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Review article

# Vitamin K and cystic fibrosis: A gordian knot that deserves our attention

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ABSTRACT

Cystic fibrosis (CF) is an inherited genetic disorder with multiorgan involvement. Gastrointestinal tract dysfunction leads to fat and fat-soluble vitamins (A,D,E,K) malabsorption and deficiency of these vitamins. Subclinical vitamin K (VK) deficiency seems to be a common problem in CF patients. However, despite the rest of fat-soluble vitamins being routinely supplemented, this is not a universal clinical practice for VK. Inefficient levels of VK may have significant effects on blood coagulation and bone formation. There are also some data indicating that VK may play a key role on regulation of inflammation. Supplementing CF patients with VK seems rational, but the appropriate dosing regimens are still a matter of debate. This review will try to delineate the problem and communicate the latest opinions on this controversial issue.

## 1. Introduction

Cystic fibrosis (CF) is the most common lethal genetic disorder in Caucasians. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is responsible for encoding chloride channels at the surface of epithelial cells [1]. CF patients carry two disease causing CFTR mutations, in trans. Nevertheless, in spite of the CFTR gene having been identified over twenty years ago, the relationship between genotype and phenotype in CF is still challenging and remains a matter of debate; many cases with non-classic CF or CFTR related disorders have also been described [2,3].

Affected individuals present with lung and pancreas insufficiency-related problems [4]. Exocrine pancreatic insufficiency (PI) affects about 90% of the CF patients leading, among other things, to fat-soluble vitamins malabsorption [5]. The supplementation of patients with pancreatic enzymes has revolutionized the CF treatment. However, the underlying pancreatic pathology is not reversible and continues to evolve over the years [6,7]; despite maximizing the supplements doses, there is always some degree of fat-soluble vitamins malabsorption [8,9]. Conway et al. reported a biochemical deficiency of vitamin K (VK) in 60–70% of CF patients [10,11].

VK is a hydrophobic vitamin discovered in 1929 by Henrik Dam. It is synthesized by plants and green vegetables and is obtained by humans through diet; it is also produced by intestinal bacteria. In general, VK exerts its action through the post-translational carboxylation of glutamic acid residues of certain proteins, to γ-carboxyglutamyl residues. This modification introduces an affinity for calcium ions [12,13]. It is well known that VK plays a pivotal role both in the liver where it is necessary for the activation of numerous proteins implicated in blood coagulation [12], and in the modulation of bone metabolism [14] where it exerts its actions mainly through the activation of osteocalcin (OC).

The role of VK in CF remains somewhat unclear, but there is a growing body of evidence suggesting that its deficiency may have serious implications on patients. The etiology of CF bone disease (CFBD) seems to be the end result of the functional abnormalities and pathologic manifestations encountered in CF, such as chronic inflammation and infection, poor nutritional intake and malabsorption, hypovitaminosis D, reduced level of physical activity, corticosteroid use, and pubertal delay. In recent years, VK deficiency has been added to the contributors of CFBD and the vitamin gained a new role as a potential treatment modality [15]. Apart from that, there are some data indicating that VK may have antibiotic-modifying activity and antibacterial effects [16], and immunomodulatory attributes[17 18].

Supplementation of fat-soluble vitamins is of utmost importance in CF and a number of consensus guidelines have been formulated to

*Abbreviations:* VK, Vitamin K; CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; PI, Pancreatic Insufficiency; OC, Osteocalcin; CFBD, Cystic Fibrosis Bone Disease; MK, Menaquinone; Gla, Gamma-carboxyglutamate; un-OC, Undercarboxylated osteocalcin; ApoE, Apolipoprotein E; BMD, Bone Mineral Density; TNF-α, Tumor Necrosis Factor alpha; PIVKA-II, Prothrombin Induced by Vitamin K Absence or Antagonist-II

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address this issue. However, the recommendations they provide are, to a large extent, based on expert opinions and solid evidence for certain issues, such as VK, is lacking [19–20].

## 2. A short note on vitamin K biochemistry

VK includes many different homologs that fall into two general categories; VK1 (or phylloquinone) and VK2 [21]. VK1 is a single compound and bears a side chain composed of four isoprene residues, three of which are saturated. It is made by plants, and is abundant in green leafy vegetables (e.g., spinach, kale) and in certain oils (soybean, canola, and olive). It is the predominant form of VK in the human diet and has full functional activity.

VK2 exists in several subtypes that differ in the length of their side chains which consist of 4–13 mostly unsaturated isoprene residues. They are called menaquinones and are denoted as MK-n, where n represents the number of isoprene residues [14,22]. VK2 is endogenously synthesized by intestinal bacteria. It is also found and consumed in a limited number of foods such as animal liver and certain fermented products. MK-4 differs from the rest of the menaquinones in that it is not synthesized by bacteria but is produced through tissue-specific conversion from dietary phylloquinone. It is also believed that longer-chain menaquinones, such as MK-7, can be converted to MK-4 as well. The exact role of each menaquinone, if any, is unclear and remains a matter of investigation [22,23].

## 3. History and physiology of vitamin K

The role of VK in human physiology became evident for the first time in 1929, through a very unusual way. Henrik Dam, a Danish scientist, was investigating the role of cholesterol in an experiment in which he was feeding chickens with cholesterol-depleted diet [24]. After several weeks, chickens started to bleed. Despite the reintroduction of purified cholesterol into diet, the bleeding did not stop. Dam concluded that an unknown compound must have been extracted from the food during the cholesterol depletion procedure. He surmised that this hypothetical compound was responsible for the coagulation problem and named it “the coagulation vitamin”. Later, the letter K was ascribed to this vitamin because Dam's study was published in a German journal, in which it was designated as “Koagulationsvitamin”. Further work from Dam showed that the active compound was a fat soluble vitamin distinct from A, D, and E [24].

Initially, VK was known to be present in green leaves and in certain animal foods. However, after finding that fishmeal putrefied by the action of bacteria had antihemorrhagic activity, it became evident that bacterial action could lead to the production of VK [25,26]. In 1939, VK1 and VK2 were isolated from alfalfa leaves and putrefied fish, respectively [25]. Nevertheless, it took until 1974 for the precise function of VK to be uncovered [26–28], when the VK-dependent coagulation factor prothrombin (Factor II) was isolated from cows receiving high doses of warfarin, a VK antagonist. The researchers found that warfarin-treated cows had a form of prothrombin that contained 10 glutamate amino acid residues near its amino terminus, whereas untreated cows bore a prothrombin molecule which carried 10 respective residues which were chemically identified as gamma-carboxyglutamate, or Gla. The extra carboxyl group in Gla implied that VK played a role in a carboxylation reaction during which glutamate is converted into Gla. The biochemistry of how VK is used to convert glutamate to Gla has been elucidated over the last thirty years [24].

Members of both forms of VK are involved as cofactors in the post-translational conversion of glutamyl to carboxyglutamyl residues in VK-dependent proteins, such as prothrombin and OC. The latter is produced by osteoblasts, and accounts for 10%–20% of non-collagenous bone proteins; it has high affinity for hydroxyapatite, and is of pivotal importance in the process of bone formation and mineralization [29]. Undercarboxylated osteocalcin (un-OC) has been associated with osteopenia and osteoporosis whereas undercarboxylated prothrombin leads to abnormal clotting and bleeding. Undercarboxylated VK-dependent proteins are not functionally effective, and their levels in serum are associated with the VK function in the tissue where each protein has been synthesized [30,31].

In CF, pancreatic insufficiency and lipid malabsorption result in fat-soluble vitamin deficiencies. However, in patients with normal liver function and adequate supplementation with pancreatic enzymes, coagulopathy caused by VK deficiency is very unusual. Liver cells possess a highly effective mechanism for VK uptake from the serum and are able to maintain sufficient tissue concentrations even when VK concentrations in serum are low. On the other hand, bone metabolism needs higher VK serum concentrations and is more vulnerable to VK deficiency [14,32]. Accordingly, chronic VK deficiency can result in osteopenia without obvious problems in coagulation mechanism.

## 4. The role of vitamin K in CF

### 4.1. Vitamin K in CF related bone disease

Over the last decades the evolution of treatment modalities has led to a dramatic increase in life expectancy in CF, and the median survival of patients has reached 50 years of age [33]. Because of this expanded lifespan some late-appearing problems have been recognized. Amongst them is the CF related bone disease (CFBD). Registry data indicate that CFBD affects about 11% of the CF population. The latter figure, however, may represent an underestimation of the actual number due to the underreporting or incomplete detection of the problem [34].

Several cross-sectional studies have shown a high incidence of fractures in CF individuals [35,36]. Henderson et al. first reported in 1994 increased fracture rates in girls with CF, aged 6–16 years [37]. In the same period, another cross-sectional study reported 12 fractures in 9 CF patients from a cohort of 71 children and adults [38]. The two previous studies attracted the scientific community's attention in a problem that had been underreported up to then. A few years later, a study from California reported an approximately twofold greater rate of fractures in CF patients (women 16-34 and men 25-45 years old) compared to the general population. Furthermore, the chest X-ray review demonstrated that in the CF patients the mean kyphosis angle was markedly abnormal, and also vertebral compression and rib fractures were 100 and 10 fold higher than expected, in women and men, respectively [39].

In 1998, Donovan et al. reported significantly reduced bone mineral density (BMD), and high vertebral fracture rate in 30 CF adults with advanced disease. However, they attributed these findings in the relatively low levels of serum 25-hydroxyvitamin D despite vitamin D supplementation [40]. Similar were the findings from the Elkin et al. study where radiographic evidence of vertebral deformity, and a history of non-vertebral fractures, were present in 17% and 35% respectively [41]. More recent studies, confirmed the reduced BMD from a young age, and the excess of fractures (21% prevalence in a Canadian and 27% in an Italian study) [42–44]. Some evidence, however, indicate that the overall improvements in pulmonary status may have helped to ameliorate the problem [45].

Dual Energy X-ray Absorptiometry has aided greatly in the investigation of CFBD. Results have demonstrated a decline in cancellous bone volume with low bone formation at both tissue and cellular level [46]. Buntain et al. [47] reported normal bone mass in 5–10 years old CF children, but significantly reduced whole body and wrist bone mass in the 11-20 years age group; this is in accordance with the assumption that CFBD starts developing from an early age but the clinical manifestations become apparent later in life.

As it has been aforementioned, the etiology of CFBD seems to be the end result of many irregularities occurring in CF. In recent years, VK deficiency has been added as another potential cause of CFBD [48].

VK is a coenzyme of glutamate carboxylase which converts

glutamate residues of certain proteins, such as OC, to Gla. The Gla compound attracts calcium ions and incorporates them into the hydroxyapatite crystals. Although there are at least three Gla proteins associated with bone tissue, OC is the most abundant and well-studied. It is secreted by osteoblasts during bone formation and constitutes the major non-collagenous protein incorporated in bone matrix. About 30% of the newly produced OC remains in circulation and can be used as a marker for the bone formation process [49,50]. In a VK deficiency state, there will be an increase in un-OC, the non-active form of the protein. Studies have demonstrated that low dietary VK intake leads to low BMD and increased susceptibility to fractures in post–menopausal women [51–53]. Additionally, VK supplementation has been shown to reduce un-OC and improve the bone turnover profile in non-diseased post-–menopausal women [53]. However, the results of the above studies should be extrapolated with caution to CF.

A low bone mass is a common finding in CF disease and often co-exists with suboptimal VK blood levels [54]. Supplementing CF patients with VK would be a reasonable approach in order to improve bone mass and prevent CFBD, at least to a certain degree. There are studies showing that low VK concentration and increased un-OC are predictors of hip fracture risk, irrespective of bone density, at least in elderly men and women [55 56]. Some other studies indicate that treating with VK, either elderly patients with established osteoporosis, or children with skeletal unloading due to corticosteroid treatment, could reduce the bone lose or even improve BMD and/or prevent the occurrence of new fractures [51,52,57–59]. Some other data point towards a synergistic action of VK with vitamin D [60–62]. The above results warrant additional and more focused research efforts on VK supplementation in CF. Beker et al. [63] recruited 18 CF patients all of whom had increased serum un-OC concentrations at enrolment. They were randomly assigned to either a four-week treatment with 5 mg phylloquinone per week or no supplementation, and then crossed over to the other treatment for a second four week period. By the end of the trial the majority of patients had achieved the normal reference mean levels (21%) of un-OC. Drury et al. [64] divided 14 pancreatic insufficient CF children, between the ages of 8–18 years old, in two groups and treated them either with 5 mg/day or 1 mg/day phylloquinone, for one month. They observed that supplementation reduced the overall percent un-OC from a median of 46.8%–29.1%. The reduction was independent of the dose but this may have been due to the small sample size. Nicolaidou et al. [54] treated with VK 20 children and adolescents with CF, and demonstrated an increase in serum OC without, however, noticing any effect on bone mineralization.

Indeed, the evidence on the role of VK in the prevention or treatment of CFBD is not enough to be considered indisputable. This has prevented experts from constructing widely acceptable guidelines on VK supplementation. Characteristically, only 18% of Pediatric CF Centers in UK have incorporated VK supplementation into their clinical practice, at doses varying from 0.3 to 10 mg/day [65]. Nevertheless, despite the drawback of evidence scarcity, European and American guidelines do recommend VK supplementation to all pancreatic insufficient patients [19,66].

### 4.2. The anti-inflammatory role of VK; could this apply to CF?

Until relatively recently, it was believed that the role of VK was restricted to the carboxylation and activation of coagulation proteins and OC. Nonetheless, it has now been recognized that the vitamin exerts some anti-inflammatory actions which seem to be completely independent of its role as an enzymatic cofactor [67].

This newly discovered action of VK has been mostly investigated in animal and experimental models and in cross-sectional studies of aging-related disorders [68]. In vitro cultured cell experiments and in vivo animal experiments have shown that VK restrains pro-inflammatory reactions induced by lipopolysaccharide (LPS) in rats and human macrophage-like THP-1 cells, via the inactivation of the NF-kappa B signaling pathway [17,67].

In an animal model study by Aoganghua et al. it was shown that VK3, (but not VK1 or VK2), exerted inhibitory effects against tumor necrosis factor alpha (TNF-α) production in a LPS-induced acute inflammation. The authors provided evidence indicating that this effect is induced through the inhibition of human mitochondrial DNA polymerase γ [69]. The same research group had also shown that VK3 can reduce pancreatic inflammation in acute pancreatitis through inhibition of the autophagic pathway [70]. They also demonstrated that the supplementation with K3 in an animal model of acute lung injury, before or after LPS administration, could attenuate the severity of lung injury. This was attained through the suppression of the LPS-induced increase in the serum TNF-a levels and the inhibition of the LPS-evoked nuclear translocation of NF-kappa B in lung tissue [71].

In a cross-sectional analysis of the Framingham Offspring Study, VK status - as it was estimated by plasma phylloquinone concentration and phylloquinone intake - was inversely associated with circulating inflammatory markers [18]. In another cross-sectional study on of multi-ethnic adults' cohort, higher serum phylloquinone was associated with lower levels of inflammation mediators [72].

Despite the above results pointing clearly towards an immunomodulatory role for VK, the true relevance of VK to inflammation remains poorly elucidated. What is more, we do not yet have any data regarding an anti-inflammatory action of VK in CF. This issue consists an important knowledge gap since the anti-inflammatory effects of VK – though it has been proven beyond doubt to be clinically important - may be more relevant to certain diseases or certain patients groups [68].

### 4.3. Vitamin K as a coagulation factor in CF

VK is necessary for the functionality of procoagulant factors (II, VII, IX, and X), as well as the anticoagulant proteins C, S, and Z [73]. Given the impaired VK status and the often-coexistent liver disease, one would expect coagulation related symptoms to be quite often in CF. However, such symptoms are only rarely encountered.

CF can only occasionally present as a bleeding disorder secondary to VK deficiency. Manifestations may include mucosal and subcutaneous bleeding or, especially in the first months of life, a more severe bleeding disorder such as intracerebral hemorrhage [74–76]. The uncommonness of the problem probably reflects the ability of the liver to absorb efficiently and use effectively circulating VK [77]. There are only a few reports in medical literature of CF patients having as an initial manifestation some type of bleeding and, as a matter of fact, some of these cases had been published before the widespread prophylactic use of postnatal VK. To the best of our knowledge, the first report was from Torstenson et al. [78] who reported on 3 infants with life threatening bleeding associated with prolonged prothrombin times and deficiencies of plasma clotting factors II, VII, IX, and X, which returned to normal with the administration of VK. All infants were subsequently diagnosed with CF and bleeding was proved to be the presenting symptom of the disease. Only a handful of similar cases have since been published [74–76,79,80].

Though in the last decades the practice of neonatal prophylaxis with VK has been universally adopted, usual doses are not adequately sufficient in preventing late VK bleeding diathesis in children with unrecognized liver disease and malabsorption syndromes [79]. Despite the fact that CF is not among the common causes of bleeding in infancy, the severity of clinical consequences necessitates the disease to be considered in the differential diagnosis whenever a bleeding diathesis is present in the first year of life. This is especially true for countries where neonatal screening programs for diagnosing CF are not available.

Recently, a number of studies have described an increased incidence of thrombophilia in CF patients [81–85]. This issue is of utmost concern due to the increasingly frequent placement of indwelling intravenous catheters in CF patients. It has to be mentioned, however, that despite

the high reported rates of prothrombotic state, the true incidence of venous thrombosis is low. The cause of thrombophilia tendency is probably multifactorial and largely unknown. Depressions of anticoagulant proteins C and S appear to be common in CF, probably as a consequence of hepatic dysfunction and presence of inflammation [81]. Since these proteins are VK dependent, the involvement of VK deficiency in the development of a thrombophilic state, is a reasonable hypothesis.

## 5. Why vitamin K is lacking in CF?

VK levels depend on two main factors; intake and absorption. VK is absorbed mainly in the terminal ileum and arrives into the lymphatic system following a pathway that is similar to most of the dietary lipids. The bile salts and pancreatic lipase have a pivotal role in this process [86]. Exocrine pancreatic insufficiency, and the resultant absence of lipase, occur very early in CF and are present in about 85% of patients by the end of the first year of life. Also, in CF there is abnormal viscosity, decreased flow, and increased concentration of bile components, and so the function of bile salts is most likely affected [87]. The above is evident from the improvement of fat digestion and absorption after treatment with the hydrophilic bile acid, ursodeoxycholic acid [88]. Bile salt secretion may also be affected in asymptomatic fibrosing liver disease which is present in as many as 25–30% of CF patients [88].

Neonates are especially vulnerable to VK deficiency because they have limited VK stores [86]; the problem worsens with decreasing gestational age. VK deficiency in the newborn is caused by the inadequate placental transfer, limited hepatic stores, and inadequate postnatal dietary sources of VK. Breast-fed infants are at especially increased risk of VK deficiency due to the insufficient amounts of VK in breast milk [89]. Additionally, it has been assumed that breast milk can further deteriorate VK status through the promotion of intestinal colonization with lactobacilli that cannot synthesize VK [89]. Postnatal administration of VK has helped to this direction, but the problem can emerge in cases of infants with CF due to the early onset pancreatic insufficiency that leads to malabsorption of fat-soluble vitamins [11].

In older children and adult CF patients there is a range of risk factors that may add to pancreatic insufficiency. These include liver disease, diarrhea, bowel resection due to meconium ileus, inadequate dietary intake of VK, and frequent antibiotic therapy. The latter is believed to alter the flora and affect the amount of VK produced there [90].

## 6. Vitamin K and antibiotics

The rationale behind the use of antibiotics in CF is to control the chronic endobronchial infection. However, despite the aggressive and long antibiotic regimens, bacteria populations continue to grow and evolve into more resistant strains, mostly in the form of biofilms. Because of this vexing issue, there have been ongoing research efforts to explore the potential of various compounds being able to work cumulatively or even synergistically with antibiotics [91,92].

It has been firmly established that lipid-soluble compounds can damage many elements necessary for bacterial membrane integrity making it more permeable to various compounds, such as antibiotics [93–95]. In connection with the above, there are some data suggesting that menadione (VK3) may have antibacterial and antibiotic-modifying qualities and being able to augment the effects of aminoglycosides against multi-resistant bacteria [16]. However, the evidence on this issue comes from only one paper, whereas another study has shown quite an opposite result, namely, that VK3 can induce efflux pumps and decrease the susceptibility to antibiotics [96]. It is clear that further research is needed in order to clarify this important topic.

## 7. Measuring VK levels

Despite VK deficiency being a fairly common problem in CF patients, there is absence of a straightforward and widely performed laboratory test to estimate its levels and/or function accurately [97]. In the usual clinical settings, prothrombin time and international normalized ratio are typically used as surrogates of VK status. However, the above indices are far from being adequately sensitive and can depict VK deficiency only when the undercarboxylated coagulation factors exceed 50% [98]. For research purposes, VK status and function are assessed by a variety of methods, none of which, however, can be considered as the gold standard [99].

Direct measurement of serum VK concentration is available only in reference laboratories. This method has some serious disadvantages; it can measure only phylloquinone and not the MK-n series, it is affected by abnormal lipid profiles, and finally, it reflects mainly the recent dietary intake of phylloquinone and not the overall amount of VK in the body [100]. It is worthy of note, that there is a considerable amount of VK stored in liver - an adult human liver contains about 200–300 nmol of VK. Whether this reserve represents the total amount of VK available resources [14,101], and if liver may or may not supply the circulation with VK when dietary intake is insufficient, remains to be elucidated [14].

Given the caveats and drawbacks of direct measurement of circulating VK, functional assessments through the estimation of the γ-carboxylation status of specific Gla proteins in liver and bones, such as prothrombin and OC, have gained ground. The prothrombin induced by vitamin K absence or antagonist-II (PIVKA-II) is a well-established method for measuring abnormal decarboxylated prothrombin. Low PIVKA-II concentrations are detectable at an early stage, before the classical coagulation factors are affected, rendering it a relatively sensitive and reliable marker of early VK deficiency [102]. The reference range of PIVKA-II is variably reported but the majority of authors consider levels above 2 ng/ml as abnormal [98,102,103]. It seems that liver can maintain a sufficient supply of VK even at low blood levels that may cause VK deficiency of bone metabolism [77].

The measurement of the fraction of total OC in its undercarboxylated state is another sensitive indicator of the VK status. In fact, it is the first functional marker to respond to VK depletion and the last to respond to. Bones are not as competent as liver in binding and using VK from the circulation and are therefore more prone to VK deficiency. This fact renders circulating level of un-OC a more sensitive indicator of VK deficiency, compared to PIVKA-II. In general, OC is the first protein to become undercarboxylated in VK deficiency, and the last to respond to supplements [98,104,105]. However, a study showed that VK deficiency could have been missed if its estimation had been based only on un-OC [98]. What this finding implies is that, at least in CF, VK deficiency can appear independently in each distinct tissue and so, ideally, it has to be assessed separately in each individual organ; PIVKA-II for liver, and un-OC for bone [98].

Relatively recently, a promising method has been developed for the measurement of the urinary 5 (5C) and 7 (7C) carbon aglycone VK metabolite. These are the common metabolites of K1 and the MK-n, and most likely reflect the total sum of the available VK. 5C and 7C excretion is ideally determined from complete 24-h urine collections, but if this is not feasible then the analysis can be performed in spot urine collections [100,106].

## 8. Do we know the right dose of vitamin K for CF patients?

There has been a considerable amount of data suggesting that CF patients are VK deficient and need supplemental intake of this vitamin. However, the absence of a convenient and universally accepted method to assess VK status hinders the accumulation of clear and robust evidence regarding VK supplementation. Available guidelines are based mostly on consensus opinion than on sound evidence. This is true not only for CF but also for many other conditions related with VK deficiency [99].

It has been suggested that VK sufficiency should be defined as the

intake required to ensure that all VK-dependent proteins in the body are fully γ-carboxylated [107]. Though the above approach seems biologically and clinically correct, it is not usually applicable in everyday clinical practice, since the related methods, e.g., PIVKA-II, and un-OC, are not readily available in most clinical settings. Given so, another more practical approach concerning the dose and frequency of administration should have to be adopted. Fortunately, there are some studies with valuable inferences and implications that can be used in the development of such guidelines.

In 1992 [20] the Consensus Committee of the CF Foundation suggested a relatively low dosage scheme (2.5–5 mg once weekly and doubling of the dose when the patient is on antibiotics) which was proved ineffective in a study performed a few years later [63]. In 2002 [66] the American Consensus Committee recommended a low-dose (0.3–0.5 mg/day) of VK supplementation independently of age. However, the authors stated explicitly that no adverse effects are expected at higher dosages. Recommendations from a European Consensus suggested that the dose of VK should be 1 mg/day to 10 mg/week [19]. The results from a study from Nicolaidou et al. [108] indicated that a weekly dose of 10 mg/week could substantially improve bone health indexes in CF patients. A more recent study assessed the results of VK supplementation in 3 groups of CF patient receiving 0.15, > 0.15 - < 1, and 1 mg/day. They concluded that only subjects in the high-dose group could achieve a VK status similar to that of healthy subjects [103].

In 2011, a group of researchers published The European CF Bone Mineralization Guidelines [15]. They indicated clearly that “studies are lacking to determine the most effective VK supplementation regimen to correct VK deficiency”. Nevertheless, they recommended that all pancreatic insufficient patients should take VK supplementation starting from a minimum dose 0.5–2 mg/day in infants, and increasing to 1–10 mg/day in children and adults.

In cases of liver disease the risk for VK deficiency increases because the dearth of bile salts in the intestinal lumen further compromises VK absorption. Wilson treated 6 patients with advanced CF liver disease with low doses of VK (mean intake 0.18 mg/d), and found that even at this low dose, PIVKA-II levels were corrected in half of them [109].

Recently, a Cochrane review concluded that although the evidence from randomized controlled trials on the effectiveness and benefits of routine VK supplementation in CF patients remains weak, there are no references indicating adverse effects. The authors suggested adherence to the current recommendations encouraging VK supplementation, though they could not advocate any particular dose regimen [110].

It is obvious that we have not yet been able to determine the ideal dose that normalizes VK status of CF patients. However, given the short turnover time of VK (about 1.5 day) it would be reasonable to prefer daily from weekly supplemental schemes [111].

## 9. Conclusions

Subclinical VK deficiency seems to be a common problem in CF patients. However, despite the fact that the rest of fat-soluble vitamins are routinely supplemented, this is not a universal clinical practice for VK. This irrational approach probably comes from the difficulties in estimating VK status, or even from a deeply rooted, historical belief in the merit of prothrombin time as being a sensitive indicator of VK.

Regardless of the insufficient available data on the proper regimen, oral VK is very safe and its supplementation should be incorporated into routine clinical practice. Most researchers agree that until more detailed evidence is available, a reasonable approach would be to provide a dose of 1 mg–10mg on a daily basis.

## Conflicts of interest

There is no conflict of interest.

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