Early Infant Vitamin K Deficiency: Extent, Health Consequences and Approaches to Prophylaxis

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Introduction

The classical role of vitamin K is as an antihemorrhagic factor which is needed for the synthesis in the liver of functional forms of prothrombin (factor II) together with factors VII, IX and X [1, 2]. After secretion into the blood, these four vitamin K-dependent proteins form part of the coagulation cascade that, once initiated, culminates in the conversion of fibrinogen to fibrin and the formation of a hemostatic plug. The biochemical role of vitamin K is to act as a cofactor for the conversion of specific peptide-bound glutamate (Glu) residues to γ-carboxyglutamate (Gla) residues. Hence vitamin K-dependent proteins are often known as Gla proteins. The subsequent discovery of many more Gla proteins with a widespread tissue distribution has led to a reevaluation of the health roles of vitamin K. Two of these Gla proteins (proteins C and S) have well-defined roles in the negative feedback control of coagulation. Osteocalcin plays a putative role in the regulation of bone turnover while matrix Gla protein is essential for normal skeletal development and the prevention of calcification in the developing artery [3]. Other suspected functions of Gla proteins are in vascular repair processes (protein S), cell cycle regulation, cell-cell adhesion (Gas6) and signal transduction (Gas6 and proline-rich Gla proteins) [3]. For the most part, the precise physiological function of these new Gla proteins is poorly understood.

Naturally occurring compounds with vitamin K activity have a common 2-methyl-1,4-naphthoquinone nucleus and a variable alkyl substituent at the 3 position. The vitamin K found in the plant kingdom is phylloquinone.
(vitamin K$_1$) and the multiple forms that are synthesized by bacteria are menaquinones (vitamin K$_2$). Phylloquinone has a phytol side chain whereas menaquinones have multi-prenyl side chains, the number of prenyl units being indicated by a suffix (i.e. menaquinone-n, abbreviated MK-n). The compound 2-methyl-1,4-naphthoquinone (trivial name menadione) is not naturally occurring but does possess biological activity in vertebrates because of their ability to add on a geranylgeranyl side chain to produce menaquinone-4 (MK-4); in this sense menadione may be regarded as a provitamin K.

Put simply, a deficiency of vitamin K results in a failure to synthesize γ-carboxyglutamic acid. The consequences of vitamin K deficiency for hemostasis are an inability to synthesize functional molecules of factors II, VII, IX and X which results in a hypocoagulable state. The hemostatic system has a considerable capacity to function adequately at low factor concentrations but as deficiency progresses a point will be reached when the procoagulatory mechanisms fail and bleeding occurs. This point is highly individual and unpredictable.

With the continuing discovery of new Gla proteins with other functions, many still unknown, there is awareness that chronic subclinical vitamin K deficiency (i.e. insufficient to cause bleeding) may be detrimental to health in other ways. For example a suboptimal vitamin K status in adults results in undercarboxylation of Gla bone proteins such as osteocalcin, and this has been implicated in the pathogenesis of osteoporosis [3]. The anti-calcification role of matrix Gla protein in skeletal development and arterial integrity also depends on the Gla residues.

**Vitamin K Deficiency in Early Infancy**

To date the only manifestation of vitamin K deficiency in early infancy representing a public health issue is a bleeding syndrome originally known as hemorrhagic disease of the newborn (HDN), but now renamed vitamin K deficiency bleeding (VKDB) in infancy [4]. VKDB in infancy is largely confined to the first 6 months of life and often occurs without warning, needing emergency treatment. Some infants die and many are left with permanent mental handicap due to bleeding into the central nervous system. The syndrome has no counterpart in adults who, in the absence of underlying disease, have virtually no risk from spontaneous bleeding due to a dietary vitamin K deficiency.

The first description of a bleeding syndrome (and the term HDN) which had all the attributes of severe vitamin K deficiency is accredited to a Boston Physician, Charles Townsend who in 1894 described 50 cases of a generalized bleeding tendency in neonates. Townsend noted that HDN could be differentiated from hemophilia by its much earlier time of presentation (usually on days 2–3), lack of family history and most importantly by its self-limiting time course. The fascinating history of VKDB in infancy has been reviewed by
Hathaway [5], a history that is littered with uncertainties and controversies on the extent of the problem and the role of vitamin K prophylaxis in public health. A major recent controversy, still ongoing, is whether the administration of vitamin K preparations by the intramuscular route increases the risk of later childhood cancer [6, 7]. The objective of this chapter is to review the evidence that relates to our knowledge of the normal physiology of vitamin K in young infants with the pathophysiology of VKDB and its incidence in different countries. The issue of vitamin K prophylaxis and its effectiveness is addressed with respect to possible risks, and cultural and epidemiological issues that have influenced policy makers, healthcare workers and parents in its implementation. An important discussion point is the problem faced by developing countries where the incidence of VKDB is often far higher than in industrialized countries.

This review will not cover the demands of vitamin K by the fetus since little is known about this. However, in the context of other noncoagulation roles of vitamin K there is one syndrome that has been attributed to a deficiency of vitamin K in utero. This is an embryopathy characterized by abnormal calcification of the cartilage and features collectively called chondrodysplasia punctata. It is rare and typically results from the use of vitamin K antagonists (e.g. warfarin) during early pregnancy. However, 3 cases were recently described in which maternal malabsorption of vitamin K seemed the likely cause [8].

Nutrition and Physiology of Vitamin K in the Healthy Infant

Vitamin K Contents of Breast Milk

Modern analyses have established that the major vitamin K component of breast milk is phylloquinone, followed by MK-4 and trace concentrations of some higher menaquinones, MKs 6–8 [9, 10].

Until the 1980s the only available values for the vitamin K content of human milk and most other foods had been obtained by Henrik Dam's curative chick bioassay. This bioassay gave values for breast milk and cows’ milk of 15 and 60 μg/l of ‘vitamin K’, respectively. These values became widely cited in the literature without regard to the fact that Dam had used ‘menadione’ as the standard in the bioassay, a form that does not occur naturally. Later measurements of the vitamin K content of human milk by a physiochemical method suggested that the bioassay values were too high [11]. With standardized sampling techniques and accurate analysis, average concentrations in colostral and mature milk are about 2 and 1 μg/l, respectively [12]. Maternal supplementation with pharmacological doses of phylloquinone substantially increases breast milk concentrations in a dose-responsive manner with a lag phase of about 12 h between the time of supplementation and the appearance of peak milk concentrations [11, 12].
The presence of MK-4 in breast milk at concentrations that are about half those of phylloquinone is of interest with respect to its origin because MK-4 is neither a common bacterial form nor a major dietary form. However, recent evidence suggests that animals possess a pathway by which phylloquinone itself can be converted to MK-4 and that the MK-4 in breast milk is derived from dietary phylloquinone, possibly by the mammary gland [10].

**Vitamin K Contents of Formula Milks**

In 1971 the Committee on Nutrition of the American Academy of Pediatrics recommended that formulas should be supplemented with phylloquinone to a level of at least 50 μg/l and possibly 100 μg/l [13]. This recommendation was based on the bioassay values for human milk (15 μg/l) and cows’ milk (60 μg/l) and the knowledge that cows’ milk offered better protection against vitamin K deficiency [14]. These early deliberations seem to be the reason why formulas came to have concentrations of phylloquinone that greatly exceeded the true value of phylloquinone in human milk. Unsupplemented formulas have variable vitamin K contents (3–16 μg/l), which are generally higher than breast milk [11].

The average intake of phylloquinone in infants fed human milk during the first 6 months of life is of the order of 1 μg/day compared to about 50 μg/day in infants fed a typical supplemented formula (table 1) [15].

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**Table 1.** Dietary intakes and plasma levels of phylloquinone in human-milk-fed versus formula-fed infants aged 0–6 months

<table>
<thead>
<tr>
<th>Age, weeks</th>
<th>Phylloquinone intake, μg/day</th>
<th>Plasma phylloquinone, μg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>breast-fed&lt;sup&gt;a&lt;/sup&gt;</td>
<td>formula fed&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>0.55</td>
<td>45.4</td>
</tr>
<tr>
<td>12</td>
<td>0.74</td>
<td>55.5</td>
</tr>
<tr>
<td>26</td>
<td>0.56</td>
<td>52.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Breast milk concentrations averaged 0.86, 1.14, and 0.87 μg/l of phylloquinone at 6, 12, and 26 weeks, respectively.

<sup>b</sup> All infants were fed a formula containing phylloquinone at 55 μg/l.

Adapted from Greer et al. [15]

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Assessment of Vitamin K Status

**Coagulation Factor Assays**

Traditional screening tests for vitamin K deficiency are based on global coagulation assays such as the prothrombin time (PT), which measure ‘blood clotting ability’. These are simple to perform but a lengthening of the PT is not specific to vitamin K deficiency and needs to be confirmed by other tests.
The most useful confirmatory test is to show that the PT can be reversed by vitamin K administration. Inherent to the PT is its insensitivity, becoming prolonged only when the prothrombin concentration drops below 50% of normal [16]. Thus while the PT is appropriate for diagnosis of overt vitamin K deficiency, it is not useful for picking up subclinical deficiency.

**Abnormal or Undercarboxylated Coagulation Factors**

When the supply of vitamin K is insufficient, abnormal, functionally defective molecules of the coagulation factors are released into the bloodstream. These abnormal molecules comprise multiple, undercarboxylated or partially carboxylated molecules but their chemical identity remains unknown. The historical collective abbreviation for these undercarboxylated species is PIVKA (proteins induced by vitamin K absence or antagonism). The development of assays to detect undercarboxylated prothrombin (PIVKA-II) has proved to be extremely useful as a functional marker of subclinical vitamin K deficiency in infants [16, 17]. The most sensitive assays are enzyme immunoassays using antibodies that recognize PIVKA-II but do not cross-react with normal factor II.

**Plasma Concentrations of Vitamin K**

The major circulating form of vitamin K is phylloquinone and its measurement in serum by HPLC is a useful indicator of vitamin K status. Reference values in healthy adults fall within the range of 0.2–1.0 µg/l with a median of around 0.5 µg/l. The common hepatic forms MKs 9–13 have not been detected in plasma although there is evidence that MK-7 and perhaps MK-8 are present at lower concentrations than phylloquinone. Dietary restriction of vitamin K leads, within a few days, to a rapid decline in plasma levels of phylloquinone.

**Vitamin K Status of the Normal Breast-Fed Infant**

**Coagulation Factor Concentrations**

The plasma concentrations of the vitamin K-dependent coagulation factors in healthy, full-term infants are about half of normal adult values although there is a 3- to 6-fold range [18]. After birth the plasma concentrations rise gradually and attain normality by about 6 months [18]. Since all the infants in these Canadian studies had received vitamin K prophylaxis (1 mg i.m. at birth) the low concentrations reflect a reduced synthesis of the core proteins due to hepatic immaturity rather than a failure of vitamin K-dependent γ-carboxylation. Aballi and de Lamerens [19] collated early published work on the natural course of prothrombin activity in breast-fed infants and showed that prothrombin activity follows a U-shaped curve falling rapidly at birth to a trough by the 2nd or 3rd day before recovering again to initial concentrations by 6 days. It is likely that this trough reflects an early nutritional deficit of vitamin K.
Early Infant Vitamin K Deficiency

The wide range of concentrations of vitamin K-dependent proteins means that the diagnosis of vitamin K deficiency using conventional coagulation assays may be problematic because the lower physiological limits overlap with those found in vitamin K deficiency. This severely limits the value of functional assays of coagulation factor for unmasking vitamin K deficiency or in testing the efficacy of vitamin K prophylaxis.

Abnormal or Undercarboxylated Coagulation Factors

The use of several methodologies for PIVKA-II with different sensitivities often makes their interpretation difficult and, in the past, has lead to false assumptions of the vitamin K status of newborns. Using sensitive PIVKA-II assays we now know that levels of undercarboxylated prothrombin are higher than adult levels in around 20–50% of cord blood samples. In babies not given vitamin K at birth, the time course of PIVKA-II in infants confirms that there is a temporary dip in vitamin K status in the 1st week of life. Thus, even using a relatively insensitive electrophoretic assay, PIVKA-II was readily detectable at 4–5 days in about 70% of breast-fed infants [20]. Use of these sensitive assays for PIVKA-II shows that there is still evidence of suboptimal vitamin K status between the ages of 1 and 2 months in infants solely fed breast milk [21]. The administration of vitamin K causes PIVKA-II to disappear at a half life of about 45 h so that its detection may reflect a retrospective vitamin K deficiency.

Plasma Concentrations and Tissue Stores of Vitamin K

Concentrations of vitamin K in cord blood are extremely low compared to adults [21]. There is no accepted reference range, but best estimates suggest that cord concentrations are generally <0.05 μg/l. Estimates of the maternal/fetal concentration gradient lie between 20:1 and 40:1 and show that vitamin K has by far the highest placental blood gradient of any of the fat-soluble vitamins [21]. Plasma phylloquinone concentrations begin to be measurable in breast-fed infants at around 12–24 h after delivery and by 3–4 days their plasma concentrations are within the same range as adults [21]. The large disparity in dietary intakes between breast- and formula-fed infants is reflected in plasma concentrations that may be an order of magnitude higher in infants who are exclusively fed with supplemented formulas (table 1).

Liver Reserves

The major difference between fetal/neonatal hepatic reserves and those of adults is that whereas the long chain MKs (mainly 7–13) make up the majority of adult reserves (~90%) they are absent, or very low, at birth and build up slowly over several weeks [21]. This slow build up of hepatic MKs would be consistent with the colonization of the neonatal gut by MK-producing bacteria although some dietary contribution cannot be ruled out. The implication that low hepatic reserves of MKs may be a major contributory factor to the
brittleness of neonatal vitamin K status is enticing but presently lacks hard evidence. In fact the larger question of the relative importance of MKs to human nutrition still remains unanswered. In reviewing the evidence, Suttie [22] concluded that although MKs may partially satisfy the human requirement for vitamin K, their contribution is probably less than previously thought.

### Classification and Etiological Factors

In 1985 Lane and Hathaway [23] gave a detailed description of three patterns of VKDB (table 2), which is still widely accepted and recognized by the Pediatric/Perinatal Subcommittee of the International Society on Thrombosis and Hemostasis [4]. It has been customary to differentiate between idiopathic and secondary VKDB. In secondary VKDB there is an identifiable underlying cause. The cause may be an undiagnosed disease such as a hereditary hepatobiliary disease (biliary atresia and α1-antitrypsin deficiency, cystic fibrosis) or the effects of drugs that have been given to the mother or infant [23]. Breast-feeding is a common risk factor for both secondary and idiopathic VKDB.

#### Early VKDB

Early VKDB is defined as bleeding attributable to vitamin K deficiency in the first 24 h of life. It is rare and is typically seen in infants whose mothers have been prescribed drugs that interfere with vitamin K metabolism, by

<table>
<thead>
<tr>
<th>VKDB syndrome</th>
<th>Time of presentation</th>
<th>Common bleeding sites</th>
<th>Etiological factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>First 24 h</td>
<td>Cephalohematoma,</td>
<td>Maternal drugs (e.g. warfarin, anticonvulsants)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intracranial,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>intrathoracic,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>intra-abdominal</td>
<td></td>
</tr>
<tr>
<td>Classical</td>
<td>Days 1–7</td>
<td>Gastrointestinal,</td>
<td>Mainly idiopathic, breast-feeding (may be related to low milk intakes)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>skin, umbilical,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>nasal, circumcision</td>
<td></td>
</tr>
<tr>
<td>Late</td>
<td>Weeks 2–26 (peak 3–8)</td>
<td>Intracranial, skin,</td>
<td>Mainly idiopathic, breast-feeding, some degree of cholestasis often present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gastrointestinal</td>
<td>Secondary cases result from malabsorption due to underlying disease (e.g. biliary atresia, α1-antitrypsin deficiency, cystic fibrosis) or chronic diarrhea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibiotic therapy sometimes implicated</td>
</tr>
</tbody>
</table>

**Table 2.** Classification of vitamin K deficiency bleeding (VKDB) in the newborn
mechanisms that are known (oral anticoagulants such as warfarin) or unknown (anticonvulsants, e.g. phenytoin; antituberculous drugs, e.g. rifampicin, isoniazid).

**Classical VKDB**

Defined as VKDB between days 2 and 7, classical VKDB typically presents between 3 and 5 days. Although often regarded as idiopathic, a probable cause (other than maternal drugs taken during pregnancy) is delayed or inadequate feeding. As already mentioned, this concept is consistent with the natural dip in prothrombin activity seen in coagulation studies in the 1940s [19]. The importance of establishing adequate breast-feeding to vitamin K status is supported by more recent studies using PIVKA-II as a sensitive marker of vitamin K status. Von Kries et al. [20] showed that vitamin K status is strongly related to the cumulative intake of human milk in the 1st week of life. Thus, fully breast-fed infants who had raised PIVKA-II and factor II activities of $<25\%$ of normal on day 5 were shown to have received $<100\, \text{ml milk/day}$ over the first 4 days. In contrast, infants who had factor-II levels of $>25\%$ on day 5 had increased their average breast milk intakes to $>100\, \text{ml/day}$ by days 3 and 4. Essentially similar findings were made by Motohara et al. [24] who used a more sensitive PIVKA-II assay to show that a breast milk intake of $400\, \text{ml/3 days}$ would be sufficient to ensure $\gamma$-carboxylation of prothrombin.

**Late VKDB**

The term late HDN (now late VKDB) is now used to denote bleeding due to vitamin K deficiency that presents between 2 weeks and 6 months [4] but has a peak incidence between 3 and 8 weeks [25–27]. The major distinguishing feature of late VKDB from the classical syndrome is the much higher prevalence of intracranial bleeding, usually as the first presenting sign.

Although late VKDB had been reported in Thailand as early as 1966 [27], it went unnoticed in developed countries until there was a flurry of case reports in the early 1980s. One of these publications reported the return of hemorrhagic disease to the UK [28]. The authors suggested that the likely reason for its reappearance in the UK had been the progressive rise in exclusive breast-feeding because of increased awareness of intolerance to cow's milk protein. This had led to a decrease in supplementary feeds with formula milk, which with their high vitamin K concentrations, had presumably been a protective influence in the absence of widespread vitamin K prophylaxis.

Late onset VKDB, which nearly always occurs in exclusively breast-fed infants, occurs at a time when lactation is fully established, and the mothers of affected infants seem to have normal concentrations of vitamin K in their milk [29]. Breast-feeding apart, the only other consistent factor is the growing evidence of an association of late VKDB with hepatobiliary dysfunction which leads to an impaired secretion of bile salts. Such is the high dependence of the intestinal absorption of vitamin K on bile salts that any reduction in their
production and/or luminal secretion will result in a degree of malabsorption of vitamin K. In many surveys, especially from the Far East [25, 27], the majority of cases have been reported as idiopathic. In the second Japanese survey, liver function tests in infants with idiopathic VKDB were often abnormal but not sufficiently so to cause noticeable jaundice [25]. In European surveys the proportion of cases deemed idiopathic declines with the increasing thoroughness of investigation, with as many as 60% having undiagnosed hepatobiliary disease [4]. This apparently increasing proportion might relate to the increased coverage of oral vitamin K prophylaxis which, while protecting most babies, exposes an underlying reservoir of infants with cholestasis who are not protected by current multidose oral regimens. Evidence for this has come from the German survey from 1995 to 1998 in which cholestasis was found to be present in 20/23 of infants with late VKDB despite them having received 3 oral doses of 2 mg of phylloquinone [30]. An important point is that this cholestasis may be mild, and its course transient and self-correcting.

**Clinical Manifestations of VKDB**

Idiopathic early VKDB is rare but may be life-threatening or fatal due to bleeding from intracranial, intrathoracic, intra-abdominal and gastrointestinal sites [23]. Sometimes the separation of early and classical VKDB as separate entities seems unwarranted. In a hospital-based Ethiopian survey of VKDB during the 1st week of life, the mean age at onset among the 20% classified as having the early syndrome was 20 h and the clinical features were no different from classical VKDB [31].

In classical VKDB, typical bleeding sites are the gastrointestinal tract, umbilicus, skin, nose and site of circumcision (table 2) [23, 32]. In a recent study of 127 cases of early/classical VKDB from Ethiopia the most common bleeding sites were the gastrointestinal tract (53%) and umbilicus (23%) [31]. Only 1 infant in this Ethiopian series was suspected of having bled into the central nervous system, in addition to having presented with gastrointestinal and umbilical bleeding. The mortality rate from classical VKDB in the developed world is extremely low but is likely to be much higher in developing countries. This may be inferred from the fact that 26% of the Ethiopian infants died after hospital admission; this high death rate is salutatory and reflects the scarcity of resources and long delay before proper treatment could be instigated [31].

As already mentioned the majority of cases of late VKDB recorded in the literature have presented with acute intracranial hemorrhage. A pooled data analysis of 131 published cases of late VKDB throughout the world up to the end of 1992 showed that 63% had presented with severe intracranial hemorrhage and 14% had died [26]. Of the 67 surviving infants in whom follow-up data were available, some 40% had long-term neurological handicaps.
Of special interest is that about one third of the infants had had previous minor ‘warning bleeds’ that had not been acted upon before the serious bleeding episode [26]. As would be expected the fatality rate is higher in developing countries. Of 691 cases of late VKDB in Thailand from 1963 to 1995, the prevalence of intracranial hemorrhage was 82% and the fatality rate was 24% with about 50% having permanent neurological deficits (table 3). Another consistent finding from population surveys is that late VKDB is about twice as likely to occur in males than in females [25, 27].

**Table 3.** The clinical findings of 691 cases of idiopathic vitamin K deficiency in Thailand from 1963 to 1995

<table>
<thead>
<tr>
<th>Total 691 cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male:female</td>
<td>2.5:1</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>2 weeks to 2 months</td>
<td>422/497</td>
</tr>
<tr>
<td>&gt;2–12 months</td>
<td>75/497</td>
</tr>
<tr>
<td><strong>Infant feeding</strong></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>567/618</td>
</tr>
<tr>
<td>Breast and formula</td>
<td>33/618</td>
</tr>
<tr>
<td>Formula</td>
<td>18/618</td>
</tr>
<tr>
<td><strong>Vitamin K prophylaxis</strong></td>
<td></td>
</tr>
<tr>
<td>Receiving IM</td>
<td>18/575</td>
</tr>
<tr>
<td>Receiving oral</td>
<td>42/575</td>
</tr>
<tr>
<td>Not receiving</td>
<td>515/575</td>
</tr>
<tr>
<td>Undetermined</td>
<td>70/691</td>
</tr>
<tr>
<td><strong>Clinical manifestation</strong></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>641/641</td>
</tr>
<tr>
<td>Intracranial</td>
<td>524/641</td>
</tr>
<tr>
<td>Skin and muscle</td>
<td>170/641</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>107/641</td>
</tr>
<tr>
<td>Anemia</td>
<td>473/641</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
</tr>
<tr>
<td>Fatality rate</td>
<td>167/691</td>
</tr>
<tr>
<td>Sequelae</td>
<td></td>
</tr>
<tr>
<td>Immediate</td>
<td>142/257</td>
</tr>
<tr>
<td>Long-term follow-up (&gt;1 year)</td>
<td>15/31</td>
</tr>
</tbody>
</table>

Adapted from Chuansumrit et al. [27].

Of special interest is that about one third of the infants had had previous minor ‘warning bleeds’ that had not been acted upon before the serious bleeding episode [26]. As would be expected the fatality rate is higher in developing countries. Of 691 cases of late VKDB in Thailand from 1963 to 1995, the prevalence of intracranial hemorrhage was 82% and the fatality rate was 24% with about 50% having permanent neurological deficits (table 3). Another consistent finding from population surveys is that late VKDB is about twice as likely to occur in males than in females [25, 27].

**Incidence of Classical VKDB**

There is little accurate national data on the incidence of classical VKDB, even in industrialized countries. In a 2-year prospective survey in the British
Isles during 1988–1990 [33] the incidence of classical VKDB of 5.4/10^5 births was similar to that of 4.4/10^5 births for late VKDB [34]. Earlier studies had suggested a higher incidence for classical VKDB: for example Salmonsén [14] in Oslo reported that the incidence of classical VKDB in Oslo during 1934–1939 was 0.8%. The later important and influential studies in the 1960s by a group in Cincinnati showed that the incidence of moderate or severe bleeding in an urban population was 1.7% among breast-fed infants who had not received vitamin K [32]. These data from Cincinnati would not have been representative of the national incidence of VKDB in the USA since the hospital served a mainly black disadvantaged population. Also, a minority of the bleeds could not be attributed to vitamin K deficiency and the high frequency of bleeds after circumcision gave an upwards bias to the incidence. The studies by the Cincinnati group are important because they reaffirmed the connection between breast-feeding and classical VKDB [32], and the link between coagulation status and feeding practices and/or vitamin K prophylaxis [35].

If economic deprivation predisposes to classical VKDB, it might be expected that there would be a greater incidence in underdeveloped countries but this has been difficult to document. A retrospective study of 14,110 admissions in the 1st week of life in a children's hospital in Addis Ababa, Ethiopia, from 1982 to 1991 showed that 0.9% had early or classical VKDB of whom 80% had the classical syndrome [31]. Most of the affected infants came from families with a very low income and poor educational background. High incidence rates of classical VKDB have also been reported in southeast Asia. A recent review of the history of VKDB in Thailand cites incidence rates of 89 and 54/10^5 births for two longitudinal field studies in Ayutthaya Province which were carried out before Thailand brought in widespread vitamin K prophylaxis [27]. In a 2-year regional, hospital-based survey in Malaysia from 1987 to 1988, the incidence of classical VKDB in Kelantan was estimated to be 25/10^5 births [36]. This is a rural state on the east coast of the Malaysian peninsular with about 40,000 live births/year, the majority (77%) at home. This Malaysian incidence appears somewhat lower than in Thailand but being a hospital-based survey may have missed infants with mild bleeding symptoms who had not been referred to hospital and perhaps some who had died before reaching hospital.

**Incidence of Late VKDB**

The first attempts to document the incidence of late VKDB in a large population were made in Japan in a series of nationwide surveys spanning the years from 1980 to 1990. The extent of the problem can be gauged by the results of the second Japanese nationwide survey (1981–1985) which reported some 484 cases of late VKDB [25]. Of these 484 cases, 427 were classified as ‘idiopathic’ and 57 cases, in which there was an identifiable cause, as ‘secondary’ late VKDB [25].
Early Infant Vitamin K Deficiency

In order to make valid comparisons of incidence rates between different populations, the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis have clarified the criteria necessary for inclusion as a case (the numerator), and defined the denominator as the proportion of the population given no prophylaxis [34]. Table 4 shows comparisons of incidence rates of late VKDB in several countries. As can be judged from the relatively wide 95% confidence intervals the ability to determine accurate incidence rates is limited by the comparative rarity of late VKDB. However, the average incidence rates in 4 Western European countries ranged from 4 to 7 cases/10^5 births with an even higher incidence in Japan.

Table 4 also shows data from Thailand taken from the review by Chuansumrit et al. [27] which suggests that before the widespread introduction of vitamin K prophylaxis, there had been a very high prevalence of late VKDB. The figure of 72 cases/10^5 births was collected from a regional field study in central Thailand (Ayutthaya Province) conducted during 1981–1984. Although this figure lacks confidence intervals, it is consistent with an even higher incidence from a previous survey in the same region and with the many case reports in the Thai literature during this period. In 1983, a nationwide hospital-base study reported an incidence of late VKDB of 35 cases/10^5 births [37]. This lower figure probably reflects the underestimation of hospital-based studies compared to studies in the field [27]. This underestimation in hospital-based surveys might have been a factor in the Malaysian survey that reported 13 cases of late VKDB/10^5 births [36].

Comparison of the incidence in Thailand and Japan, both with good surveillance systems, suggests that emerging countries have much higher rates than developed countries. New evidence from China suggests that there is a very high incidence of late VKDB in this country. A review of the Chinese literature from 1982 to 1998 by Dr. H.F. Zhang revealed over 4,000 suspected cases in the Chinese literature of which 3,970 fitted acceptable diagnostic criteria for late VKDB. As in other countries, nearly all had intracranial hemorrhage (92%) and had been breast-fed (89%), the peak incidence was 4–8 weeks (79%), and the male/female ratio was 2.6. All regions of China were represented. In 1993 a 1-year prospective study was conducted in 3 regions of the Yantai area of Shandong province, which were classified as urban, plain or mountainous districts (Wan GT, personal communication). None of the infants born in these regions were given vitamin K and the mountainous district was economically much the poorest. Using trained investigators some 40 cases of late VKDB were diagnosed among 4,413 infants enrolled, and which equated to incidence rates of 315, 348 and 1,428 cases/10^5 births for the urban, plain and mountainous regions, respectively. These incidence rates are the highest so far reported from any country. Although a relatively small study it had the advantage that the data were obtained by active surveillance in the field by trained investigators. The diagnosis was confirmed by stringent
<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Prophylaxis interruption</th>
<th>No. of infants with late VKDB</th>
<th>Incidence rate per 10^5 infants</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>British Isles</td>
<td>McNinch et al. [33], 1991</td>
<td>Nil</td>
<td>9</td>
<td>4.4</td>
<td>2.0–8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral phylloquinone (1–2 mg; 1 × dose)</td>
<td>7</td>
<td>1.5</td>
<td>0.6–3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM phylloquinone (1–2 mg; 1 × dose)</td>
<td>0</td>
<td>0</td>
<td>0.0–0.4</td>
</tr>
<tr>
<td>Sweden</td>
<td>Ekelund [49], 1991</td>
<td>Oral phylloquinone (1–2 mg; 1 × dose)</td>
<td>16b</td>
<td>6</td>
<td>3.7–9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM phylloquinone (1 mg; 1 × dose)</td>
<td>0</td>
<td>0</td>
<td>0.0–5.6</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Tönz et al. [50], 1988</td>
<td>Oral phylloquinone (1–3 mg; 1 × dose)</td>
<td>7</td>
<td>6.4</td>
<td>2.5–13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM phylloquinone (1 mg; 1 × dose)</td>
<td>0</td>
<td>0</td>
<td>0.0–5.3</td>
</tr>
<tr>
<td>Germany</td>
<td>Von Kries et al. [51], 1992</td>
<td>Nil</td>
<td>10</td>
<td>7.2</td>
<td>3.5–13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral phylloquinone (1–2 mg; 1 × dose)</td>
<td>2</td>
<td>1.4</td>
<td>0.2–5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.m. or s.c. phylloquinone (1 mg; 1 × dose)</td>
<td>1</td>
<td>0.25</td>
<td>0.01–1.32</td>
</tr>
<tr>
<td>Japan</td>
<td>Hanawa [39], 1992</td>
<td>Nil</td>
<td>20.4c</td>
<td>10.5</td>
<td>7.0–15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oral menaquinone-4 (2 mg; 1 to 3 × dose)</td>
<td>29.5c</td>
<td>2.8</td>
<td>2.0–3.78</td>
</tr>
<tr>
<td>Thailand</td>
<td>Chuansumrit et al. [27], 1998</td>
<td>Nil</td>
<td>Not given</td>
<td>72d</td>
<td>Not given</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.m. (1 mg) or oral (2 mg) phylloquinone (1 × dose)</td>
<td>Not given</td>
<td>4.2–7.8e</td>
<td>Not given</td>
</tr>
</tbody>
</table>

With the exception of Thailand, incidence rates are based on the criteria of von Kries and Hanawa [34], and with the exception of Japan, vitamin K prophylaxis consisted of a single dose of phylloquinone given by intramuscular injection or by mouth. CI = Confidence interval; i.m. = intramuscular; s.c. = subcutaneous.

* Except for Thailand the incidence rate denominators are the proportion of infants who either received no prophylaxis or designated regimen.

* The original publication includes a case of bleeding with a prothrombin activity of 24%, which was omitted from this table.

* Estimated number of cases. Modified from the original publication to include idiopathic and secondary VKDB but not those cases detected by mass screening. Cases without clear information on vitamin K prophylaxis were included in the vitamin K prophylaxis group.

* Approximate incidence based on regional field study and estimated total births during 1981–1984. ~10–15% of population received prophylaxis.

* Based on nationwide hospital surveys and estimated total births from 1988 to 1995 when prophylaxis coverage rose from ~70 to >90% of the population.
clinical criteria and in 29 cases was supported by a prolonged PT. The series fitted the typical pattern of late VKDB in time of presentation, high incidence of intracranial hemorrhage (88%).

**Effect of Vitamin K Prophylaxis on Incidence Rates**

In 1961 the American Academy of Pediatrics recommended phylloquinone as the compound of choice for vitamin K prophylaxis and concluded that ‘a single parenteral dose of 0.5–1.0 mg or oral dose of 1.0–2.0 mg is probably adequate for prophylaxis’ [38]. In practice parenteral injections became the common route of administration in the USA.

The intramuscular injection of a single dose of phylloquinone has long been regarded the gold standard against which other prophylactic regimens should be judged. In a review up to 1988 only 4 infants with VKDB had been reported to have received vitamin K by intramuscular injection, and none from the USA where a large number of infants are exposed to this route of administration [29].

The implementation of surveillance programs in different countries has greatly improved our knowledge of how vitamin K supplementation protects against late VKDB [6, 34]. The data given in table 4 show some of the first results from these surveys at a time when most countries (Japan is the exception) were giving single doses of phylloquinone by intramuscular injection or by mouth. The figures from the British Isles, Sweden, Switzerland and Germany confirm that the intramuscular injection of 1 mg of phylloquinone gives almost complete protection but that the oral route is less effective than the parenteral route when equivalent (or even greater) amounts of phylloquinone are given as a single bolus.

In Thailand, the Ayutthaya Province field study of 1981–1984 indicated a very high incidence rate of late VKDB of about 72 cases/10^5 births when vitamin K prophylaxis extended to less than 20% of the population. However, based on nationwide hospital surveys from 1988 to 1995 the incidence was reduced at least tenfold by the administration of a single dose of phylloquinone given intramuscularly or by mouth [27]. It is unclear from this publication [27] what proportion of the newborn population of Thailand had received each regimen but by 1995 the coverage of vitamin K prophylaxis had extended to nearly the entire Thai population.

Japan differs from other countries in the use of MK-4 rather than phylloquinone which is administered orally in a syrup formulation [39]. The introduction of oral prophylaxis in Japan with 1–3 oral doses of MK-4 (2 mg) reduced late VKDB substantially and by the early 1990s the incidence (2.8/10^5 births) was similar to that seen in Europe after 1 oral dose of phylloquinone (table 4). Moreover, no cases of late VKDB were reported in the one third of infants who had received 3 oral doses [39]. This suggested that this 3-dose oral regime with MK-4 was similar in efficacy to parenteral prophylaxis,
although as with parenteral prophylaxis there have been occasional reports of failure.

Following the controversy of the possible link between intramuscular vitamin K with childhood cancer there has been a notable shift towards oral prophylaxis and it has therefore become important to validate the effectiveness of different oral regimens. In a systematic approach to this problem, pediatricians in 4 countries with an active surveillance program for VKDB instigated a joint assessment of the efficacy of the prophylactic regimens being used in their respective countries [6, 40]. A summary of their findings is given in table 5. Two countries, Germany and Australia, originally followed a 3-dose oral regimen with 1 mg phylloquinone. Although the occurrence of late VKDB in infants who had received this regimen was low (~2/10^5 births) it was still higher than that seen after intramuscular prophylaxis. In Germany an increase of the dose from 1 to 2 mg in a 3-dose regimen significantly reduced but did not eliminate VKDB [30]. The strength of these German data lie in the large population of 3.2 million infants exposed to the regimen and the resulting close confidence intervals of the incidence data (table 5). Although based on much smaller population sizes, the figures from Denmark and Switzerland indicate the importance of dose and frequency of administration. In Denmark the incidence after a single oral dose of 1 mg at birth was similar to that seen in Europe without any prophylaxis, while in Switzerland a 2-dose (2-mg) regimen did not prevent all cases. However, when Denmark changed to a regimen of a 2-mg dose at birth followed by a 1-mg dose given at weekly intervals for 3 months, no case was observed over a 4-year surveillance interval [41]. The Netherlands have adopted yet another approach by giving a single dose of 1 mg phylloquinone at birth followed by a daily 25-μg dose for 3 months to breast-fed infants [40]. This daily dose mimics the approximate daily intakes of vitamin K obtained by formula-fed infants and over a 2-year surveillance period, no infant who followed this regimen developed VKDB [40].

**Bioavailability Issues in Vitamin K Prophylaxis**

Measurements of plasma concentrations of phylloquinone have been used to assess the effectiveness of different doses and routes of administration as well as to compare different formulations. Comparative studies have shown that a single dose of 1 mg of phylloquinone given by the intramuscular route gives higher average plasma levels at 14, 30, and 90 days than the same single dose given orally [42]. The higher concentrations at 90 days after intramuscular injection are consistent with a depot effect arising from the slow release of this highly lipophilic phylloquinone from the injection site. In another study Greer et al. [43] compared the same intramuscular protocol against 3 doses of 2 mg phylloquinone given orally. The timings of the 3 oral doses at birth, 7 and 30 days matched the recommendation of a number of European countries and
<table>
<thead>
<tr>
<th>Country</th>
<th>Oral dose regimen</th>
<th>Observation period</th>
<th>Birth population</th>
<th>Incidence rate per 10^5 infants(^a)</th>
<th>Incidence rate for cases who had received regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Netherlands</td>
<td>1 mg at birth, 25 µg daily for weeks 1–13 (breast-fed infants only)</td>
<td>October 1992 to December 1994</td>
<td>439,000</td>
<td>0.5 (0.1–1.6)</td>
<td>0 (0–0.7)</td>
</tr>
<tr>
<td>Germany</td>
<td>3 × 1 mg on days 1, 4–10 and 28–42</td>
<td>April 1993 to December 1994</td>
<td>1,400,000</td>
<td>1.9 (1.3–2.8)</td>
<td>1.5 (0.9–2.3)</td>
</tr>
<tr>
<td></td>
<td>3 × 2 mg days 1, 4–10 and 28–42</td>
<td>January 1995 to December 1998</td>
<td>3,200,000</td>
<td>0.7 (0.6–1.5)</td>
<td>0.5 (0.3–0.8)</td>
</tr>
<tr>
<td>Australia</td>
<td>3 × 1 mg days 1, 3–5 and 21–28</td>
<td>January 1993 to March 1994</td>
<td>325,000</td>
<td>2.5 (1.1–4.8)</td>
<td>1.5 (0.5–3.6)</td>
</tr>
<tr>
<td>Denmark</td>
<td>1 mg (once)</td>
<td>April 1990 to November 1992</td>
<td>134,500</td>
<td>4.5 (1.6–10.3)</td>
<td>4.5 (1.6–10.3)</td>
</tr>
<tr>
<td></td>
<td>2 mg at birth + 1 mg weekly for 3 months</td>
<td>December 1992 to January 1996</td>
<td>163,000</td>
<td>0 (0–2.0)</td>
<td>0 (0–2.0)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>2 × 2 mg on days 1 and 4(^c)</td>
<td>January 1995 to December 1995</td>
<td>83,000</td>
<td>4.7 (1.3–11.9)</td>
<td>1.2 (0–6.5)</td>
</tr>
</tbody>
</table>

The 95% confidence intervals are shown in parentheses. Sources: von Kries [6] and von Kries et al. [30].

\(^a\) Infants with late VKDB in weeks 2–12 and no underlying condition known before the bleeding.

\(^b\) Estimated number exposed.

\(^c\) Mixed micellar phylloquinone preparation available and widely used.
plasma phylloquinone concentrations after the oral regime were significantly higher than after a single intramuscular injection at 14 and 56 days, and equivalent at 30 days. Thus, in order to match the plasma concentrations attained by intramuscular administration over the major risk period, it is necessary to administer larger and/or multiple oral doses of phylloquinone.

**Intramuscular Vitamin K Prophylaxis and Childhood Cancer**

Over the last 10 years there has been a major controversy concerning a putative association between the practice of intramuscular injection of phylloquinone and later childhood cancer [6, 7]. The controversy began with a case-control epidemiological study in the UK, which suggested an association of intramuscular (but not oral) vitamin K for all cancers but leukemia in particular [44]. This spawned a series of studies in several countries to test the validity of this association. A major review of the evidence from all these studies is beyond the scope of this remit and the reader is referred to two recent reviews by two experts in the field [6, 7] as well as a monograph on the deliberations of a WHO expert working group [45]. Based on the evidence of all these studies von Kries [6] concluded that a small risk for leukemia could not yet be excluded but emphasized that these epidemiological studies had been set up to show ‘evidence of risk’ rather than to prove the ‘absence of risk’. Ross and Davies [7] thought that the evidence of risk was unconvincing and, from the USA public health point of view, did not believe that any change in pediatric practice was warranted. The WHO have standard categories for evaluation of carcinogenic risk and the conclusion of this working group was that ‘there is inadequate evidence in humans and experimental animals for the carcinogenicity of vitamin K substances’ (group 3) [45]. This evaluation included both epidemiological evidence and biochemical plausibility for carcinogenicity [45].

**Vitamin K Deficiency Bleeding and Its Prevention: Global Perspectives**

It is evident from this review that VKDB is still a significant concern in the developed world and a bigger problem in the developing world. In many developing countries the problem is unseen or overshadowed by more common and more visible deprivation and disease. One reason for the lack of information from developing countries is that bleeding in infancy can have different causes and VKDB can only be properly diagnosed by laboratory tests or, failing this, showing that bleeding responds to vitamin K. Perhaps another is that the bleeding in the late onset form of vitamin K deficiency may be sudden in onset and the infant may die before reaching hospital. The differences
in mortality rates for developed and developing countries reflects the different access of their respective populations to intensive care facilities.

Victora and van Haecke [46] have made a detailed analysis of the public health issues and global implications of vitamin K prophylaxis including cost-effectiveness and feasibility. An assumption was made that the possible carcinogenicity was unproven [46]. With the high rate of mortality and long-term disabilities in survivors they conclude that the costs of vitamin K prophylaxis in industrialized and middle-income countries (where most births take place in hospitals) are considerably smaller than the economic consequences of the disease [46]. For developing countries, the introduction of a prophylactic program needs to be made on a national basis, after considering mortality levels and causes, health resources, budgets and feasibility. Victora and van Haecke [46] also made some calculations based on the World Bank model of disability-adjusted years (DALYs). In a model of an intermediate incidence of 28 cases of VKDB/10⁵ births it could be calculated that late VKDB had much less impact on lost DALYs than iodine or vitamin A deficiency. On the other hand if the incidence rates were 10-fold higher, as seems to be the case in China, the consequences of late VKDB on lost DALYs would be similar to that for vitamin A deficiency in this model. Although breast-feeding is a risk factor for VKDB, concern to prevent the syndrome should not affect the promotion of breast-feeding in the developing world [46].

With respect to the efficacy of different vitamin K compounds, the preparation used in most countries is phylloquinone and this is clearly effective when an appropriate protocol is followed. The Japanese experience also shows MK-4 to be an effective alternative to phylloquinone. In some developing countries, such as India, the only widely available vitamin K preparation is menadione sodium bisulfite [47]. Although effective in the short-term against classical VKDB, nothing is known of the ability of menadione salts to protect against late VKDB, which depends on the in vivo conversion to MK-4. Another major concern is that menadione, unlike phylloquinone, has an unsubstituted 3-position which makes it highly reactive with sulfhydryl compounds and is cytotoxic [45]. This reactivity is responsible for the well-known hemolytic toxicity of menadione [38] which may lead to severe hemolysis in infants with a congenital deficiency of glucose 6-phosphate dehydrogenase [45]. Of even greater concern is recent evidence that menadione is also mutagenic causing DNA damage and sometimes chromosomal aberrations in cultured mammalian cells [45]. Although cheap, menadione and its derivatives should be avoided for prophylaxis.

**Key Research Questions and Problems**

One big problem for developing countries is how best to deliver prophylaxis given the high proportion of home deliveries. Intramuscular
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injections have inherent risks and should only be given by qualified medical personnel. Some large cities in China already have programs in place using the parenteral route but prophylaxis is not available in most rural areas. Oral options are being considered but are presently hampered by the lack of availability of a low cost, easy to administer neonatal vitamin K solution that can be distributed to health workers, and to parents. One innovative solution for breast-fed infants which is being promoted by Dr. H.F. Zhang is to give the already available vitamin K (phyloquinone) tablets (5–10 mg) to mothers on a daily basis for about 20 days after delivery. Such large doses given to mothers are known to raise breast milk concentrations by some two orders of magnitude [11, 12]. Given the long-standing tradition of oral remedies in China giving vitamin K by mouth to mother or baby is more in keeping with their cultural traditions. Vitamin K deficiency in infancy differs from many other micronutrient deficiencies in that substantial protection against the devastating consequences of VKDB can be prevented by minimal supplementation. In the population context, even one oral dose at birth gives considerable protection. Thailand is a good example of how an initially very high incidence of VKDB can be reduced by the integration of vitamin K prophylaxis into the national healthcare program so that by 1994 Thailand achieved almost complete coverage of the population [27]. Even in Thailand, however, the incidence in 1995 was still similar to the baseline incidence seen in Europe before the widespread introduction of prophylaxis [27].

Further research is needed into the etiology of late VKDB. The apparent extremely high incidence rates of late VKDB in China of up to 1.4% in a poor mountainous region raises the question of whether Chinese infants are exposed to additional risk factors. One possibility is a low maternal dietary intake of vitamin K that may stem from a cultural tradition for Chinese mothers to avoid eating green vegetables in the first month after delivery. In developed countries green vegetables account for some 50% of the dietary intakes of vitamin K. Interestingly, a similar explanation of a restricted maternal diet and avoidance of fresh vegetables was suggested as a possible reason for the higher than expected prevalence of late VKDB among the Chinese population of Singapore in 1969 [48]. The importance of maternal nutrition has come from recent studies by Dr. H.F. Zhang and coworkers using PIVKA-II as a biochemical marker of vitamin K status. They found that up to 40% of women living in an economically poorer, mountainous region of Qixia county had evidence of subclinical vitamin K deficiency at delivery compared to 2–5% of women from Shijiazhuang and Zezhou cities. This was mirrored by a doubling of the prevalence of a raised PIVKA-II in the newborns in the Qixia region, which is known to have a high incidence of late VKDB.

Further field studies are needed in developing countries to determine the incidence of classical and late VKDB and similar surveys to document the effectiveness of prophylaxis regimens as they are introduced. As outlined by Dr. C. Bates the use of biochemical indices of status can be of enormous

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benefit in assessing micronutrient deficiencies and their response to supplementation. As described earlier, the assay of PIVKA-II offers excellent specificity for the coagulation function of vitamin K and allows deficiencies to be detected before any change in conventional clotting tests. Their use can short-circuit the need for expensive clinical trials in which overt deficiency is the endpoint. In the hands of Dr. H.F. Zhang the use of PIVKA-II is already providing valuable information on the demographics of VKDB in China.

References

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Discussion

Dr. Bates: Am I right in thinking that it is very unlikely that these very high prevalences you are seeing in parts of China are due to cholestasis and therefore there must be other reasons for the high prevalence?

Dr. Shearer: There is a possibility that cholestasis may be a factor in China as well as other places.

Dr. Bates: 1.4% of the population is affected, is that possible?

Dr. Shearer: There are probably cultural reasons as well. I was talking to my friends in China and they said that after birth women avoid green vegetables for about 1–3 months and take foods which are quite low in vitamin K. Now in Western societies 50% of our vitamin K intake comes from green leafy vegetables. The rest comes from oils, for instance rape seed oil, and olive oil, but 50% of most people’s dietary sources comes from green vegetables. The same dietary tradition among Chinese mothers was also reported in the Singapore population in the late 1960s where Hoh et al. [1] reported the incidence of vitamin K deficiency in different groups, and the one with the highest incidence was the Chinese, and Hoh et al. [1] reported this cultural avoidance of green vegetables in his paper as well. But regarding cholestasis, there are viruses of hepatitis, cytomegalovirus (CMV) which can actually cause cholestasis. There was a case in the 1980s from Germany [2] where there was good evidence of subclinical vitamin K deficiency due to a transient cholestasis which actually went on to present as clinical vitamin K deficiency bleeding. The baby had CMV, and it was shown that the absorption of vitamin K was low but then several weeks later absorption was normal. I spoke to some Chinese pediatricians yesterday and they said that CMV is quite common in China, about 15% was mentioned, but I am not sure of the prevalence in Europe.

Dr. Bates: Is there any possibility of genetic differences in susceptibilities? Is there any evidence?

Dr. Shearer: We know very little about that. I didn't show the now classical vitamin K epoxide cycle which is one way that we conserve our vitamin K stores. When we make carboxylated prothrombin and other Gla proteins, the vitamin K cofactor goes through a cycle, it is recovered and there are two enzymes as you know, vitamin K epoxide reductase and another one called vitamin K reductase, but it is probably the same vitamin K epoxide reductase enzyme doing the both reactions. But it recycles the vitamin K in the liver. We know virtually nothing about vitamin K epoxide reductase, the enzyme has not even been isolated, it is very difficult, and being microsomally bound, it is almost impossible to isolate, so we know nothing about the genetics of the enzyme, and perhaps there are genetic susceptibilities in that enzyme and perhaps other enzymes involved in vitamin K metabolism.
**Dr. al Frayh:** To go back to the observation made on the association of vitamin K used in the parenteral route and cancer, I have two questions. First, is that observation made on clinical studies or is it based on animal experiments? Secondly, are you aware of any association of excessive use or excessive intake of micronutrients in the form of vitamins, trace elements and minerals, and the increased risk of tumor in human subjects, because there have been reports which in fact make a sort of question mark on the long-term use of micronutrients in healthy subjects.

**Dr. Shearer:** The cancer story originated in 1990 from Golding et al. [3] in Bristol, UK. They were looking at risk factors for cancer in the perinatal period, so they were looking at smoking and other risk factors in pregnancy, pethidine and drugs given to the babies. Now the only drug to my knowledge given routinely to babies is vitamin K, so that was the only drug they could actually look at. They came up with an unexpected association with an odds ratio of >1 in that group. So they did a subsequent study in 1992 which also showed an association with vitamin K but only when given parenterally. Unfortunately when it was given as a small abstract at a pediatric meeting and the press got hold of it. It became spread over the UK newspapers, the Department of Health panicked and convened a meeting of experts. Golding's data was later published in the *British Medical Journal* [4]. It was a case-control study, if I remember, also showing a >1 odds ratio risk of cancer. In the USA there was a study by Klebenoff [5] and there were studies in Denmark, Sweden and elsewhere [6,7]. None of those studies confirmed the association. These were human epidemiological studies, not animal studies. Then there were some further inconclusive studies in the UK [8–12]. I should say that the risk for cancer in Golding's study [4] was very strange, it was for all tumors, not just leukemia, it was for solid tumors also, and that is apparently quite unusual. I am not an expert in the science of cancer but apparently something that causes a risk of all cancers is very unusual. The only thing I should say is that the risk was found when vitamin K was given in the intramuscular form and this preparation of vitamin K contains a solubilizer and also contains phenol in the UK, about 5 mg compared to 1 mg of vitamin K. So it was impossible to show whether it was the vitamin K or the excipients in the preparation. The WHO recently looked at all the evidence. They also looked at the animal data, and came out with a statement that there was inadequate evidence for the carcinogenicity of vitamin K. It is very difficult to prove from the epidemiological studies that there is no risk, so we still have this doubt and the latest epidemiological studies still don’t discount an association with leukemia although the solid tumors have apparently been completely discounted [12]. I should also say it is impossible to do control trials because firstly it is not ethical and secondly you would need a huge population because the incidence in the West is quite low, about 5/100,000, so it needs an impossibly high population to do appropriate control trials. Now what was the second question?

**Dr. al Frayh:** The second question concerned the excessive use of multivitamins in healthy subjects and its possible association with the development of tumors.

**Dr. Shearer:** There is certainly no evidence for that at all. If it was then you might expect a higher incidence of cancer in formula-fed babies and that has not been shown to my knowledge.

**Dr. West:** Thank you for your nice talk, I have been waiting to hear a talk like that for a long time on vitamin K. I have a few questions for you and then one for the group at large. What do we know about colostrum in terms of vitamin K content?

**Dr. Shearer:** About twice as much as mature milk.

**Dr. West:** So in breast-feeding populations if colostrum is given that may be an important protective factor.

**Dr. Shearer:** Yes, I think it is like vitamin A, colostrum has high concentrations of vitamin K.
**Dr. West:** In the studies in Ethiopia and China do you recall what their case definitions were for developing the estimates of the incidence of nasal bleeding and/or bruising at a particular age for a child? What would you recommend as a case definition to epidemiologists who do large population-based studies in the developing world? And then the third question for the rest of the group is, does anybody working in hospitals in the developing world see nasal bleeding and bruises and these kinds of features when they examine infants?

**Dr. Shearer:** You have to differentiate between classical and late. Classical occurs in the 1st week of life and the warning bleeds are certainly there, the umbilicus is certainly one of the major lesions. The Ethiopian study was a hospital-based one. One clinician looked back at the hospital records and he tried to look at the bleeding sites. There is some doubt about some of the cases, but I think that it is pretty easy to differentiate vitamin K deficiency bleeding from disseminated intravascular coagulation in most cases. Now for late onset of vitamin K deficiency bleeding, in the Chinese prospective study they were trained to investigate this. They were trained to look for the signs of late onset, seizures, central nervous system collapse, and they were also actually trained to do the prothrombin times. In 8% of those cases they showed that the prothrombin time was long, and that is the gold standard, if the prothrombin time is long and it is corrected by vitamin K that is a pretty good case definition. But it is quite difficult to do that in the field.

**Dr. Azizi:** Is there any response to the Dr. West’s third question from the audience?

**Dr. Al Lamki:** I am from the University Hospital in Muscat, Oman. It being the referral center for hematological problems we tend to have recognizable numbers of infants with late hemorrhagic disease of the newborn and basically they come very late, at the age of 6 or even 9 months a few of them. Now all these children received vitamin K1 injections at birth. At the same time they had no recognizable or confirmed liver disease or cholestasis. So I don't know what explanation is behind this, whether it is genetic or inadequate vitamin K in the mothers. All of them were exclusively breast-fed, so that is quite an interesting observation.

**Dr. Shearer:** It is certainly very interesting because to my knowledge nearly all the reports from various parts the world say that it is quite rare beyond the age of 6 months. We know that the prophylaxis, given intramuscularly lasts for at least 3 months, forms a depot in the muscle and we think that is why it has this longevity. Being fat-soluble it leaches out slowly, and that is why we rarely see vitamin K deficiency bleeding in a baby given an intramuscular injection.

**Dr. Sacy:** I have two questions. First, what would be your recommendation for premature babies with mainly bleeding problems?

**Dr. Shearer:** The recommendation for premature babies is usually to give it intramuscularly in most countries. In developed countries, in Europe and USA, any baby born prematurely will be given, depending on the birth weight, 0.5 mg or even less, 0.2 mg by intramuscular injection, and that is standard practice, or an intravenous injection in those cases where you don't want to give it intramuscularly. That is standard practice in the West.

**Dr. Sacy:** But don't you repeat it?

**Dr. Shearer:** You don't usually need to repeat it, if you give it intramuscularly. If you give it orally you would do so.

**Dr. Sacy:** Because they are usually not fed, they don't have gut production.

**Dr. Shearer:** That is all right, parenteral feeds usually have vitamin K added to them.

**Dr. Sacy:** The second question: in a family with a genetic problem, 1 patient died at 1 week from intracranial bleeding, and 3 other children with a prothrombin time at 30%, and we found a genetic problem, and this gene is being determined now in
Germany. Do you have any recommendation for treating these patients because they all live with 30% prothrombin?

Dr. Shearer: There are a few cases of defects in carboxylation and the carboxylase enzyme, and also the one I was talking about earlier, the vitamin K epoxide reductase. There are several cases in the literature [13,14], quite rare diseases where they have low prothrombin and other factor levels. Did you find that if you give vitamin K it increased the levels?

Dr. Sacy: No, we have given huge amounts of vitamin K and repeatedly.

Dr. Shearer: You can keep them going with large doses.

Dr. Sacy: We arrived at a high number of vitamin K but without any effect on prothrombin time and none of them had any cancer.

Dr. Shearer: Another interesting point is that some of these babies are born with skeletal defects and we think that is down to the role of a vitamin K-dependent protein called matrix Gla protein in fetal development. There are 3 cases of maternal vitamin K deficiency actually resulting in babies born with the skeletal defect chondrodysplasia punctata. It is abnormal calcification of the cartilage and results in a very specific phenotype. This has also been shown in babies who have a defect in the vitamin K epoxide reductase enzyme. So there is some evidence that vitamin K adequacy in pregnancy is quite important also in terms of skeletal problems.

Dr. Pettifor: You really didn't discuss the problem in developing countries. You mentioned Ethiopia and China and we have scattered data to suggest that in fact it is quite common in the mother. Do you have any evidence that vitamin K deficiency of the mother is a substantial contributor to vitamin K deficiency in the infant, and if so, what effect does the dietary preparation have on vitamin K? How stable is it in foods in fact?

Dr. Shearer: Vitamin K is quite stable in foods.

Dr. Pettifor: So cooking isn't an issue.

Dr. Shearer: No, only light is a problem. We haven't got enough evidence on maternal deficiencies, as seen in the first slide I have shown from China. There is very little out there in developing countries, that is why we need more work in this area.

Dr. Kupka: Is it possible to raise breast milk levels by supplementing the mother, and secondly, how effective are oral forms? Are there now licensed forms of oral vitamin K that can be administered to infants? I remember that for a long time the intramuscular form was given orally.

Dr. Shearer: Yes, if you give vitamin K to the mother the breast milk levels rise substantially. One of my colleagues in China is giving 20 mg to the mothers every day and the breast milk levels go up 10-fold. That may be one way you could look at the problem of delivery. The other question, there is an oral preparation in the UK which you can give every day. In Holland they give a preparation of 25 μg/day which mimics the amounts obtained by formula-fed babies. So there are preparations.

Dr. Young: Our Institute of Medicine expert panel, in which Dr. Suttie participated as the vitamin K expert, had great difficulty in arriving at an estimate of the vitamin K requirement for any age group. One of the problems was the difficulty in determining or establishing subclinical vitamin K inadequacy, and there was an insufficient number of studies that focused on the time-dependent change in the under-carboxylated proteins and so on. Would you care to comment on the evaluation of vitamin K adequacy, and in the adult in particular?

Dr. Shearer: There are not many studies but those out there just suggest that if you are taking 1 μg/kg body weight/day it is certainly an adequate intake, but I don't think there is enough evidence to set up a recommended daily allowance or an estimated average requirement. The problem with using the carboxylation of osteocalcin as an indicator, for instance, is we don't know what osteocalcin does, we don't know
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whether it is necessary to have it carboxylated, and if you did want full carboxylation you could not possibly achieve it from normal diets because everybody in this room has an under-carboxylated osteocalcin, that is for sure. You can take in 400 μg of vitamin K/day, and you still do not fully carboxylate your osteocalcin, and it seems to take about 1 mg/day which is unachievable by normal diets. But under-carboxylated osteocalcin does respond to dietary intakes so it is a very useful interesting marker, the one that needs further study I think.

Dr. Fawzi: I was going to ask the same question about plasma levels of various markers, how good are they as markers of vitamin K status and how stable are these installed specimens?

Dr. Shearer: I think a low vitamin K is quite a good indicator of a low vitamin K status. In the normal range vitamin K levels do vary a lot individually and they also reflect the recent intake of diets. They go up and down more than vitamin A or vitamin D, so they are not that good. I would suggest that those functional markers of coagulation, such as PIVKA-II, which represents undercarboxylated prothrombin is very sensitive and it is also a functional marker, so it is what you need, a functional marker.

Dr. Zlotkin: This may seem like a naïve question but I noticed in your slides you said that in general the etiology of the classic and late onset of vitamin K deficiency is idiopathic and babies who are breast-fed seem to be at higher risk. When I think of breast milk the only micronutrient that I would be concerned about in terms of inadequacy would be vitamin D, but babies are probably meant to be exposed to sunshine, in which case it doesn’t matter if they don’t have vitamin D from milk. From a teleological perspective why do infants get vitamin K deficiency?

Dr. Shearer: The answer is that not many do, only a very small percentage gets vitamin K deficiency and a normal healthy breast-fed baby is very unlikely to get vitamin K deficiency. So I have heard the teleological argument before and people say there must be an evolutionary reason why the placental transport is blocked. I would tend to look at it in another way, that vitamin K, being very lipophilic, does not easily cross the placenta and the evolutionary problem is to solve that.

Dr. Zlotkin: So you are suggesting that every breast-fed baby who gets vitamin K deficiency has some pathological condition that we haven’t identified?

Dr. Shearer: I would think so, yes.

Dr. El Haddadin: The late type of hemorrhagic disease is a problem in Syria. We see many cases and have done a study.

Dr. Shearer: Late onset?

Dr. El Haddadin: Late onset, and we could not find cholestasis as you did in China.

Dr. Shearer: I did not find it in China, just in the West.

Dr. El Haddadin: Many of them were breast-fed, some of them got vitamin K after birth, but most of them had diarrhea for more than 2 weeks or they were given antibiotics for a week, therefore we recommend giving vitamin K each time.

Dr. Shearer: But diarrhea is certainly a risk factor as are antibiotics, and antibiotics may wipe out the intestinal flora as well, so that may be a contributing reason.

Dr. Allen: What is the current thinking about the impact of antibiotics on vitamin K status of adults?

Dr. Shearer: In hospitalized patients antibiotics seem to be a risk factor but that was confused in the 1980s because some antibiotics were shown to actually be inhibitors of vitamin K metabolism. There are certain cephalosporins which were shown to actually inhibit the vitamin K hypoxide reductase. I remember Dr. Suttie organized a very interesting conference on that in the USA, and antibiotics can perhaps interfere with vitamin K metabolism or the gut flora.

Dr. El Hodhod: My observation in Egypt is similar to that in Syria, and there was a question about the cases needing gut decontamination therapy as in some cases of
leukemia and small bowel bacterial overgrowth. We need to use an adjuvant vitamin K therapy in such cases.

References