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Chair: _____

Prevalence of Subclinical Vitamin K Deficiency in Cholestatic Liver Disease

A thesis submitted to the

Division of Research and Advanced Studies of the University of Cincinnati

In partial fulfillment of the
Requirements for the degree of

MASTER OF SCIENCE

In the Division of Epidemiology and Biostatistics,
Department of Environmental Health
of the College of Medicine

2004

by

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Abstract

Current practice is to monitor prothrombin time as an indicator of vitamin K sufficiency in cholestatic liver disease. Since prothrombin time is a surrogate marker, it may underestimate the actual prevalence of vitamin K deficiency in this population. This study investigates the frequency of vitamin K deficiency among a convenience sample of children and adults with cholestatic liver disease by determining plasma levels of protein induced in vitamin K absence II (PIVKA-II), and assesses the relationship between PIVKA-II levels and markers of cholestasis, measured prothrombin time, and vitamin A, E and 25-hydroxyvitamin D levels. **Methods:** Subjects with cholestatic liver disease were recruited from the Cincinnati referral area. Subjects with decompensated cirrhosis, malignancy, concurrent disease that results in fat malabsorption and AIDS were excluded. All subjects had blood collected for liver function tests, prothrombin time (PT), INR, bile acids, 25-hydroxyvitamin D, vitamin A, vitamin E and PIVKA-II levels. Plasma PIVKA-II concentrations were measured using a commercially available ELISA kit. **Results:** 31 subjects were enrolled (age range 0.5-54 years). Nine subjects (29%) had prolonged prothrombin times, while 21 (68%) had elevated PIVKA-II levels. All patients with prolonged PT had PIVKA-II elevations. Of the 21 patients with PIVKA-II elevation, 15 were on supplemental vitamin K therapy (range 7.8-700 $\mu\text{g/kg/day}$). PIVKA-II levels were positively correlated with serum conjugated bilirubin, bile acids, AST, ALT and measured PT and negatively correlated with serum 25-hydroxyvitamin D levels. Seven subjects (22.5%) had vitamin A deficiency, 5 subjects (16%) had vitamin D deficiency and 2 subjects (8%) had vitamin E deficiency. **Conclusions:** Despite vitamin K supplementation, vitamin K deficiency, as measured by PIVKA-II, is common

in cholestatic liver disease. Better strategies for vitamin K supplementation and dosing guidelines are needed.

Acknowledgments

The author would like to acknowledge the following individuals for their thoughtful comments, advice and their assistance in this project.

James E. Heubi, M.D., thesis chair and advisor and research mentor, Paul Succop, Ph.D., thesis committee member, Division of Gastroenterology, Hepatology, and Nutrition at Cincinnati Children's Hospital Medical Center, Kenneth Sherman, M.D., Steve Zucker, M.D., Glenda Lovell, and the GCRC staff. This research is supported by USPHS GCRC Grant #M01 RR 08084 from the National Center for Research Resources, NIH.

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Introduction

Fat malabsorption is well recognized in children and adults with cholestatic liver disease. Decreased bile flow and subsequent decrease in intraluminal bile salt concentrations below critical micellar concentration result in malabsorption of fat-soluble molecules, with the degree of malabsorption generally correlating with the degree of cholestasis.¹ Not surprisingly, deficiencies of fat-soluble vitamins A/E/D/K may occur with resultant skin and visual changes, spinocerebellar syndrome, rickets and osteoporosis, and coagulopathy. Such vitamin deficiencies are present in between 20-35% of patients with cholestatic liver disease. Many of these patients are asymptomatic at presentation stressing the importance of early supplementation to help prevent long-term consequences.¹⁻⁴

Vitamin K is present naturally as phyloquinones synthesized by green plants and menaquinones produced by intestinal bacteria. Dietary phyloquinones provide the main source of vitamin K in humans and, like other fat-soluble vitamins, require incorporation into micelles within the intestinal lumen for adequate absorption. Vitamin K is rapidly metabolized so malabsorption may lead to rapid depletion of limited stores, leading to clinically significant deficiency. Vitamin K serves as a cofactor for the enzyme γ -glutamylcarboxylase, which is involved in the post-translational carboxylation of glutamic acid residues in many proteins, including coagulation factors such as factors II (prothrombin), VII, IX, X, protein C, protein S, and proteins involved in bone homeostasis, specifically osteocalcin and matrix Gla protein. Most of these proteins are multiply carboxylated, and these carboxylated glutamic acid residues have the ability to bind calcium, making these proteins functionally active.^{1,5} In vitamin K deficiency, there

is an increase in abnormal undercarboxylated or partially carboxylated forms of these proteins, which are functionally defective. If significant, this deficiency results in coagulopathy, as measured by prolongation of prothrombin time. Vitamin K deficiency may also contribute to bone disease due to inadequate carboxylation of bone matrix proteins, specifically osteocalcin, which represents 15-20% of non-collagenous bone, and in several large cohort studies, an association between low vitamin K intake and decreased bone mineral density⁶ and increased risk of hip fractures^{7,8} has been identified.

Traditionally, vitamin K status has been assessed by surrogate markers such as the prothrombin time, which measure activity, rather than vitamin K presence. This may greatly underestimate the actual incidence of vitamin K deficiency, as the prothrombin time is a late indicator of vitamin K deficiency and can be normal even when prothrombin concentrations fall to half normal values.⁹ Thus many patients with cholestasis may have subclinical vitamin K deficiency, placing this group at potential risk for bleeding complications as well as having long-term impacts on skeletal health. New methods measuring undercarboxylated vitamin K dependent proteins, such as protein induced in vitamin K absence-II (PIVKA-II), prove to be a more sensitive measure of vitamin K status, enabling the clinician to identify early deficiency and subsequently correct vitamin K nutritional status.^{10,11}

There have been a few studies in adults with cholestatic liver disease demonstrating elevated abnormal prothrombin or decreased vitamin K levels. In a study of 52 patients with primary biliary cirrhosis, Kaplan et al identified 8 patients (15%) with vitamin K deficiency as measured by prolonged prothrombin time; however, 29 of these 52 patients (56%) had elevated abnormal prothrombin (undercarboxylated prothrombin),

with the degree of elevation directly correlating with stage of disease and serum bilirubin. Subsequently, 11 of these 29 patients were treated with intramuscular vitamin K. Repeat abnormal prothrombin decreased in 10 of 11 of these patients, suggesting the presence of subclinical vitamin K deficiency.¹² O'Brien et al measured vitamin K₁ levels in 11 patients with cholestatic jaundice of whom 7 (64%) had levels below normal; however, prothrombin time was only prolonged in 2 of these 11 patients.¹³ A similar study by Kowdley et al found significantly lower vitamin K₁ levels in 77 patients with primary biliary cirrhosis compared with normal controls with 18 patients (23%) having vitamin K levels below normal range. Only one of these diseased patients with decreased vitamin K levels had an elevated prothrombin time again illustrating the poor sensitivity of prothrombin time as a surrogate marker for vitamin K deficiency.¹⁴ Although these later studies demonstrated discrepancies between vitamin K levels and prothrombin time, a limitation in these studies is that plasma vitamin K levels may not be representative of total body vitamin K stores. Additionally, the majority of the adult studies focus on one subset of liver disease (primary biliary cirrhosis). Therefore, the findings may not be generalizable to other cholestatic diseases, particularly those prevalent in pediatric patients since clinical management may be markedly different. Pediatric data is limited. A preliminary study by Mager et al examined 43 cholestatic children and found significantly elevated mean PIVKA-II levels when compared to non-cholestatic children and healthy controls. Twenty-one of the 43 cholestatic children (49%) had PIVKA-II levels compatible with vitamin K deficiency, but only 2 of these patients had a prolonged prothrombin time.¹⁵ These results suggest vitamin K deficiency is relatively common in pediatric cholestatic liver disease. The primary aim of this work was to determine the

frequency of vitamin K deficiency in cholestatic children and adults as measured by protein induced in vitamin K absence-II (PIVKA-II) and assess the relationship between PIVKA-II and serum conjugated bilirubin, various serum markers of liver function (GGT, alkaline phosphatase), serum bile acids, and prothrombin time.

Methods

Study Design: This is a cross-sectional study of patients with cholestatic liver disease.

The study was approved by the institutional review boards of Cincinnati Children's Hospital Medical Center and the University of Cincinnati. The study was also approved by the Scientific Advisory Committee of the General Clinical Research Center of Cincinnati Children's Hospital Medical Center.

Overview of Patient Population: Subjects represented a convenience sample of available children and adults with cholestasis defined as a direct bilirubin > 1 mg/dl and/or serum bile acids > 30 umol/L. Subjects of either sex were eligible. Inclusion diagnoses were: biliary atresia, primary sclerosing cholangitis, alpha-1 antitrypsin deficiency, neonatal hepatitis, progressive familial intrahepatic cholestasis (PFIC I, II, or III), Alagille's syndrome, primary biliary cirrhosis, chronic hepatitis, and idiopathic cirrhosis. Exclusion criteria were: 1. Presence of concurrent disease known to result in fat malabsorption (**Table 1**); 2. Malignancy; 3. Age < 6 months or > 65 years; 4. AIDS; 5. Decompensated cirrhosis characterized by intractable ascites and severe coagulopathy; 6. Weight < 6 kg.

Table 1. Exclusion Criteria--Etiologies of Fat Malabsorption

Pancreatic Disorders	Cystic Fibrosis Shwachman syndrome Johanson-Blizzard syndrome Isolated pancreatic enzyme deficiency Chronic pancreatitis
Bile Acid Disorders	Terminal ileum resection Crohn's disease Bacterial overgrowth Bile acid sequestrants
Defective Chylomicron Formation	Abetalipoproteinemia
Altered Immune Function	Autoimmune enteropathy AIDS Combined immunodeficiency
Altered Surface Area	Celiac disease Short Bowel Syndrome Microvillus inclusion disease
Altered Lymphatics	Intestinal Lymphagiectasia
Altered Anatomy	Hirschsprung's disease Partial small bowel obstruction

Recruitment: Subjects were identified from records of outpatient and inpatient encounters with members of the Division of Pediatric Gastroenterology, Hepatology and Nutrition at Cincinnati Children's Hospital Medical Center (CCHMC) and the Division of Digestive Diseases of the University of Cincinnati College of Medicine. Subject recruitment began October 31, 2002 with the first patient enrolled January 7, 2003.

Experimental Methods (Figure 1): Eligible subjects were given a verbal explanation of the study followed by completion of a written consent form. For subjects less than 18 years old, parents provided informed consent and subject's assent was obtained from minors greater than 11 years old. A brief questionnaire, including such items as age of diagnosis, specific diagnosis, medications, including vitamin supplementations, and hospitalizations and surgeries was administered by the primary investigator (**Appendix**

A). Supplemental information was obtained by reviewing records at the Cincinnati Children's Hospital and by having subjects release information from their current gastroenterologist.

The subject's blood was drawn for liver function tests (AST, ALT, alkaline phosphatase, GGT, albumin, total protein, conjugated and unconjugated bilirubin), prothrombin time, INR, serum bile acids (normal range: 7-9 months: 1-28 $\mu\text{mol/L}$; 10-12 months: 1-37 $\mu\text{mol/L}$; >1 year: 1-7 $\mu\text{mol/L}$), 25-hydroxyvitamin D (normal range: 9-46 ng/mL), vitamin A (normal range: <1 year: 200-490 $\mu\text{g/L}$; >1 year: 140-520 $\mu\text{g/L}$), vitamin E (normal range: 3-15 $\mu\text{g/mL}$), and PIVKA II. Liver function tests, prothrombin time (PT), INR, serum bile acids, 25-hydroxyvitamin D, vitamin A, and vitamin E levels were measured using standard laboratory techniques. PIVKA-II concentrations were measured using a commercially available enzyme-linked immunosorbent assay kit (Diagnostica Stago; Asnier, France). This assay uses a mouse monoclonal antibody specific for undercarboxylated prothrombin, but without cross-reactivity to fully carboxylated prothrombin. The assay was standardized at our institution by using discarded plasma from previously stored samples with all identifiers stripped and by recruitment of healthy adults to serve as controls. The lowest limit of detection for this assay is 2 ng/mL. Values of less than 2 ng/mL are considered normal; however, for the purposes of this research protocol, values less than two times the lower limit of detection (4 ng/mL) were considered normal. All control samples fell greater than two standard deviations below this value. All PIVKA-II samples were run in duplicate on two separate days. Within our institution, intraassay variability was 6.2% and interassay variability was 10%. Additional blood was collected for measurement of undercarboxylated

osteocalcin. Cumulative blood drawn was 20 mL, which was less than 5% of subjects total blood volume based on subject's weight greater than 6 kilograms.

Figure 1. Overview of Experimental Methods

Data Management: To ensure subject confidentiality, each subject was given a unique numeric identifier. Research medical records were stored in a locked file cabinet and results generated from this study were kept in a password protected database. All database results were coded by subject's unique numeric identifier.

Data Analysis:

Sample Size: A sample size of 30 was calculated to be required to detect a correlation of at least 0.5 between PIVKA-II and markers of cholestasis. This sample size calculation was based on a two-sided test with an alpha level of 0.05 and power of 0.80.

Statistical Analysis: All statistical analyses were performed using SAS[®] software (version 8.3, SAS Institute, Cary, NC). As the data were not normally distributed, Spearman correlations were determined between PIVKA-II and other variables. Partial correlations were used to assess the relationship between PIVKA-II and other measured parameters while controlling for vitamin K intake. For the purpose of analysis, all laboratory values below the limit of detection for a particular assay were recorded as the lowest value for that assay. For example, the lowest limit of detection for the Vitamin E assay is 3 ug/mL, therefore, values < 3 ug/mL were recorded as 3 ug/mL. All p-values were two-sided.

Results

Thirty-six patients were enrolled in this study. Two patients (one with biliary atresia, one with idiopathic cholestasis) withdrew due to difficulty obtaining a sufficient quantity of blood for study laboratories. Three additional patients were ineligible based on inclusion/exclusion criteria (1 subject with history of necrotizing enterocolitis and small bowel resection, 2 subjects with biliary atresia did not meet eligibility requirements for cholestasis based on conjugated bilirubin or serum bile acid levels). Of the 31 subjects who fulfilled eligibility criteria, 24 were children (<18 years old) and 7 were adults. Seventeen subjects were female. The most common diagnoses were Alagille's syndrome and biliary atresia, with 10 and 9 subjects respectively, accounting for 60% of the study population. Other diagnoses included primary biliary cirrhosis (4), Progressive Familial Intrahepatic Cholestasis (2), congenital hepatic fibrosis (1), primary sclerosing cholangitis (1), α -1 antitrypsin deficiency (1), hepatitis C (1) and idiopathic cholestasis (2). The mean age of the study subjects was 12.8 years (median 5.7, range 0.7-54.1

years) (**Table 2**). Mean serum conjugated bilirubin and bile acids were 1.8 mg/dL and 276 μ mol/L, respectively. When pediatric and adult data were separated, there was a trend for the pediatric subject to be more cholestatic, with higher mean serum conjugated bilirubin, alkaline phosphatase, and bile acids. Other laboratory values for study subjects are listed in Table 2.

Table 2. Clinical and Laboratory Characteristics of Subjects*

	<i>Pediatric Subjects</i>	<i>Adult Subjects</i>	<i>All Subjects</i>
N	24	7	31
Age (Years)	4.4 (0.7-14.5)	41.5 (20.2-54.1)	12.8 (0.7-54.1)
AST (U/L)	155 (50-352)	122 (57-227)	148 (50-352)
ALT (U/L)	100 (25-292)	81 (26-192)	96 (25-292)
Albumin (g/dL)	3.8 (2.9-4.6)	3.4 (2.3-4.3)	3.7 (2.3-4.6)
Conjugated Bilirubin (mg/dL)	2.0 (0-12.3)	1.1 (0-6.8)	1.8 (0-12.3)
Alkaline Phosphatase (U/L)	637 (301-1098)	349 (197-646)	572 (197-1098)
GGT (U/L)	406 (18-2529)	437 (159-1386)	413 (18-2529)
Bile Acids (μmol/L)	316 (29-928)	138 (48-335)	276 (29-928)
PT (Secs)	13.3 (10.9-21.6)	12.7 (10.7-14.1)	13.1 (10.7-21.6)
INR (Secs)	1.32 (0.87-3.45)	1.18 (0.83-1.46)	1.29 (0.83-3.45)
PIVKA-II (ng/mL)	743.2 (2.0-5831.4)	72.5 (2.44-413.5)	591.7 (2.0-5831.4)
Vitamin A (μg/L)	501 (99-1263)	312 (84-868)	458 (84-1263)
Vitamin E (μg/mL)	10.5 (3-24.2)	10.0 (5.8-15.9)	10.4 (3-24.2)
25- OH vitamin D (ng/mL)	25.4 (6.1-55.7)	20.5 (5-32)	24.3 (5-55.7)

*Values expressed as mean (range)

Of the 31 subjects, 28 had all laboratories drawn at the time of study enrollment. Three patients did not have prothrombin time or INR results available on the day of study due to insufficient quantity of blood for PT/INR analysis (1 subject) and clotting of the prothrombin sample (2 subjects). One of these subjects had a prothrombin time and INR

obtained two days after initial laboratory assessment and this value was used in the analysis. Laboratory records were reviewed for the other two subjects. Both subjects had normal PT/INR before and after study laboratories and a stable medical history without change in medications or clinical status. For these subjects, the prothrombin time and INR values obtained closest to the time of study enrollment were used for analysis. Of the 31 subjects, 9 (29%) had elevated prothrombin times, defined as greater than 13.3 seconds (**Table 3, Table 4**). Most prothrombin times were mildly elevated with only 3 values being greater than 15 seconds, with the most prolonged prothrombin time being 21.6 seconds. Twenty-one subjects (68%) had an elevated (abnormal) PIVKA-II, defined as greater than 4 ng/mL (**Table 3, Table 4**). All subjects with prolongation of their prothrombin times had elevated PIVKA-II values. The highest PIVKA-II value of 5831.4 ng/mL was observed in a pediatric patient with Alagille's. This subject also had the most prolonged prothrombin time and was on supplement vitamin K therapy at a dosage of 162.3 µg/kg/day at the time of study enrollment. Of the 21 subjects with PIVKA-II elevation, 15 (71%) were on supplemental vitamin K therapy, with dosages ranging from 7.8 to 700 µg/kg/day. In subjects with normal PIVKA-II levels, 9 (90%) were on supplemental vitamin K therapy (dosage range 10-289 µg/kg/day). Seven patients (6 adults, 1 child) had no supplemental vitamin K intake. Within this subgroup, 6 (86%) had elevated PIVKA-II levels.

Other fat-soluble vitamin deficiencies were observed in 35% of the study participants. The most common was Vitamin A deficiency, observed in 7 (22.5%) of subjects (**Table 3**). Five subjects (16%) had decreased serum 25-hydroxyvitamin D levels, whereas 2 subjects (8%) had decreased serum Vitamin E levels (**Table 3**). One

subject, a pediatric patient with idiopathic cholestasis, had both Vitamin D and Vitamin E deficiency, while two subjects, a pediatric patient with biliary atresia and an adult patient with primary sclerosing cholangitis, had Vitamin D and Vitamin A deficiency. In total, 43% of subjects with Vitamin A deficiency and 100% of subjects with Vitamin D and E deficiency had elevated PIVKA-II values.

Table 3. Abnormal Laboratory Values

	<i>Pediatric Subjects N=24</i>	<i>Adult Subjects N=7</i>	<i>All Subjects N=31</i>
PT Prolongation	7 (29%)	2 (29%)	9 (29%)
PIVKA-II Elevation	16 (67%)	5 (71%)	21 (68%)
Vitamin A Deficiency	5 (21%)	2 (29%)	7 (23%)
Vitamin D Deficiency	4 (17%)	1 (14%)	5 (16%)
Vitamin E Deficiency	2 (8%)	0	2 (6%)

Table 4. PT Prolongation and PIVKA-II Elevation by Diagnosis

<i>Diagnosis</i>	<i>PT Prolongation</i>	<i>PIVKA-II Elevation</i>
Alagille's syndrome (N=10)	4 (40%)	7 (70%)
Biliary Atresia (N=9)	2 (22%)	5 (56%)
Primary Biliary Cirrhosis (N=4)	0	3 (75%)
PFIC (N=2)	1 (50%)	2 (100%)
Other (N=6)	2 (33%)	4 (67%)

To assess whether markers of the severity of an individual's cholestatic liver disease such as conjugated bilirubin and prolongation of prothrombin time were associated with elevated PIVKA-II levels, Spearman correlations were performed between PIVKA-II levels and other measured parameters. PIVKA-II levels directly

correlated with prothrombin time ($p < 0.02$), INR ($p < 0.02$) and the severity of cholestasis as measured by conjugated bilirubin ($p < 0.001$) and serum bile acids ($p < 0.002$) (**Table 5, Figure 2**). Additional positive correlations were observed between plasma PIVKA-II levels and AST, ALT, and alkaline phosphatase. There was an inverse correlation between PIVKA-II values and serum 25-OH vitamin D levels ($p = 0.01$). No significant correlations were found between PIVKA-II levels and other measured laboratories (**Table 5**).

Table 5. Correlations of PIVKA-II with Liver Function Tests and Vitamin Levels

<i>Variable</i>	<i>Spearman r</i>	<i>P-value</i>
AST	0.46	0.01
ALT	0.41	0.02
Albumin	0.07	0.73
Conjugated Bilirubin	0.66	0.001
Alkaline Phosphatase	0.36	0.05
GGT	0.15	0.43
Bile Acids	0.53	0.002
PT	0.42	0.02
INR	0.42	0.02
Vitamin A	0.24	0.18
25-OH vitamin D	-0.47	0.01
Vitamin E	-0.10	0.59

Figure 2. Correlation between PIVKA-II and Conjugated Bilirubin (A), Bile Acids (B) and PT (C)*

* PIVKA-II value 5831.4 data point removed for graphical purposes

As the majority of subjects (77%) were on some form of vitamin K therapy, Spearman correlations were also determined between supplemental vitamin K intake and other measured parameters. Vitamin K intake was positively correlated with serum bile acids and alkaline phosphatase, and the correlation between vitamin K intake and conjugated bilirubin approach significance (**Table 6**), indicating patients with more significant cholestatic liver disease were more likely to be on higher dosages of supplemental vitamin K. However, no significant correlation was observed between vitamin K intake and prothrombin time or PIVKA-II levels (**Table 6**). Subsequently, Spearman correlations between PIVKA-II and other laboratories were re-analyzed, controlling for vitamin K intake. Apart from the correlation between PIVKA-II and alkaline phosphatase, all previously correlated parameters persisted, with the correlations between PIVKA-II and conjugated bilirubin and bile acids remaining most significant (**Table 7**).

Table 6. Correlations of Vitamin K Intake with Liver Function Tests and PIVKA-II

<i>Variable</i>	<i>Spearman r</i>	<i>P-value</i>
Conjugated Bilirubin	0.34	0.06
Alkaline Phosphatase	0.41	0.02
GGT	-0.06	0.74
Bile Acids	0.46	0.01
PT	0.25	0.18
INR	0.25	0.18
PIVKA	0.15	0.43

Table 7. PIVKA-II Correlations, Controlling for Vitamin K Intake

<i>Variable</i>	<i>Spearman r</i>	<i>P-value</i>
AST	0.44	0.02
ALT	0.39	0.03
Albumin	0.06	0.74
Conjugated Bilirubin	0.65	0.001
Alkaline Phosphatase	0.33	0.07
GGT	0.16	0.41
Bile Acids	0.52	0.003
PT	0.40	0.03
INR	0.40	0.03
Vitamin A	0.23	0.22
25-OH vitamin D	-0.50	0.005
Vitamin E	-0.13	0.49

Discussion

Fat-malabsorption in cholestatic liver disease results from decreased intraluminal bile salts below the critical micellar level and fat-soluble vitamin deficiencies are a well-known complication of this process. Our study indicates that vitamin K deficiency, as determined by elevated PIVKA-II levels, is common in cholestatic liver disease, affecting 68% of our study population. This deficiency occurred despite oral vitamin K supplementation in 71% of subjects with elevated PIVKA-II levels. Prothrombin time, a surrogate marker of vitamin K status, identified only 42% of these individuals, thus greatly underestimating the prevalence of this vitamin deficiency.

Deficiencies of other fat-soluble vitamins were also present, occurring in 35% of the study population. Vitamin A deficiency was present in 23% of subjects, while vitamin D and Vitamin E deficiency occurred less frequently. These findings are

consistent with previously reported frequencies of vitamin A, D, and E deficiencies in patients with cholestatic liver disease.^{2,4,12,14,16} However, others have reported higher prevalence of fat soluble vitamin deficiencies.^{3,17-21} The reasons for these differences may relate to the methods used to assess deficiency states. Cholestatic liver disease can lead to hyperlipidemia, which may artificially raise vitamin E levels to normal values since vitamin E is carried in the HDL and LDL fraction of lipoproteins, thus masking true vitamin E deficiency.^{22,23} The measurement of the ratio of serum vitamin E to total lipids is a more accurate indicator of vitamin E status²³, and as serum total lipids were not measured in our study, vitamin E deficiency may be underestimated in our study population. Since neither serum cholesterol, triglycerides or total lipids were measured in this study, it is unclear how large an impact the presence of hyperlipidemia might have had on serum vitamin E levels. Additionally, in the present study, vitamin A deficiency may be overestimated in our population because vitamin A levels were not normalized for serum retinol binding protein concentrations. In the presence of advanced liver disease, the major transport protein for vitamin A, retinol binding protein, may be reduced, and therefore, some investigators have suggested that the molar ratio of vitamin A to retinol binding protein may better reflect vitamin A sufficiency.²⁴ Since few patients had evidence of advanced hepatic decompensation as assessed by reduced serum albumin concentrations, this may not represent a significant problem in our study population.

Previous studies have found positive associations between vitamin K deficiency, either measured by low plasma vitamin K levels¹⁴ or elevated undercarboxylated prothrombin^{12,15}, and the severity of cholestasis as determined by elevated conjugated

bilirubin, serum bile acids and alkaline phosphatase. This study confirmed these findings, and in addition we identified correlations between PIVKA-II and serum aminotransferases. Since PIVKA-II may be elevated in hepatocarcinoma²⁵⁻²⁷, the correlation between PIVKA-II and markers of liver cell injury raises the question of whether the PIVKA-II elevation found in our study may have been due to liver inflammation with impaired γ -glutamylcarboxylase rather than deficiency of vitamin K, a cofactor for this enzyme. In fact, patients with non-cholestatic liver disease may have either normal^{15,26} or elevated^{26,27} PIVKA-II levels. In the present study, the 100% concordance between prolonged prothrombin times and elevated PIVKA-II levels and the presence of other fat-soluble vitamin deficiencies in 33% of the subjects with PIVKA-II elevation, strongly suggest that the abnormal PIVKA-II levels were the result of vitamin K deficiency.

Although dietary intake was not assessed, supplemental vitamin K intake was quantified. Not surprisingly, vitamin K intake was positively correlated with the severity of cholestasis suggesting that clinicians recommend supplementation in the presence of increasing cholestasis; however, PIVKA-II elevation was common in these patients despite vitamin K therapy. It would be expected that negative correlations of vitamin K intake with prothrombin time and PIVKA-II levels would be identified; but in fact, there was no correlation among these parameters. This suggests that even with supplementation, intake is not sufficient to overcome the absorptive defect in these patients and current practices of oral vitamin K supplementation may be inadequate to maintain vitamin K nutriture in cholestatic liver disease. A mixed micellar preparation of vitamin K₁ now is available (Kanakion MM) in Europe, but unfortunately, absorption of

this formulation in cholestatic infants remains poor and unpredictable.²⁸ In addition, few clinicians recommend parenteral vitamin K unless extremely prolonged prothrombin times develop. Future studies examining the dosage and route of vitamin K administration are therefore indicated.

This study evaluated subjects with a variety of cholestatic conditions and had a large proportion of pediatric patients, a subgroup for whom data on vitamin K deficiency is lacking. However, one limitation of this study is the sample size, particularly in regards to the adult population. Clinical characteristics and medical management may differ between adult and pediatric subjects. In the present study, there was a trend for the pediatric patients to be more cholestatic, with higher conjugated bilirubin and serum bile acids, factors that are highly correlated with PIVKA-II elevation. Additionally, pediatric patients were more likely to be on supplemental vitamin K replacement. Only one adult subject, a patient with Alagille's Syndrome followed at the pediatric center, was on vitamin K therapy. Although the study had significant power to evaluate correlations between PIVKA-II and markers of cholestasis within the study population, differences between various cholestatic conditions and adult and pediatric subjects and predictors of PIVKA-II elevation within these subgroups could not be assessed. A second limitation of this study might be whether the results from this small group of children and adults with cholestasis would be generalizable to a larger group of patients. There is, however, no reason to believe that our subjects would not be representative of all pediatric and adult cholestatic patients.

In summary, despite supplementation, vitamin K deficiency, as determined by elevated PIVKA-II levels, is common in cholestatic liver disease and frequently

accompanies other fat-soluble vitamin deficiencies. Patients with advanced cholestasis, with high conjugated bilirubin and serum bile acids, are at highest risk for vitamin K deficiency. Prothrombin time underestimates the prevalence, and better strategies for vitamin K assessment and guidelines for specific dosing in cholestatic liver disease should be developed.

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APPENDIX A: Subject Questionnaire

**VITAMIN K DEFICIENCY IN CHOLESTASIS
Patient Data Form**

NAME: _____
DOB: _____
AGE: _____
HEIGHT: _____

DATE: _____
ID#: _____
SEX: ___ Male ___ Female
WEIGHT: _____

RACE/ETHNICITY: ___ CAUCASIAN ___ AFRICAN AMERICAN
___ NATIVE AMERICAN ___ ASIAN
___ HISPANIC ___ NON-HISPANIC

HISTORY OF LIVER DISEASE:

DIAGNOSIS: _____ AGE OF DIAGNOSIS: _____

PERTINENT PHYSICAL FINDINGS: _____

CURRENT MEDICATIONS:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____

PAST MEDICAL HISTORY:

- OTHER MEDICAL PROBLEMS: 1. _____
2. _____
 3. _____
 4. _____

HOSPITALIZATIONS: _____

SURGERIES: _____

LABORATORY:

AST _____	C BILI _____	PT _____	VIT A _____
ALT _____	U BILI _____	INR _____	25-OH D _____
ALB _____	ALK P _____	PIVKA II _____	VIT E _____
GGT _____	BILE ACIDS _____	VIT K1 _____	T. LIPIDS _____
			E/Lipid _____