

Screening for late neonatal vitamin K deficiency by acarboxyprothrombin in dried blood spots

K MOTOHARA, F ENDO, AND I MATSUDA

Department of Pediatrics, Kumamoto University Medical School, Japan

SUMMARY Acarboxyprothrombin (protein induced by vitamin K absence or antagonist-II (PIVKA-II)) concentrations in dried blood spots were determined in 19 029 infants at about 1 month of age as an indicator of vitamin K deficiency. We observed 51 cases with raised blood concentrations of PIVKA-II (>4 AU/ml), nine of whom showed very high concentrations (>20 AU/ml).

For infants who did not receive vitamin K prophylaxis at birth, the incidence of the PIVKA-II test yielding positive results was significantly higher in those solely breast fed (0.51%) compared with those fed formula milk (0.18%). Among solely breast fed infants, the incidence of a very high result of the PIVKA-II test was 0.14% in those who had not received vitamin K prophylaxis at birth, 0.04% in those who received 2 mg orally, and 0.03% in those who received 2 mg orally plus a further dose of 2-4 mg orally at 7 days.

Thus vitamin K prophylaxis at birth did not completely prevent vitamin K deficiency at 1 month. We administered vitamin K therapeutically to all infants whose PIVKA-II test yielded a positive result at 1 month. Only one infant with a positive result developed late neonatal intracranial haemorrhage.

Severe intracranial haemorrhage related to vitamin K deficiency in infants aged 1 month may occur suddenly and lead to death or severe central nervous system dysfunction. Almost all of the affected infants who have been reported had not received vitamin K at birth.¹⁻⁸ The American Academy of Pediatrics recommends that all newborn infants receive vitamin K (a single parenteral dose of 0.5-1.0 mg or 1.0-2.0 mg orally).⁹ In Japan newborn infants are not given routine vitamin K prophylaxis. This might be one of the reasons for the high incidence of late neonatal vitamin K dependent intracranial haemorrhage in our country. This disorder occurs in one in every 4500 infants and one in every 1700 breast fed infants who are not given vitamin K prophylaxis.¹

Recently, the study group of the Ministry of Health and Welfare of Japan recommended that all newborn infants should receive vitamin K orally,¹ but intramuscular administration of the vitamin was not recommended. In this study we report a mass screening system for acarboxyprothrombin (protein induced by vitamin K absence or antagonist-II (PIVKA-II)) as an indicator of vitamin K deficiency at 1 month. PIVKA-II is a precursor of active

prothrombin and converted to the active form by vitamin K and carboxylase.¹⁰ This study aimed to evaluate the effect of oral vitamin K that had been administered after birth and to detect patients with severe vitamin K deficiency at 1 month before symptoms occurred.

Patients and methods

Plasma PIVKA-II concentration was measured by means of an assay using a monoclonal, monospecific antibody.¹¹ The PIVKA-II concentration in dried blood spots was determined as follows. Blood was spotted on filter paper, four discs of 6 mm diameter were punched out for each individual, and then the blood was eluted from the discs for one hour in 0.3 ml of phosphate buffered saline (10 mM phosphate buffer and 150 mM sodium chloride, pH 7.4), with horizontal shaking. Then 100 µl of the eluate was subjected to the PIVKA-II assay as described previously.¹¹ Each measurement was performed in duplicate. The PIVKA-II concentrations determined for the same blood sample with different assay systems were compared to evaluate the reliability of the dried blood assay system.

Monitoring of PIVKA-II concentrations by the blood spot test was carried out in 19 029 young infants aged about 1 month in the Kumamoto region of Japan between April 1984 and January 1986. Measurements were made within 24 hours of receiving the samples. Roughly 90% of the breast fed infants in this area were included in this survey. The infants consisted of 11 107 who were breast fed, 7157 who were both breast and formula fed (mixed fed), and 765 who were formula fed. As breast feeding is known to be a risk factor for vitamin K deficiency most of the women who participated in the monitoring were breast feeding their infants. The incidence of haemorrhagic disease in the first week of life among these infants was not known and infants who showed early neonatal bleeding were not excluded. Those infants allocated to receive vitamin K were given either 2.0 mg orally within 24 hours of birth (one dose) or this initial dose plus 2-4 mg orally at 1 week of age (two doses). The decision to administer vitamin K and the manner of administration were determined by the obstetricians. The vitamin was given as vitamin K₂ syrup (Kay Two Syrup (menaquinone 4, 2-methyl-3-all-trans-tetraprenyl-1, 4-maphthoquinone), Eisai Co Ltd, Tokyo, Japan) because vitamin K₁ was unavailable as a syrup in Japan.

In Japan all infants are examined under the child health survey program at 1 month of age. At this examination we obtained informed consent from all the parents and then took blood samples from the infants by means of a heel puncture, which were then spotted onto filter paper, dried, and sent to our laboratory by post.

The cut off point for PIVKA-II on the blood spot test was set at 4.0 AU/ml. One arbitrary unit (AU) corresponds to 1 µg purified prothrombin. Our preliminary study suggested that in breast fed infants aged 1 month who had not received vitamin K prophylaxis the 0.6 centile for PIVKA-II might be about 4.0 AU/ml.¹² This cut off point was anticipated to select 10-fold the number of infants with late onset bleeding due to vitamin K deficiency, based on an estimated incidence of 0.06%.¹ When the result was above this the plasma PIVKA-II concentration and normotest activity were measured by methods previously described¹¹ and then 5 mg vitamin K₂ given orally.

When the PIVKA-II concentration was higher than 20 AU/ml according to the blood spot test 1-2 mg vitamin K₂ was injected within six hours of the diagnosis being made. Infants with normotest activities of under 20% had a computed tomogram taken. The normotest activity reflects the total coagulant activities of factors II, VII, and X.¹³ The normal mean (SD) normotest

value in breast fed infants at 1 month of age is 71.4 (17.0)% (n=218).¹⁴

The comparison of incidences between various groups was analysed by the χ^2 test with Yates's correction.

Results

Measurement of PIVKA-II in dried blood spots.

Figure 1 shows the correlation between PIVKA-II values on dried blood spots and those obtained by measuring PIVKA-II in plasma samples from the same subjects. Blood samples with high PIVKA-II concentrations were obtained from 27 adults receiving warfarin as it was difficult to obtain many samples from the infants with vitamin K deficiency at the same time. The high correlation indicated that the dried blood method was reliable for measurement of PIVKA-II concentrations. The detection limit for blood PIVKA-II was 1.0-32.0 AU/ml with the dried blood method. The coefficients of variance of intra- and interassays were less than 0.06. The recovery of PIVKA-II from dried blood spots was monitored after spotting. A mean of 87.7% (n=27) of the initial PIVKA-II concentration was found at room temperature on the fifth day after the blood had been obtained.

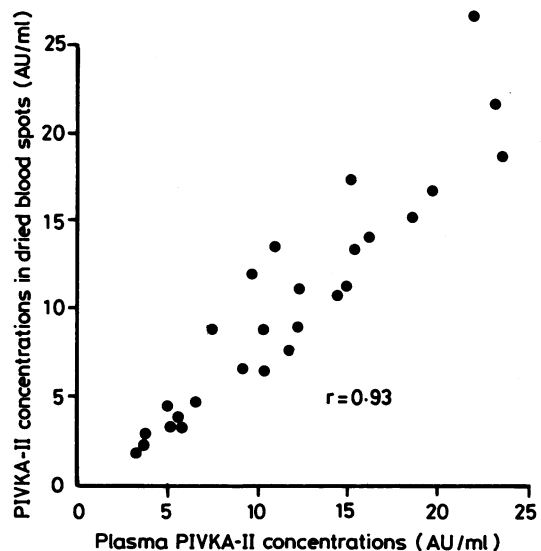


Fig. 1 Correlation between PIVKA-II concentrations in dried blood spots and in plasma samples from the same subjects. The equations for the regression lines for the PIVKA-II values for dried spots v those for plasma are $y=0.97x-1.07$, $r=0.93$, $p<0.005$.

Table 1 Infants with positive results of PIVKA-II test according to method of feeding and vitamin K prophylaxis at birth

Group	Vitamin K prophylaxis	No	PIVKA-II >4AU/ml (No (%))†		PIVKA-II >20 AU/ml (No (%))†	
			Solely breast fed	Mixed or formula fed	Solely breast fed	Mixed or formula fed
1	None	8346 (5090)*	26 (0.51)	6 (0.18)	7 (0.14)	0 (0)
2	2 mg orally on day 1	5169 (2811)*	10 (0.36)	3 (0.13)	1 (0.04)	0 (0)
3	2 mg orally on day 1 and 2-4 mg on day 7	5514 (3206)*	6 (0.19)	0 (0)	1 (0.03)	0 (0)
Total		19 029	42 (0.38)	9 (0.11)	9 (0.08)	0 (0)

*No of solely breast fed infants.

†AU=Arbitrary unit.

PIVKA-II >4 AU/ml=Group 1 v group 3, $p<0.01$; group 1 v group 2, not significant; group 1 solely breast fed v group 1 mixed or formula fed, $p<0.05$; total solely breast fed v total mixed or formula fed, $p<0.01$.

PIVKA-II >20 AU/ml: Total solely breast fed v total mixed or formula fed, $p<0.05$.

Table 2 Clinical and laboratory data of infants with very high PIVKA-II concentrations (>20 AU/ml)

Case no	Age (days)	Sex	PIVKA-II concentrations		Normotest activity (%)
			Dried blood	Plasma	
1	31	M	>32.0	40.1	<10
2	32	M	>32.0	54.4	<10
3	36	F	>32.0	65.5	<10
4	39	M	>32.0	ND	ND
5	31	M	>32.0	ND	ND
6	33	M	>32.0	38.0	<10
7	31	F	>32.0	54.4	11.0
8	37	M	>32.0	69.7	12.5
9	35	M	16.7	23.24	15.0

ND=Not done.

All but case 9 had blood spot values above the upper detection limit (>32.0 AU/ml). Case 9 was included because plasma PIVKA-II was above 20 AU/ml.

Results of screening for vitamin K deficiency. The results of the screening survey are shown in Table 1. Fifty one cases with high PIVKA-II concentrations (>4.0 AU/ml) were found among the 19 029 infants who were monitored. Of these, 42 were solely breast fed, nine mixed fed, and none solely formula fed.

For infants who did not receive vitamin K prophylaxis, the incidence of vitamin K deficiency was higher in those who were entirely breast fed compared with those who received formula or mixed feeds ($p<0.05$). When all the infants were classified into three groups according to vitamin K prophylaxis—group 1 (none), group 2 (2 mg orally at birth), and group 3 (2 mg orally at birth and 2-4 mg orally at 1 week)—the incidence of vitamin K deficiency was higher in group 1 (32 of 8346, 0.38%) compared with group 3 (6 of 5514, 0.11%) ($p<0.01$).

All nine cases of severe vitamin K deficiency (PIVKA-II >20 AU/ml) were entirely breast fed. Seven belonged to group 1 for vitamin K prophylaxis

and one to each of the other two groups. Although the incidence of severe vitamin K deficiency did not differ significantly between these groups, there was a trend towards a decreasing incidence of severe vitamin K deficiency with increasing vitamin K prophylaxis (from group 1 to group 3) (Table 1).

Clinical and laboratory data. Laboratory data for the nine infants with severe vitamin K deficiency are shown in Table 2. After the intramuscular injection of 1-2 mg vitamin K normotest activities returned to normal within 24 hours and plasma PIVKA-II concentrations decreased gradually and reached normal within eight to 14 days (Fig. 2).

Only one of the 51 infants with a positive result of the PIVKA-II test had evidence of late onset haemorrhage, and that was a baby with severe vitamin K deficiency who had an asymptomatic small subdural haemorrhage apparent on computed tomography (Table 2, case 2). Among the other infants with severe vitamin K deficiency, a diagnosis of congenital bile duct atresia was later established in case 6. Other forms of hepatic dysfunction, manifest by raised serum transaminase activities, were observed in cases 2 and 4.

In the present study only one infant with a negative result of the PIVKA-II test had late onset haemorrhage (intracranial bleeding), and this occurred 21 days after the blood spot test had yielded negative results. This infant had congenital bile duct atresia.

Discussion

Direct determination of plasma vitamin K may lead to a greater understanding of the role of this vitamin,¹⁵ but the direct assay is not practical for a

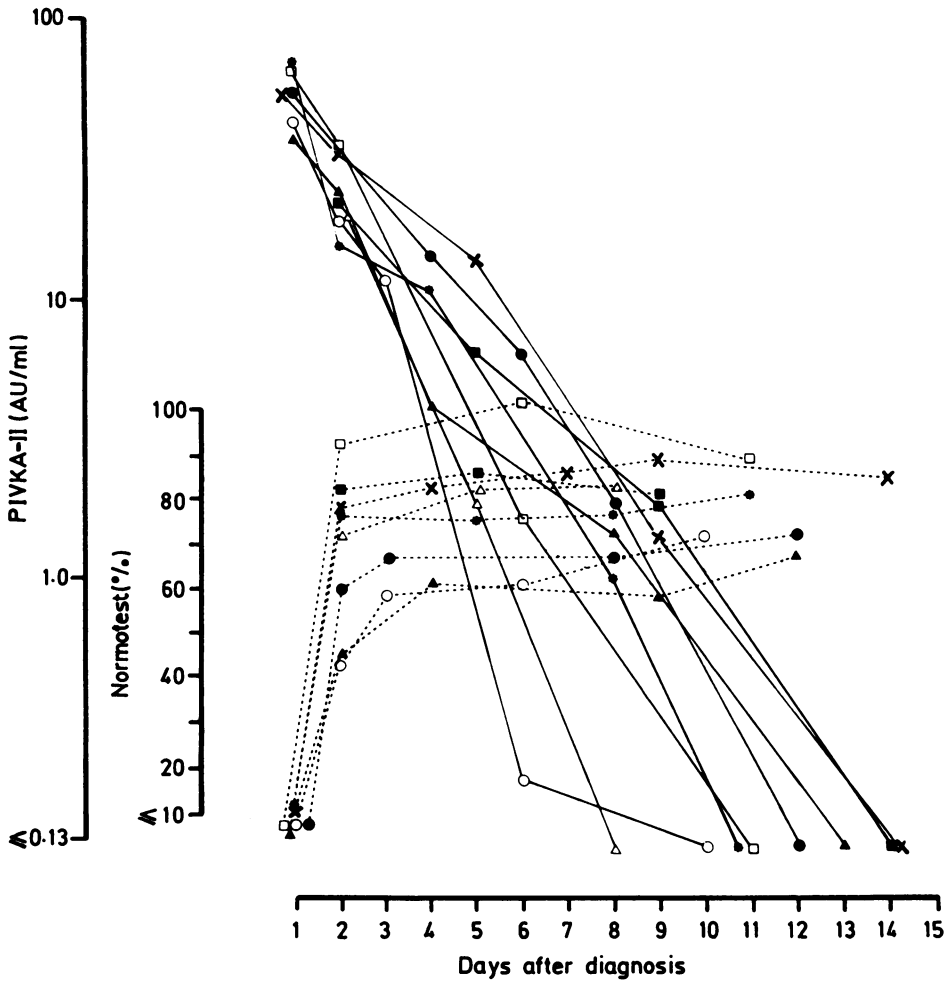


Fig. 2 Effects of intramuscular administration of vitamin K₂ (1–2 mg) on plasma PIVKA-II and normotest activity in infants with vitamin K deficiency. The solid lines represent plasma PIVKA-II, the broken lines the normotest activity. The eight infants represented here are those with blood spot PIVKA-II concentrations >20 AU/ml. All had values above the upper limit of detection (>32 AU/ml).

hospital service. PIVKA-II occurs in peripheral blood in vitamin K deficiency or patients treated with warfarin but is not present in normal adults.^{10 16–18} Thus measurement of the plasma PIVKA-II concentration is useful for evaluating vitamin K deficiency. Previously, we measured PIVKA-II concentrations in newborn infants as an indicator of vitamin K deficiency and concluded that oral vitamin K prophylaxis at birth was effective in preventing early neonatal bleeding.¹⁹

Faced with the need for prophylaxis for all neonatal infants, physicians have been reluctant to

use the usual intramuscular injection routinely in Japan. McNinch *et al* and Sann *et al* reported that serum vitamin K concentration increased considerably after oral administration of vitamin K (1–2 mg) and concluded that oral administration of vitamin K was sufficient to prevent neonatal bleeding due to vitamin K deficiency.^{20 21} Oral prophylaxis with vitamin K in newborn infants is more economical and simpler than intramuscular prophylaxis. The oral route is most practical when routine administration is required for all newborn infants.

It is not clear at present whether oral administra-

tion of vitamin K in the neonatal period is effective in preventing late onset infantile bleeding due to vitamin K deficiency at about 1 month of age.²² We have been unable to find any published data relating to this problem. The purpose of this survey was to obtain both a scientific basis for an effective protocol for preventing vitamin K deficiency and a means of identifying this disease before clinical symptoms occurred. To achieve this a large number of subjects was necessary as the incidence of the disease is fairly low. We therefore developed the mass monitoring system for PIVKA-II described in this paper. The reliability of the simplified method using dried blood spots was sufficient for these purposes.

In this study the infants were divided into three groups according to vitamin K prophylaxis. We could then evaluate the prolonged effect of administration of vitamin K in the newborn period. We showed that breast feeding was a risk factor for the disease, supporting the results of retrospective surveys.¹⁻⁸ The incidence of infants with a PIVKA-II test that yielded positive results (>4 AU/ml) among untreated infants was higher compared with those given two doses of vitamin K, whereas the incidences of very high PIVKA-II concentrations (>20 AU/ml) in the two treated groups were similar. We are unable to conclude at present whether or not a double dose of vitamin K offers more protection than a single dose. It was hoped that both neonatal bleeding and late onset infantile bleeding could be prevented with a single administration of vitamin K at birth. Our results, however, suggested that vitamin K prophylaxis once or twice at birth was not sufficient completely to prevent vitamin K deficiency at 1 month.

We found 51 cases of vitamin K deficiency, nine of whom had very high concentrations of PIVKA-II on the blood spot test or plasma assay. Seven of these cases had critically low clotting activity based on the normotest. Of the nine cases with severe vitamin K deficiency, one had congenital bile duct atresia and two mildly raised serum transaminase activities, whereas the remaining six cases had no predisposing factors such as chronic diarrhoea, malnutrition, or broad range antibiotics. Vitamin K dependent coagulant activities promptly improved and the plasma PIVKA-II concentration decreased gradually in all cases on an injection of vitamin K. Only one of these cases had intracranial haemorrhage. It can be speculated that the monitoring system and treatment was effective.

Over three years (1979-81) 16 cases of late onset vitamin K deficiency were reported in the area where this study was performed¹⁴ and almost all these infants did not receive vitamin K prophylaxis.

During the period of this study (21 months), based in the same area, only three cases of vitamin K deficiency were observed. In one of these cases the deficiency occurred at 28 days of age—that is, before the PIVKA-II concentration was measured—and was therefore not included in this report. The others were case 2 in Table 2 and the case of congenital bile duct atresia mentioned above.

A recent survey by the study group of the Ministry of Health and Welfare of Japan indicated an incidence of three in 1000 infants with critical normotest activities (below 20%) for 1 month old breast fed infants who did not receive vitamin K prophylaxis. The study group speculated that one in four or five infants with normotest values <20% might develop a bleeding disorder due to vitamin K deficiency as the incidence of the disease was 1 in 1700 in breast fed infants.¹ The incidence of critical normotest activities is close to the incidence of infants with high PIVKA-II concentrations (above 20 AU/ml).

We speculate that some infants with high PIVKA-II concentrations might have suffered from the disease if they had not received vitamin K. The decrease in the number of patients seemed to be due to the prophylactic administration of vitamin K which was performed for roughly 60% of the infants in this area, or the selective administration of vitamin K to infants with high PIVKA-II concentrations found by screening, or both.

Because screening for vitamin K deficiency by means of this method costs much more than the routine administration of vitamin K, both economically and socially, we hope to prevent the disease by means of prophylactic administration of the vitamin to all newborn babies. If the oral administration of vitamin K is continued as a routine method of preventing the disease the optimal dose of the vitamin needs to be determined.

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Correspondence to Dr K Motohara, Sumoto Amakusa, Kumamoto 861-63, Japan.

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