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Vitamin K insufficiency and the prophylaxis strategy in term healthy infants: A multicentre study

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Abstract

Background/Aim: Late vitamin K deficiency bleeding (VKDB) during early infancy is a serious problem worldwide. Vitamin K (VK) deficiency commonly occurs in newborns who are exclusively breastfed. Protein Induced by VK Absence (PIVKA-II) has been identified as an early indicator of subclinical VK deficiency in neonates, surpassing prothrombin time. To assess PIVKA-II levels at 48 h, 1 and 3 months of age in full-term newborns who were exclusively breastfed and received varying VKDB prophylaxis regimens.

Methods: A prospective observational study was conducted in four hospitals, enrolling 105 newborns. PIVKA-II levels were measured using a sandwich-type enzyme-linked immunosorbent assay.

Results: At 48 h of age, there was no significant difference in PIVKA-II concentrations between newborns who received intramuscular administration of 1 mg of phylloquinone (VK1) and those who received oral administration of 2 mg of VK1 at birth. At 1 and 3 months of life, infants who received any supplementation regimen between 2 and 14 weeks exhibited significantly lower PIVKA-II concentrations compared to infants who received only 1 mg of intramuscular VK1 at birth. The prophylaxis involving a dose of 1 mg of intramuscular VK1 at birth followed by oral administration of 150 μg/day of VK1 from the 2nd to the 14th week of life showed the lowest PIVKA-II blood concentrations.

Conclusions: Oral supplementation of VK1 after discharge significantly reduced PIVKA-II concentrations in exclusively breastfed term infants. These findings suggest the importance of oral VK1 supplementation in exclusively breastfed infants during their first 3 months of life to avoid the risk of VK insufficiency.

KEYWORDS
neonatal haemorrhagic disease, newborn, prophylaxis, protein induced by vitamin K absence, vitamin K

1 | INTRODUCTION

Vitamin K (VK) is classified as a fat-soluble vitamin, and its significance lies in its pivotal role as a cofactor for VK-dependent carboxylase. This particular enzyme utilizes the oxygenation process of VK to facilitate the carboxylation of glutamyl residues within VK-dependent proteins. This process, in turn, activates these proteins, contributing to a diverse array of physiological functions.

Among these functions are haemostasis, apoptosis, bone development, arterial calcification, and growth control.[1] Notably, VK is vital for the synthesis of functional coagulation factors II, VII, IX, X, as well as protein C and protein S within the liver.

Beyond the liver, VK-dependent proteins such as osteocalcin, matrix Gla protein (MGP) and growth arrest-specific protein 6 (Gas 6) are synthesized and play important roles in different physiological functions.[2]

Insufficient levels of VK may lead to haematological complications, resulting in the impaired production of active coagulation molecules.[3,4]

VK deficiency is more common in neonates compared to adults due to factors such as inadequate bacterial colonization in the intestines, limited placental transport of VK and lower concentrations of the vitamin in breast milk.[5] Exclusive breastfeeding is considered a risk factor for VK deficiency bleeding (VKDB) as human milk contains significantly lower VK concentrations (.85–9.2 μg/L) compared to formula milk (4.24–175 μg/L).[3,5,6]

VKDB is characterized by bleeding disorders caused by insufficient activity of VK-dependent coagulation factors. It is classified into early, classical and late forms.[7,8] The early form occurs within the first 24 h of life and involves bleeding in the head, intrathoracic region, intra-abdominal area or gastrointestinal tract. Classical VKDB is usually mild and occurs between 24 h and 7 days of life.[4,9] Late VKDB develops between 8 days and 6 months of age, most commonly in exclusively breastfed infants who did not receive VK prophylaxis or those affected by malabsorption disorders and cholestasis.[10,11]

Late VKDB is associated with severe haemorrhagic manifestations, including central nervous system involvement, leading to high mortality and significant morbidity, particularly neurologic impairment.[12]

Studies have shown evidence of subclinical VK deficiency in a considerable number of exclusively breastfed preterm infants at 2–5 months of age, which is related to the duration of breastfeeding.[13]

There is a near-global consensus that VK prophylaxis should be provided to all newborn infants to prevent VKDB and its life-threatening consequences.[13] Various regimens of parenteral and oral prophylaxis have been implemented over the years.[14–18] Phylloquinone, the vitamin K1 (VK1), is the only form used therapeutically in humans, synthesized by plants and algae.[19] Healthy newborns can be treated with either a single intramuscular injection of 1 mg of VK1 at birth or three oral doses of 2 mg of VK1 administered at birth, at 4–6 days and at 4–6 weeks. Another approach involves an oral dose of 2 mg at birth followed by a weekly dose of 1 mg for 3 months.[16] Both intramuscular and oral administration of VK1 at birth effectively prevent classical VKDB but could not completely protect infants from late VKDB, especially if they are breastfed and their oral intake of VK is low.[18–21] However, there is substantial worldwide variability in recommendations for subsequent VK prophylaxis strategies after the initial dose at birth, and insufficient evidence supports any specific clinical practice.

It is therefore imperative to establish evidence-based recommendations and achieve universal consensus regarding the best strategy to protect exclusively breastfed infants from the detrimental consequences of VKDB.

Traditional screening tests for VK deficiency bleeding (VKDB) include prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT). However, these assays have limited sensitivity in detecting subclinical deficiencies of VK. PT and INR only become prolonged when prothrombin levels drop below 50% of the normal concentration, making them ineffective for identifying subclinical deficiency.[7,22] APTT, on the contrary, detects the presence of coagulation factors that are not VK-dependent.[23]

VK deficiency leads to the synthesis of undercarboxylated proteins known as protein-induced by VK absence (PIVKAs), which are unable to bind calcium and therefore remain inactive. The levels of inactive PIVKAs increase with the severity of VK deficiency.[6] PIVKA-II, an undercarboxylated form of prothrombin, is often used as a functional marker for detecting VK deficiency. PIVKA-II levels can be measured by detecting antibodies specific to undercarboxylated prothrombin.[23–25] Interestingly, there is a significant linear correlation between INR and PIVKA-II, suggesting that PIVKA-II levels can serve as an alternative to PT/INR in assessing the VK deficient status in neonates.[26] In addition, elevated PIVKA-II precedes any subsequent change in PT. Elevated PIVKA-II levels can be detected before any changes in PT become apparent. In cases of overt VK deficiency where PT is significantly prolonged, PIVKA-II levels are invariably very high.[13]

Limited data are available regarding the effects of VK supplementation in breastfed newborns on PIVKA-II levels. Studies have shown that exclusively breastfed infants and infants who did not receive VK prophylaxis have suboptimal VK status, as indicated by PIVKA-II measurements, even at 1–2 months of age.[13,24,27,28] Dituri et al.[29]

investigated PIVKA-II concentrations in newborns receiving prolonged oral VK1 supplementation, after IM VK1 administration at birth.

The aim of this study is to assess PIVKA-II concentrations at 48 h, 1 and 3 months of age in healthy term exclusively breastfed newborn infants who were administered different regimens of VKDB prophylaxis. The objective is to determine which prophylaxis regimen is associated with higher levels of PIVKA II, indicating evidence of VK deficiency, and consequently a higher risk of developing late VKDB. The findings from this study would contribute valuable evidence to establish effective recommendations for preventing late VKDB in exclusively breastfed infants.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was a prospective multicentric observational study conducted on exclusively breastfed, healthy term newborn infants who were consecutively enrolled from 1 October 2021 to 20 January 2023.

The study was prospectively registered on clinicaltrial.gov with the clinical trial registration no. NCT05713045.

Ethical approval for the study was obtained from the following research institutes: Research Institute's Committee on Human Research—Comitato Etico Area Vasta Emilia Nord, Prot. n. 9620 (04 March 2020); Regional Ethical Committee for Clinical Experimentation of the Tuscany Region, Pediatric Ethical Committee Section—Prot. n. 321/2020 (22 December 2020); Lazio Ethical Committee—Comitato Etico Lazio, Prot. n. 1440/CE Lazio1 (18 November 2020); Independent Committee of the University Hospital Policlinico Consorziale di Bari—Prot. n. 6661 (16 December 2020).

Written informed consent was obtained from parents of all newborns.

The study compared four different regimens of VKDB prophylaxis based on the local protocols of four participating centres. PIVKA-II levels were measured at 48 h, 1 and 3 months of age. The inclusion criteria were as follows: gestational age >37 weeks, birth weight >2500 g and Apgar score >9 at 5 min. The exclusion criteria were as follows: (1) gestational age less than 37 + 0/7 weeks or more than 42 + 0/7 weeks, (2) formula milk or mixed milk feeding, and (3) withdrawal of informed consent.

All enrolled newborns were clinically followed until 6 months of life. Clinical, auxological and neurodevelopmental evaluation was performed at 1, 3 and 6 months of life.

The STROBE checklist for observational studies was utilized for reporting the study.[30]

2.2 | VK prophylactic administration regimens

The four different VK prophylactic administration regimens were as follows:

Group 1: Only 1 mg of intramuscular (IM) injection of VK1 at birth.

Group 2: IM injection of 1 mg VK1 at birth, followed by 50 μg/day (4 drops of microdosed VK1 product) orally from the second to the fourteenth week of life.

Group 3: IM injection of 1 mg VK1 at birth, followed by 150 μg/day (12 drops of microdosed VK1 product) orally from the second to the fourteenth week of life.

Group 4: Oral dose (PO) of 2 mg VK1 at birth, followed by a second oral dose (2 mg) in the first week of life, and a third oral dose (2 mg) at 4 weeks of life.

The VK1 formulation used at birth was Konakion, phytomenadione, 2 mg/.2 mL MM paediatric, a solution for injection or oral administration manufactured by Cheplapharm Arzneimittel GMBH. Microdosed VK product (PediaKplus® Gocce, Pediatrica, Italy) was administered orally by the caregiver using a spoon in Group 2 and in Group 3. The product is registered as a food supplement on the Ministry of Health website (http://www.ministerosalute.it/alimenti/dietetica) and is classified under the code 46737. This product complies with the European Directive on foods according to DL n. 169 of 21 May 2004.

2.3 | Data collection and Plasma PIVKA-II levels measurement

Data were prospectively recorded in a dedicated database using MICROSOFT® EXCEL® FOR MICROSOFT 365 MSO (Version 2302 Build 16.0.16130.20332). Clinical data, including sex, gestational age (GA), mode of delivery, Apgar score, birth weight (BW), premature rupture of membranes (PROM), blood cord gas and VK prophylaxis regimen, were documented. Newborns were discharged within 48–72 h of life. Post-discharge follow-up was conducted at 1 and 3 months of age, which involved measuring PIVKA-II levels and evaluating medication adherence.

The timing of blood sampling visits was the same for all recruitment centres: 48 h of life with compulsory blood samples for metabolic diseases screening, 30 ± 3 days (1 month of life) and 90 ± 3 days (3 months of life).

Blood samples (.5 mL) were collected through a heel prick and placed in Lithium Heparin tubes. The samples were then centrifuged at 1000 revolutions for minute for 15 min, and the plasma was subsequently stored at −20°C for further analysis. Samples were collected and stored at each participant centre. They were then sent by express courier to the Siena University Hospital Laboratory for analysis.

Medication adherence was assessed using the Brief Medication Questionnaire, which was administered to the parents. Patients were excluded from the study if there were protocol violations, such as not receiving VK1 administration, concurrent use of drugs other than vitamins, or formula milk or mixed milk feeding.

Plasma PIVKA-II concentrations were quantified using a sandwich-type Enzyme-Linked Immunosorbent Assay (ELISA) kit (PIVKA-II ELISA Kit, Catalogue Number: MBS700977, MyBiosource, San Diego, CA, USA). This kit exhibited a detection range spanning from .312 to 20 ng/mL. The assay's characteristics, as detailed by the manufacturer, were as follows: the analytical sensitivity was .078 ng/mL. The Lower limit of detection was established as the minimum protein concentration distinguishable from zero. This was determined by computing the mean optical density value from 20 repetitions of the zero standard, coupled with three times their respective standard deviations. The assay showcased notable sensitivity and exceptional specificity in identifying human PIVKA-II, without any significant cross-reactivity or interference noted with analogues.

To evaluate accuracy, we subjected three samples with known concentrations to 20 repeated tests on a single plate. Furthermore, we tested three samples of known concentration in 20 distinct assays. The intra-assay coefficient of variation was found to be below 8%, indicating consistent results within a single assay. Similarly, the inter-assay coefficient of variation was less than 10%, illustrating the consistency of results across different assay runs. The measurement of plasma PIVKA-II concentrations was performed in accordance with the operational instructions furnished by the manufacturer.

Briefly, 100 μL of diluent (blank), standard substances and plasma samples were added to pre-coated 96-well plates, and they were incubated for 30 min at 37°C. After three washes, a Horseradish Peroxidase (HRP)-conjugated antibody specific to PIVKA-II was added to the wells, followed by another 30-min incubation at 37°C. Subsequently, after five additional washes, 90 μL of TMB (3,3′,5,5′-Tetramethylbenzidine) substrate solution and 50 μL of stop solution were added to each well. The absorbance at 450 nm for the blank, standard substances, and samples was measured using a microplate reader, specifically the DYNEX DSX® (Bouty S.p.a., Sesto San Giovanni, Milan, Italy). Each sample was measured in duplicate. A standard curve was constructed by plotting the mean absorbance for each standard on the *x*-axis against the concentration on the *y*-axis. The data were linearized by plotting the logarithm of the PIVKA-II concentrations (ng/mL) against the logarithm of the optical density, and the best line of fit was determined using regression analysis.

For samples that yielded values above the highest standard, dilutions (1:100, 1:250 or 1:500) were prepared using the sample diluent, and the test was subsequently repeated. In cases where samples were diluted, the concentration obtained from the standard curve was adjusted by multiplying it with the appropriate dilution factor. To maintain consistency with previous studies,[13,24,31] PIVKA-II concentrations were converted from ng/mL to AU/mL. This conversion was based on the equivalence of .05 AU/mL to a gravimetric serum PIVKA-II concentration of 50 ng/mL.[13] Notably, a PIVKA-II concentration of .05 AU/mL signifies the upper limit of the adult reference range.[13] Reference values for clinically stable adults undergoing warfarin therapy are documented as 6.9–99.5 AU/mL,[13] as well as 6.81–13.81 AU/mL.[32] For preterm infants receiving formula milk, reference values stand at .02–.03 AU/mL at 8 weeks of postnatal corrected age.[13]

2.4 | Statistical analysis

G*Power 3.1.9.2 for Windows was used to estimate the sample size. Due to lack of data, the sample size was calculated using Cohen's rule of thumb, defining the following conventional effect sizes in case sufficient data were not available to calculate it without overestimating the power: small $f = .14$; medium $f = .25$; large $f = .40$. Assuming effect size = .14, alpha = .05, beta = .10, number of groups = 4, number of measurements = 3, correlation between repeated measurements = .5, correction for nonsphericity = 1, the sample size was equal to 148. Considering possible dropouts during the study and estimating an overall loss of 22% of patients, 180 patients would therefore have been recruited, that is 45 for each group.

Statistical analysis was performed using SPSS 23.0 (IBM, Chicago, IL, USA). Data were tested for normality with the Shapiro–Wilk test, with the results expressed as mean and standard deviation, median and interquartile range, or frequency and percentage. Data were analysed using chi-square or Fisher's exact test, one way ANOVA test, Kruskal–Wallis test and repeated-measures ANOVA test followed by pairwise comparison with post hoc Bonferroni correction, as appropriate. A two-tailed p value $<.05$ was considered significant.

3 | RESULTS

Figure 1 reports flow diagram for participants. Parental informed consent was obtained from 180 eligible patients. A 48 h of life, 8 newborns were excluded due to withdrawal of informed consent, formula or mixed milk feeding, sample haemolysis and mothers with SARS-Cov2 infection. Out of 172 subjects, 29 newborns were furtherly excluded at 1 month visit and sample collection due to formula or mixed milk feeding, sample haemolysis, lack of VK1 administration. One hundred and forty-three newborn infants admitted to 3 months follow-up visit and sample collection. Thirty-eight infants were excluded due to formula or mixed milk feeding, sample haemolysis, and lack of VK1 administration.

Clinic characteristics of enrolled population are reported in Table 1. There were no differences in clinical data between groups. During the study period, no patients reported bleeding or other symptoms related to VKDB.

Table 2 displays the PIVKA-II concentrations at 48 h, 1 and 3 months of age for the different groups. The data are presented in AU/mL to ensure consistency with previous studies.[12,20,30]

At 48 h of life, there was no difference in PIVKA-II level in patients who received IM administration at birth (Group 1+2+3) versus patients who received oral administration (Group 4) (median 4.595 IQR [2.955–4.780] AU/mL vs. median 3.0 IQR [1.887–5.026] ng/mL, $p = .908$) (Figure 2A).

At 1 and 3 months of life, Group 1 had higher PIVKA-II level than Group 2, Group 3 and Group

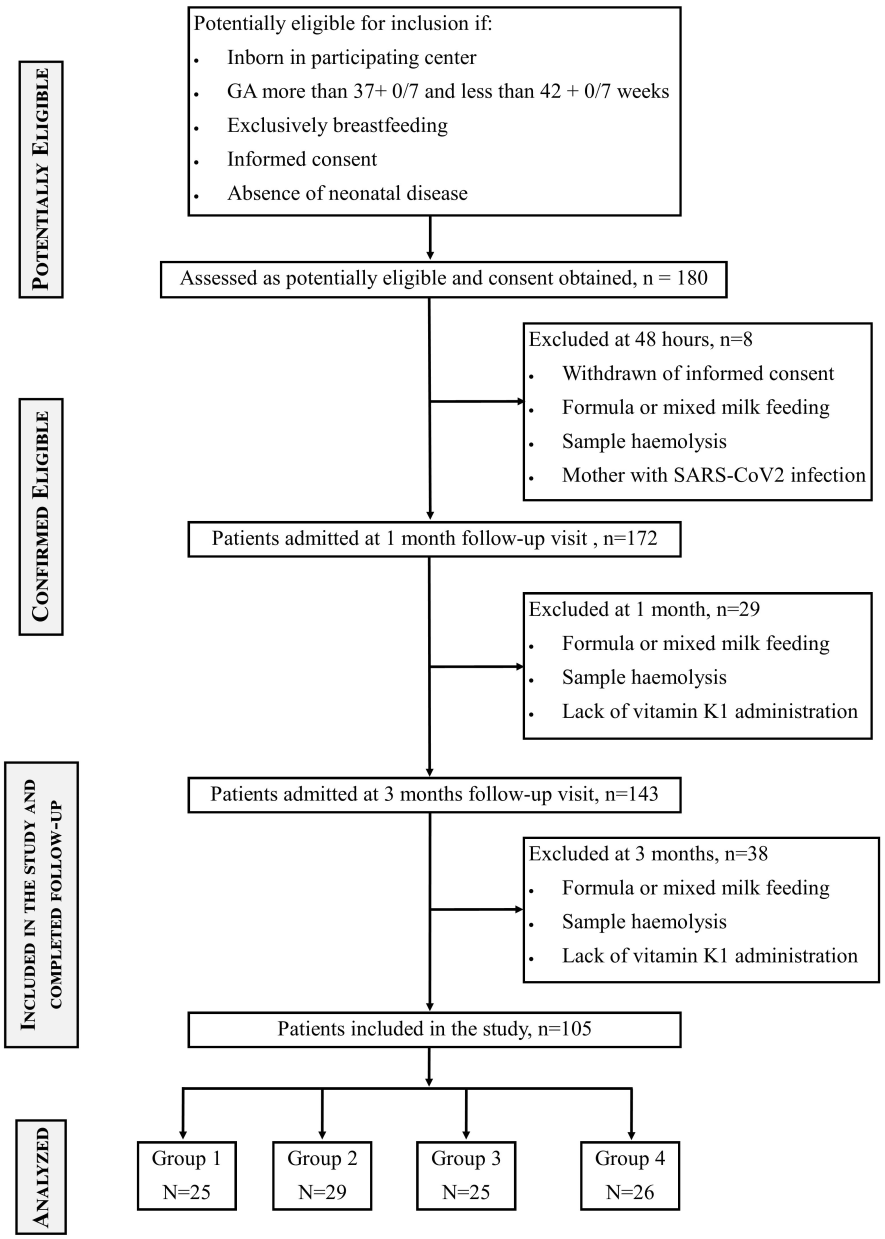

FIGURE 1 Flow diagram for participants enrolment.

TABLE 1 Basic and clinical population details.

	Group 1 ($n=25$)	Group 2 ($n=29$)	Group 3 ($n=25$)	Group 4 ($n=26$)	p Value
GA (Weeks)	40 [39–41]	40 [39–41]	40 [39–41]	40 [38.7–41]	ns
Caesarean section	4 (16%)	5 (17.2%)	7 (28%)	5 (19.2%)	ns
BW (g)	3438 (±296)	3249 (±347)	3505 (±478)	3303 (±466)	ns
Apgar score 5′	10 [10–10]	10 [10–10]	10 [10–10]	10 [10–10]	ns
Gender (male)	20 (80%)	16 (55.2%)	16 (64%)	15 (57.7%)	ns
DoH	2 [2–2]	2 [2–3]	2 [2–2.5]	2 [2–2.5]	ns
PROM	8 (32%)	4 (13.8)	7 (28%)	7 (26.9)	ns
Arterial cord blood Ph	7.26 (±.07)	7.27 (±.06)	7.27 (±.06)	7.29 (±.06)	ns

Note: Data are given as or *n* (%), or media (standard deviation) or median [interquartile range]. Chi-square or Fisher, Mann–Whitney test were used for the statistical analysis. Group 1 VK: 1 mg im at birth; Group 2 VK: 1 mg im at birth, 50 μg/day po from 2 to 14 weeks; Group 3 VK: 1 mg im at birth, 150 μg/day po from 2 to 14 weeks; Group 4 VK: 2 mg po at birth, at 4 and 12 weeks.

Abbreviations: BW, birth weight; DoH, days of hospitalization; GA, gestational age.

TABLE 2 PIKVA levels at 48 h of life, 1 and 3 months of life.

PIVKA-II 48 h (AU/mL)	4.6 [2.96–4.78]	2.31 [1.54–3.93]	2.36 [1.75–6.77]	3 [1.89–5.03]	
PIVKA-II 1 month (AU/mL)	.68 [.52–.76]	.23 [.18–.29]	.10 [.09–.14]	.3 [.27–.31]	*, **, ***, ****, *****
PIVKA-II 3 month (AU/mL)	.06 [.05–.06]	.03 [.02–.04]	.01 [.008–.01]	.04 [.03–.06]	*, **, ****, *****

Note: PIKVA level at 48 h of life, 1 month and 3 months of life in different VK regimens groups. PIVKA: Protein induced by VK absence. Data are given as median [interquartile range]. Group 1 VK: 1 mg im at birth. Group 2 VK: 1 mg im at birth, 50 μg/day po from 2 to 14 weeks. Group 3 VK: 1 mg im at birth, 150 μg/day po from 2 to 14 weeks. Group 4 VK: 2 mg po at birth, at 4 and 12 weeks.

*$p < .05$ for comparison between Group 1 versus Group 2; **$p < .05$ for comparison between Group 1 versus Group 3; ***$p < .05$ for comparison between Group 1 versus Group 4; ****$p < .05$ for comparison between Group 2 versus Group 3; *****$p < .05$ for comparison between Group 3 versus Group 4.

4; Group 2 had higher PIVKA-II level than Group 3 and Group 4 had higher PIVKA-II level than Group 3 (Figure 2B,C, respectively).

Both at 1 and 3 months of life infants who received any supplementation regimen between 2 and 14 weeks (Group 2, Group 3 and Group 4) exhibited significantly lower PIVKA-II concentrations compared to infants who received only 1 mg of VK1 at birth (Group 1) (Figure 3A,B, respectively).

At 3 months of age, Group 3 exhibited the lowest proportion of neonates surpassing a PIVKA-II concentration >.05 AU/mL when compared to the other groups (Group 1: 17/25 (68%), Group 2: 6/29 (20.6%), Group 3: 0/25 (0%) and Group 4: 11/26 (42.3%); $p < .05$).

Additionally, the PIVKA-II percentage difference (Δ) at 1 month of age and 3 months of age was significantly lower in Group 3 compared to Group 1, Group 2 and Group 4 (as illustrated in Figure 4A,B, respectively). At 1, 3 and 6 months of life, no haemorrhagic events were observed or recorded in any enrolled infant.

4 | DISCUSSION

The findings of this study contribute to the understanding of VKDB prophylaxis regimens and their potential protective effects based on PIVKA-II assessment. Currently, there is insufficient evidence to establish standardized recommendations for the dosage and routes of administration of VK prophylaxis worldwide, as practices vary significantly.

The study aimed to compare different regimens of VKDB prophylaxis to determine which one had the most favourable impact on PIVKA-II levels. The results indicated that after 48 h, there was no significant difference in PIVKA-II concentrations between oral and intramuscular administration. Data suggest that both intramuscular and oral intake of VK1 may be effective only in preventing classic VKDB, which can occur within the first week after birth.

At 1 and 3 months of age, neonates who received only a single intramuscular injection of VK1 at birth showed higher PIVKA-II levels compared to other regimens. This suggests that a single intramuscular injection may be inadequate for preventing late forms of VKDB.[17] The study found that the subsequent oral dosages, particularly 150 μg/day from Weeks 2 to 14, provided the lowest PIVKA-II concentrations during the period when susceptibility to late VKDB is highest.

In neonates who received a daily dose of 150 μg/day from Weeks 2 to 14, the median PIVKA concentrations at 3 months of age were .01 AU/mL. These values align with the data reported for formula/mixed-fed preterm newborn infants.[13]

To address the vulnerability of newborns to VK deficiency, we explored the hypothesis that the decremental

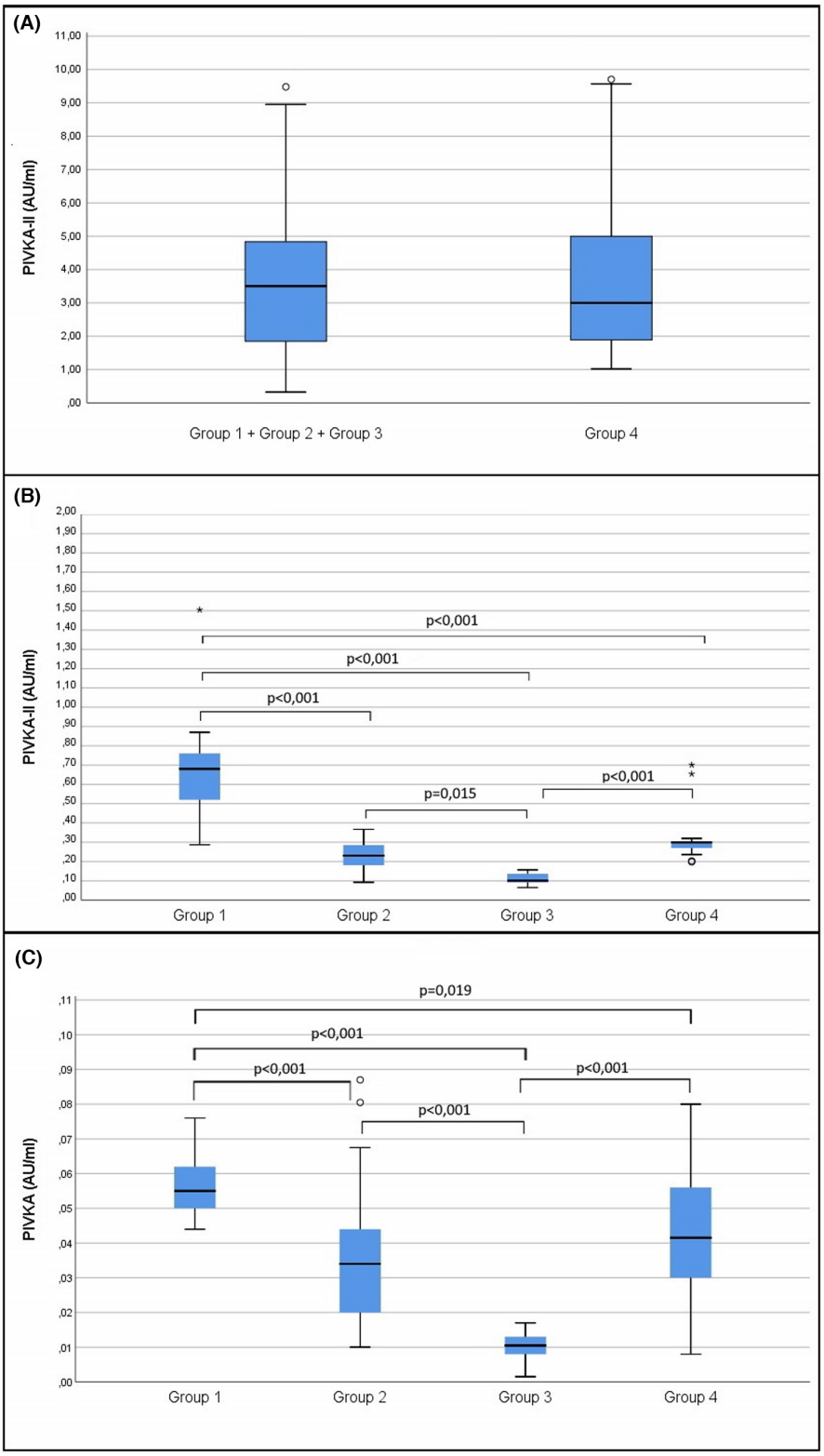

FIGURE 2 PIVKA-II concentrations at different time points. PIVKA-II concentrations at 48 h of life (A), PIVKA-II concentrations at 1 month of life (B) and 3 months of life (C) in different vitamin K1 regimens groups. The black centre line denotes the median value (50th percentile), while the blue box contains the 25th to 75th percentiles of dataset. The black whiskers mark the 5th and 95th percentiles, and values beyond these upper and lower bounds are considered outliers.

percentage of PIVKA II over time was significantly correlated with the amount of VK intake. Specifically, our findings revealed a substantial reduction in the PIVKA II decrement percentage, closely linked to the administered dose of VK1.

Hence, a proactive prevention strategy involving daily supplementary oral VK1 supplements at the point of hospital discharge emerges as the most rational and secure approach, irrespective of the route of initial prophylaxis.

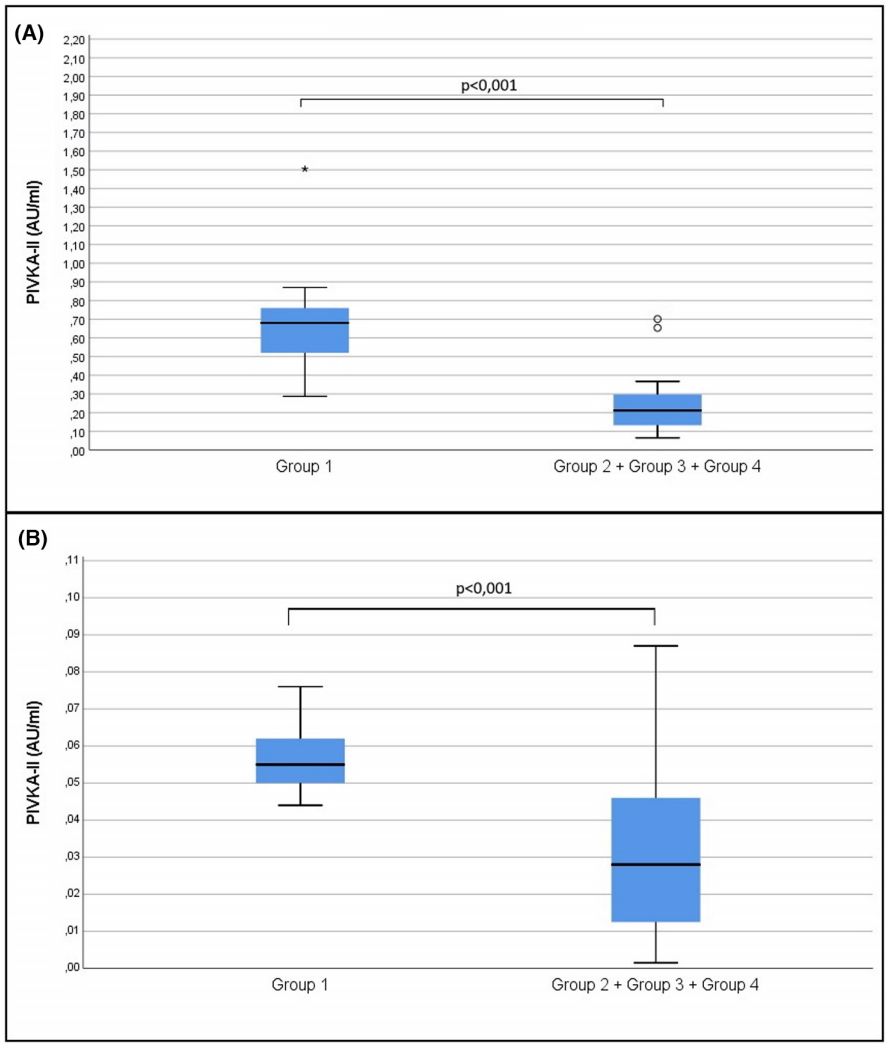

FIGURE 3 PIVKA-II concentrations between two different modalities of administration of vitamin K1. PIVKA-II concentrations at 1 month of life (A) and 3 months of life (B) between newborns who had received a single dose of intramuscular vitamin K1 at birth and those that continued to receive Vitamin K1 per os following the initial intramuscular dose of vitamin K1. The black centre line denotes the median value (50th percentile), while the blue box contains the 25th to 75th percentiles of dataset. The black whiskers mark the 5th and 95th percentiles, and values beyond these upper and lower bounds are considered outliers.

PIVKA-II measurement serves as an effective biomarker for diagnosing VK deficiency and represents an early indicator of the risk of developing VKDB.[24,29]

However, there is currently no established target range correlating PIVKA-II levels with clinical manifestations of VKDB. PIVKA-II levels have been shown to be a sensitive alternative to PT/INR for assessing VK deficiency in neonates and diagnosing subclinical VK deficiency.[7,23]

PIVKA-II concentrations of .05 AU/mL, which represent the upper limit of the adult reference range according to the Abbott method, are indicative of early Vitamin K insufficiency. Clarke et al.[13] discovered that 67% of exclusively breastfed preterm newborns, who received a single dose of .4 mg/kg VK1 prophylactic dose, exceeded PIVKA-II levels of .05 AU/mL. In our study, 68% of newborns who received a single intramuscular injection of 1 mg VK1 without ongoing oral supplementation exceeded PIVKA-II levels of .05 AU/mL, at 3 months of life. Additionally, in these neonates, the PIVKA-II decremental percentage at 1 month of age and 3 months of age was significantly lower than in the other groups.

More recently, Ilyés et al.[33] have suggested that VK insufficiency is reflected in the under-carboxylation of extrahepatic Gla proteins more than hepatic proteins. It is possible that the carboxylation status of extrahepatic vitamin K-dependent proteins is more susceptible to suboptimal VK levels compared to PIVKA-II.

These findings imply the necessity of safeguarding exclusively breastfed newborns from risk factors that can influence the progression of subclinical deficiency to VKDB. This can be achieved through prolonged oral administration of VK1, especially in regions where only intramuscular prophylaxis and surveillance programs are in place and spontaneous cases of VKDB continue to be reported.[34,35]

It is essential to consider international recommendations and practical considerations when implementing VK prophylaxis regimens. The availability of approved oral VK formulations, cost considerations and the feasibility of implementing new strategies should be taken into account when planning VK prophylaxis policies.[36] It is worth noting that the lack of adherence to recommended regimens for oral prophylaxis has been associated with

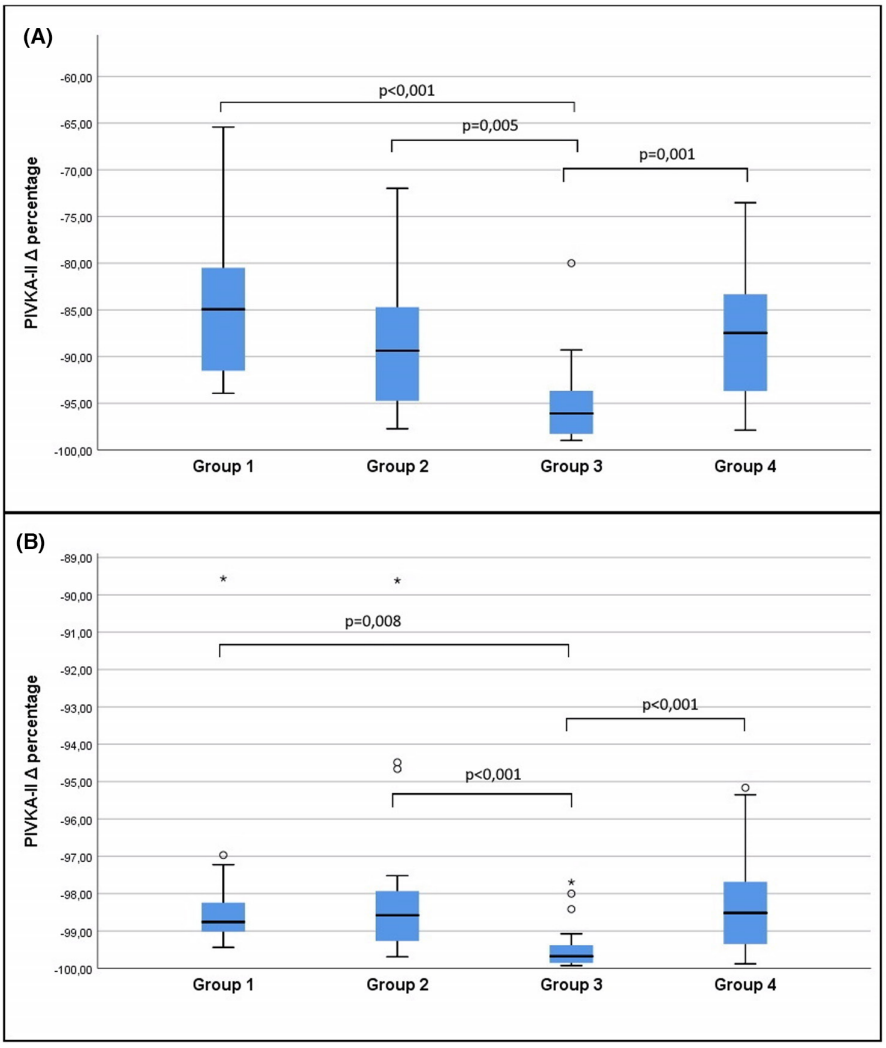

FIGURE 4 PIVKA-II Δ percentage at different time points. PIVKA-II Δ percentage at 1 month of life (A) and 3 months of life (B) in different Vitamin K1 regimens groups. The black centre line denotes the median value (50th percentile), while the blue box contains the 25th to 75th percentiles of dataset. The black whiskers mark the 5th and 95th percentiles, and values beyond these upper and lower bounds are considered outliers.

an increased prevalence and incidence of late VKDB in infants.[37,38]

This study represents the first investigation reporting PIVKA-II measurements on four different regimens of VK prophylaxis in exclusively breastfed, term healthy newborns after hospital discharge. We achieved a high rate of study completion and demonstrated a significant reduction in the risk of high PIVKA-II levels (indicating low levels of VK) and the subsequent development of VK deficiency during the first 3 months of life by supplementing with 150 μg of VK1 from the second to the fourteenth week. To the best of our knowledge, these findings present the first set of data reporting PIVKA-II assessment from birth until the third month of life, examining various regimens of VKDB prophylaxis.

Some limitations of this study should be acknowledged. Firstly, it was a relatively small observational study that relied on biochemical markers of VK deficiency rather than assessing actual haemorrhagic outcomes. Given the worldwide incidence of VKDB is approximately 1 in 100,000 live births, only large and very expensive prospective studies would have the capacity to detect these rare but significant clinical outcomes associated with VK deficiency. Secondly, we did not include measurement of prothrombin time (PT) in the study infants. PT is known to be an insensitive marker for diagnosing subclinical VK deficiency, and a prolonged PT lacks specificity for VK deficiency in term infants.

Thirdly, the inclusion of another group comprising formula-fed infants could have provided valuable data on typical PIVKA II levels, facilitating a comparison with breastfed infants. Moreover, the introduction of a fourth evaluation time point, extending the observation period to 6 months, might have yielded further insights and information.

The administration of VK prophylaxis through intramuscular injection has a similar effect as oral administration on PIVKA II levels at 48 h of life. Additionally, routine oral supplementation of VK1 after discharge until 14 weeks of life, with a dosage of 150 μg per day, has been found to significantly reduce PIVKA-II levels in exclusively breastfed term infants. This supplementation

regimen is believed to help prevent the risk of developing VK insufficiency in early infancy.

Interestingly, prior studies have explored the impact of VK supplementation on bone mineral density and quality, suggesting that VK deficiency might serve as a significant predictive factor for bone fractures.[39,40] Additionally, it is important to highlight the potential role of VK insufficiency in early infancy, which could potentially affect the future bone quality and bone strength of infants fed exclusively with breast milk.[13] Clarke et al.[13] demonstrated inverse correlation between dietary VK1 intakes and undercarboxylated osteocalcin in exclusively with breast milk preterm infants. This aspect deserves special consideration and attention.

Further studies with a larger sample size are necessary to provide more robust evidence and to determine the most effective and appropriate regimen for VK supplementation.

In the meantime, for specific recommendations regarding VK prophylaxis and supplementation in infants, it is advisable to follow national and international guidelines based on the most up-to-date medical knowledge.

5 | CONCLUSIONS

The administration of VK prophylaxis through intramuscular injection and oral administration has been found to have similar effects on PIVKA-II concentrations at 48 h of life. Additionally, routine oral supplementation of VK1 from discharge until 14 weeks of life, with a daily dosage of 150 micrograms, has been shown to significantly decrease PIVKA-II concentrations in exclusively breastfed term infants. This supplementation regimen effectively reduces PIVKA-II concentrations, which indicates a risk of low plasma VK, thereby helping to prevent the risk to develop late VKDB features in early infancy. Further studies are needed to specifically address the effectiveness of post-discharge VK1 supplementation in groups of infants with underlying risk factors for malabsorption like cholestasis.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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