these observations, as the vast majority of patients in our cohort exhibited folic acid levels above the upper reference limit and a substantial proportion had elevated vitamin B12 concentrations. The present study was designed to characterize serum vitamin levels within a standardized routine supplementation protocol in a contemporary hemodialysis population.

Although no statistically significant differences were observed between dialysis modalities, patients treated with high-flux hemodialysis exhibited a numerically higher proportion of elevated vitamin B12 levels compared with those undergoing hemodiafiltration. This observation may be explained by differences in the clearance of middle-molecular-weight compounds, as hemodiafiltration provides enhanced convective removal compared with high-flux hemodialysis. However, given the small sample size and the limited number of patients in each subgroup, the study may have been underpowered to detect significant differences, and these findings should be interpreted cautiously.[1] Although some baseline differences between dialysis modalities were observed, these were not associated with serum folic acid or vitamin B12 concentrations, supporting the comparability of the study groups.

In this cohort, serum folic acid and vitamin B12 concentrations were not significantly associated with clinical, nutritional, or inflammatory variables that could potentially influence circulating vitamin levels. Under these conditions, measured serum concentrations may be interpreted primarily as reflecting current supplementation practices in this setting.

These results suggest that routine, non-individualized supplementation practices may lead to serum vitamin concentrations that exceed physiological requirements. While supplementation aims to prevent deficiency, persistently elevated levels of folic acid and vitamin B12 may not be biologically neutral, as suggested by guideline-based concerns regarding excessive folic acid intake and by observational data linking high vitamin B12 concentrations with adverse outcomes in chronic kidney disease populations.

Beyond the biological implications, routine and uniform supplementation with folic acid and vitamin B12 in hemodialysis patients may also have relevant economic consequences. Although individually inexpensive, the chronic administration of these supplements to the entire dialysis population represents a cumulative cost for dialysis units and healthcare systems, particularly when administered in the absence of documented deficiency and when resulting in supraphysiological serum levels. In an era increasingly focused on value-based care, these findings highlight the importance of reassessing long-standing supplementation practices to ensure that they are both clinically justified and cost-effective.

Importantly, our findings suggest that current vitamin supplementation regimens may be amenable to safe dose

reduction or individualization in a substantial proportion of hemodialysis patients. Avoiding unnecessary pharmacological interventions may significantly reduce treatment burden and improve medication adherence in this population, which is characteristically exposed to high levels of polypharmacy.

Several limitations of this study should be acknowledged. The single-center design and relatively small sample size may limit generalizability and reduce statistical power, particularly for subgroup analyses according to dialysis modality. The cross-sectional nature of the analysis precludes assessment of longitudinal changes and clinical outcomes. In addition, functional markers of vitamin status, such as homocysteine or methylmalonic acid, were not available. Further studies are warranted to better define optimal supplementation strategies for folic acid and cobalamin in contemporary hemodialysis practice. Larger, multicenter cohorts are needed to confirm our findings, to explore potential differences between dialysis modalities, and to establish appropriate serum targets. Additionally, prospective studies assessing different supplementation doses, withdrawal strategies, and the use of functional biomarkers of vitamin status may help to identify patients who truly benefit from supplementation while avoiding unnecessary exposure in others.

CONCLUSION

Routine supplementation with folic acid and vitamin B12 in maintenance hemodialysis patients was associated with high achieved serum vitamin levels, particularly for folic acid, and with elevated vitamin B12 concentrations in a substantial proportion of patients. A numerically higher prevalence of elevated vitamin B12 levels was observed among patients treated with high-flux hemodialysis.

Although the clinical consequences of these findings remain uncertain, they raise important questions regarding the need for uniform, non-individualized supplementation strategies and suggest that a more tailored approach to vitamin supplementation may be warranted in hemodialysis care.

Ethical Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Financing Support: This work has not received any contribution, grant or scholarship

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of patient data.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2024).

Provenance and Peer Review: Not commissioned; externally peer-reviewed.

Contributorship Statement

ARR, MM, FT, RVA, PB, KL, FS and PS: Study conception and design; data collection; data analysis and interpretation; drafting of the manuscript; critical revision of the manuscript; and final approval of the version to be published.

REFERENCES

1. Soohoo M, Ahmadi SF, Qader H, Streja E, Obi Y, Moradi H, et al. Association of serum vitamin B12 and folate with mortality in incident hemodialysis patients. Nephrol Dial Transplant. 2017;32:1024-32. doi: 10.1093/ndt/gfw090.
2. Saifan C, Samarneh M, Shtaynberg N, Nasr R, El-Charabaty E, El-Sayegh S. Treatment of confirmed B12 deficiency in hemodialysis patients improves Epogen® requirements. Int J Nephrol Renovasc Dis. 2013;6:89–93. doi: 10.2147/IJNRD.S44660.
3. Amini M, Khosravi M, Baradaran HR, Atlasi R. Vitamin B12 supplementation in end-stage renal disease: a systematic review. Med J Islam Repub Iran. 2015;29:167.
4. Alp Ikizler T, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. Am J Kidney Dis. 2020;76:S1-S107. doi: 10.1053/j.ajkd.2020.05.006. Erratum in: Am J Kidney Dis. 2021;77:308. doi: 10.1053/j.ajkd.2020.11.004.
5. Sarvari GR, Naseri M, Esmaeeli M, Azarfar A. Serum folate and vitamin B12 levels in hemodialysis patients: Is there any correlation with plasma homocysteine levels? J Pediatr Nephrol. 2014;2:140-6.
6. Schwotzer N, Kanemitsu M, Kissling S, Darioli R, Benghezal M, Rezzi S, et al. Water-soluble vitamin levels and supplementation in chronic online hemodiafiltration patients. Kidney Int Rep. 2020;5:2160–7. doi:10.1016/j.ekir.2020.09.009
7. Yango A, Shemin D, Hsu N, Dworkin L, Selhub J, Bostom AG. L-folinic acid versus folic acid for the treatment of hyperhomocysteinemia in hemodialysis patients. Kidney Int. 2001;59:324–7. doi: 10.1046/j.1523-1755.2001.00507.x.