SELECTED LABORATORY BASED BIOMARKERS FOR ASSESSING VITAMIN A DEFICIENCY IN INFANTS AND CHILDREN

A SYSTEMATIC REVIEW AND META-ANALYSIS

A Thesis

Presented to the Faculty of the Graduate School

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Master of Science

by

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ABSTRACT

Background

In 2013, the World Health Organization estimated 190 million preschool aged children (6-59 months), and 19 million pregnant women were affected by vitamin A deficiency. It is difficult to identify subclinical vitamin A deficiency before overt clinical symptoms develop, as serum retinol levels are homeostatically regulated until liver stores become significantly depleted. Therefore, common blood tests will not indicate a subclinical deficiency. In order to improve diagnostic accuracy of vitamin A status indicators, we need non invasive biomarkers that are also sensitive and specific.

Methods

This systematic review focuses on infants and children under the age of 5 years. We searched for studies that measured vitamin A status by at least one reference standard (liver biopsy or isotope dilution) and one index test (serum/plasma retinol, serum/plasma retinol binding protein, or relative dose response/modified relative dose response). Data was extracted from studies that met the inclusion criteria via Jotform. Methodological quality of included studies was assessed using QUADAS 2 (Whiting 2011). 2x2 tables were constructed to calculate sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. Forest plots were created to compare sensitivity and specificity between studies.

Results

The initial search returned 34,574 references. After title/abstract and full text screening, 11 were included in the final review. 4 of these studies were included in meta-analysis. Due to small sample sizes, sensitivity and specificity estimates for each study had large confidence intervals. The most reliable estimates of sensitivity/specificity were from Zaklama 1972, the only included study that had participants in each category (TP, FP, TN, FN) for calculation.

Discussion

More research is needed to truly assess the diagnostic accuracy of common biomarkers used in this population. Researchers should focus on using isotope dilution as a reference standard to allow more diverse populations and clinical conditions to be studied.

BIOGRAPHICAL SKETCH

Heather Marie Falise is a Registered Dietitian, originally from Syracuse, NY. She completed her undergraduate degree in dietetics at Syracuse University. Heather then completed her dietetic internship at Cornell University in 2019. She became a Registered Dietitian in July 2020. Heather volunteers her spare time with the New York State Academy of Nutrition and Dietetics as a Student Public Policy Coordinator, working to pass nutrition-related legislation in New York State.

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Background

Vitamin A is an essential nutrient needed for gene expression, vision, immune function, and growth. There are two main forms of vitamin A found in the body, 11-cis-retinal, and retinoic acid. The retinal form is required by the eye as part of the visual cycle, where it helps to transform light into vision through neural signalling (Saari 1994). The retinoic acid form activates RAR and RXR receptors in the nucleus, which regulate gene expression. The genes regulated by these receptors code for structural proteins such as keratin in skin, conjunctival membranes, enzymes, retinol binding proteins and receptors, and more, making vitamin A essential for maintenance of intestinal integrity and other immune barriers as well as enzymatic functions (Gudas 1994). These activities are important to keep in mind when it comes to the implications of vitamin A deficiency.

Vitamin A is found in foods in two different forms, preformed vitamin A (retinol or retinyl esters) or as provitamin A carotenoids that can be converted to vitamin A in the retinol form. These pro-vitamin A carotenoids include alpha and beta-carotene, and alpha and beta-cryptoxanthin. Preformed vitamin A is more efficient at maintaining vitamin A status, while pro-vitamin A precursors are needed in larger amounts to achieve the same impact. Preformed vitamin A is most commonly found in animal products, such as liver, fish, eggs, and dairy products, while provitamin A is often found in vegetables and some fruits. There are several challenges to efficient absorption and utilization of carotenoids. Since provitamin A carotenoids are found in plant foods (high in fiber), they are embedded in the food matrix . In order to access and absorb them, the food matrix must be broken down, and fat must be consumed with the meal (van het Hof 2000). However, this can be achieved with small amounts of fat, as little as 3-5g.

Because of the differences in vitamin A bioavailability between preformed sources and provitamin A sources, recommended intake of vitamin A is reported in retinol activity equivalents (RAE). RAE aims to mitigate the discrepancy between the bioavailability of retinol compared to carotenoids. Specifically, the RAE's for provitamin A are the following:

- B-carotene-12 ug
- A-carotene-24 ug
- β-cryptoxanthin-24 μg

This means that, for example, 12 ug of beta carotene must be ingested in order to produce 1 ug of retinol in the body. (National Academy of Sciences & Panel on Micronutrients, 2001)

In addition to natural food sources of vitamin A, fortified foods make a significant contribution to vitamin A intake in both developed and developing countries. Many developed countries fortify margarine, dairy products, ready-to-eat cereals, and other miscellaneous items with vitamin A, which contributes to the relatively low rates of vitamin A deficiency in these areas. In developing countries, there are limited vitamin-A fortified foods, which include sugar, flours and oils predominantly (Dary 2002). WHO and other agencies have established guidelines for food fortification to ensure that it is biologically efficient, including dosing, food matrix, and avoidance of fortifying processed foods that may introduce further public health issues (WHO/FAO 2006).

Many people in developing countries rely on plant sources of vitamin A to meet their needs, which relies on conversion to retinol in the body. This creates challenges with meeting vitamin A needs in children, who often cannot consume the volume of food needed to obtain sufficient vitamin A from plants alone (Akhtar 2013). Breastfeeding is a major source of vitamin A in infants, and helps build initial vitamin A stores. However, the mother must consume

sufficient vitamin A in order to produce breast milk with sufficient vitamin A. In areas where vitamin A deficiency is common, mothers are more likely to produce breast milk with low levels of vitamin A. This puts offspring at a disadvantage during this critical period. Because of this, studies have attempted to decrease risk of childhood morbidity and mortality through maternal vitamin A supplementation. However, current evidence does not show improvements in risk of illness or death in mothers or their offspring with VA supplementation (Oliveira‐Menegozzo 2010). Therefore, current supplementation programs focus on infant supplementation rather than postpartum maternal supplementation. Because of limited resources, and the likelihood to consume vitamin A from almost exclusively plant sources, children in low-income countries are at risk of developing vitamin A deficiency.

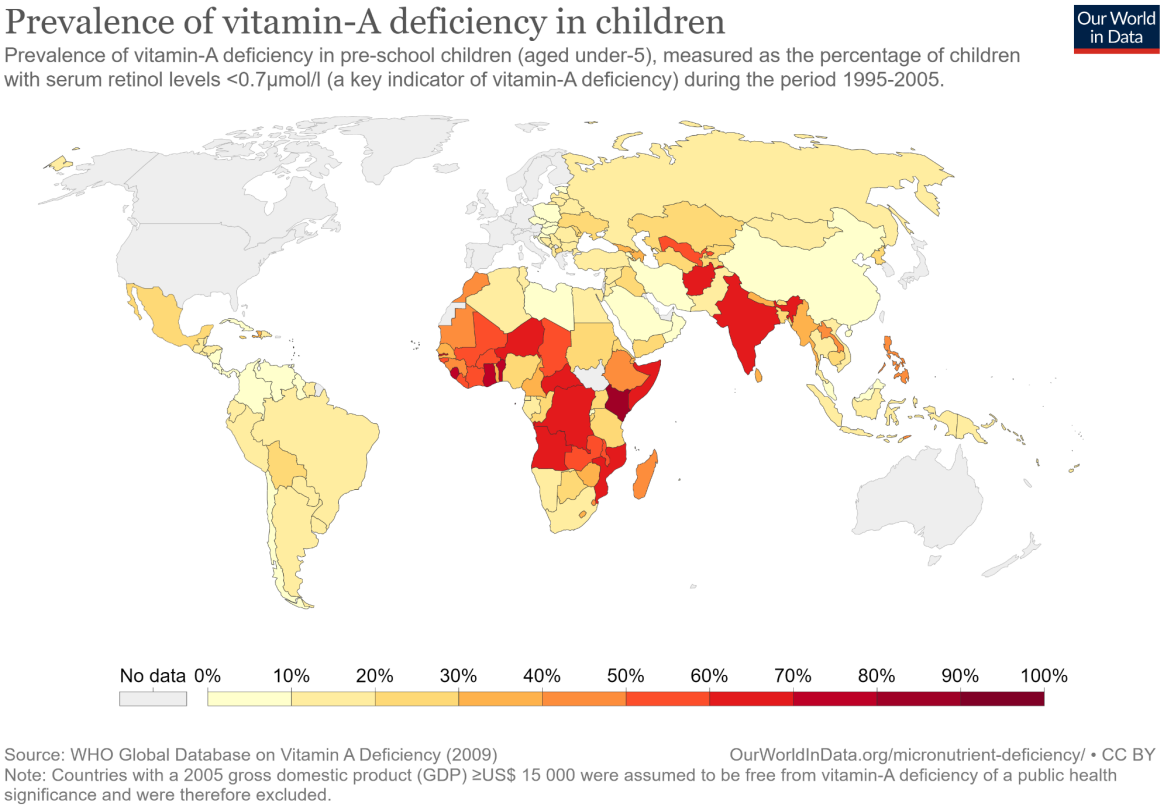
Vitamin A Deficiency

Vitamin A deficiency can present in both clinical and subclinical forms. Clinical vitamin A deficiency can lead to progressive health issues including xerophthalmia, night blindness, impaired immunity, and keratinization of mucous membranes and skin. Long-term clinical vitamin A deficiency eventually leads to scarring of the eyes and permanent blindness. In fact, progressive vitamin A deficiency is the world's leading cause of blindness in children. Unaddressed deficiency can increase risk of child mortality, specifically from measles and diarrhea (Imdad 2011, West 2002, Scrimshaw 1997, WHO 2009).

Subclinical vitamin A deficiency is more difficult to identify, as overt clinical symptoms are not present, and serum retinol levels are homeostatically regulated until liver stores become significantly depleted. Therefore, common blood tests are not reliable for detecting subclinical deficiency. However, subclinical deficiency is still associated with impaired immunity, poor

growth, increased susceptibility to infectious disease, and mortality, and should be paid attention to (Russell 2000). Identifying ways to screen for subclinical vitamin A deficiency will allow for treatment prior to development of overt clinical symptoms.

In 2013, the World Health Organization estimated 190 million preschool aged children (6-59 months), and 19 million pregnant women were affected by vitamin A deficiency. The majority of the children impacted with vitamin A deficiency reside in Africa and Southeast Asia (2). From 1991 to 2013, the prevalence of vitamin A deficiency in low and middle income countries decreased from 39% to 29%, according to a pooled analysis from population-based surveys. This analysis showed decreases in prevalence in Latin America and the Caribbean, as well as East and Southeast Asia and Oceania regions. However, as of 2013, 95% of child and infant deaths attributable to vitamin A deficiency occurred in Sub-Saharan Africa and Southeast Asia, making these regions areas of focus for addressing vitamin A deficiency in children and infants (3). Vitamin A deficiency remains a significant public health problem.



Source: WHO Global Database on Vitamin A Deficiency 2009

Sub-Saharan Africa and Southeast Asia also face high burdens of infectious disease, including diarrhoea, which is a major contributor to childhood mortality (Lancet 2016). In these areas, vitamin A deficiency is exacerbated by infections. Infections can decrease appetite and dietary intake, while also decreasing absorption of vitamin A and other nutrients. While this further worsens vitamin A deficiency, insufficient vitamin A stores can worsen disease through decreasing immune function, and decreased production of structural proteins which uphold the mucosal layer in the digestive tract. In addition, inflammatory processes in infection make diagnosing vitamin A deficiency difficult, as serum retinol and retinol binding protein transiently decrease and can cause misclassification of status (Suri 2021).

Vitamin A Supplementation

Vitamin A deficiency is treated by supplementation with high-dose vitamin A in the form of retinyl esters (WHO 1997). This is considered a cost-effective method for reducing childhood morbidity and mortality. A 2017 Cochrane review investigated the impact of vitamin A supplementation for prevention of morbidity and mortality in children aged 6 months to 5 years. The meta-analysis found that Vitamin A supplementation showed a 12% reduction in the risk of all‑cause mortality for those supplemented with vitamin A compared with control. Of those trials that reported outcomes for mortality due to diarrhea, there was a 12% overall reduction with vitamin A supplementation. Lastly, the review found no significant effect of vitamin A supplementation on mortality due to measles, respiratory disease, or meningitis. Overall, this meta-analysis provides supporting evidence for vitamin A supplementation as an effective intervention to reduce risk of childhood mortality in low and middle income countries (Imdad 2017).

Widespread public health interventions for vitamin A supplementation are implemented in areas with a substantial prevalence of deficiency. WHO defines cut-off values for prevalence for identifying whether deficiency is of public health significance in an area. However, these prevalence estimates are based on serum retinol, which is not a direct indicator of vitamin A status until stores are depleted. This undermines the intention of using vitamin A supplementation programs as a preventative measure, as populations will only meet these thresholds once the problem is significant enough to present on serum retinol screening.

Cut-off values for public health significance

Indicator	Prevalence cut-off values for public health significance	
Serum or plasma retinol <0.70 µmol/L in preschool-age children	< 2%:	No public health problem
	2-9%:	Mild public health problem
	10-19%:	Moderate public health problem
	≥ 20%:	Severe public health problem
Night blindness (XN) in pregnant women	≥ 5%:	Moderate public health problem

Reference: WHO, 2009.

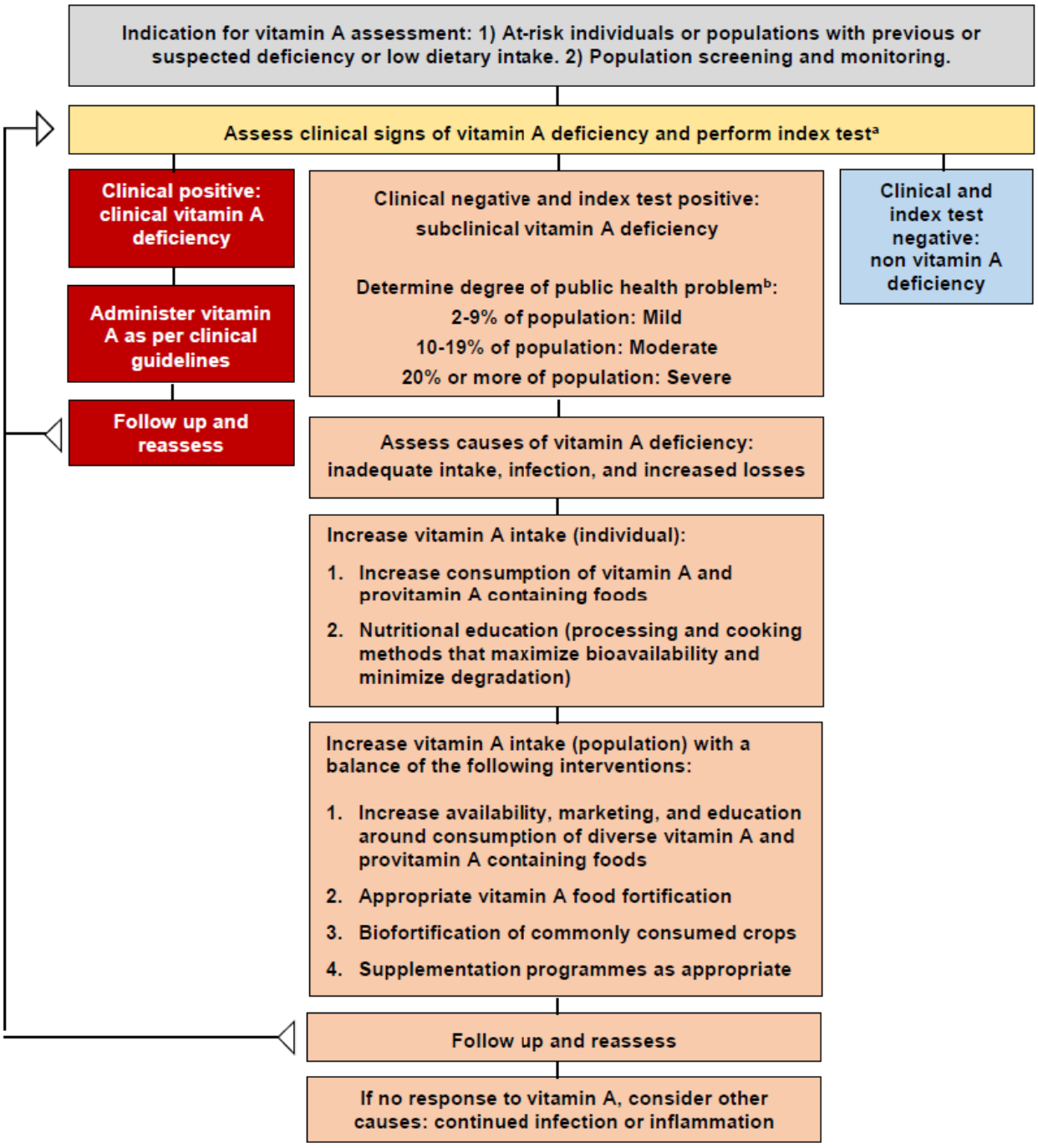
In countries that meet the criteria for public health significance of vitamin A deficiency, high-dose vitamin A supplementation is recommended for children 6 to 59 months of age every 4 to 6 months for the prevention of morbidity and mortality. (WHO 2011, Imdad 2017)

Clinical Pathway

In order to appropriately recommend interventions to treat vitamin A deficiency, we need accurate, non-invasive biomarkers. Vitamin A status can be assessed both clinically or biochemically, however, there is need for a biochemical test to identify subclinical deficiency before overt symptoms develop. In order to accurately identify population prevalence of both clinical and subclinical deficiency, a sensitive and specific test is needed. On the individual level, vitamin A status may be routinely assessed in individuals known to possess risk factors such as a

history of deficiency, malnutrition, inadequate dietary intake of vitamin A foods, or infections. If appropriate, this assessment can be applied as a standard of care screening method.

Clinical Pathway from Original Review Protocol:



[a]Adjusted for inflammation as appropriate
[b](WHO 1996, WHO 2011)

(Gannon 2020)

Index Tests for Vitamin A Status

Serum/Plasma Retinol

Serum or plasma retinol is an accurate indicator of vitamin A status when liver stores are depleted (Tanumihardjo 2016). However, if liver stores are not severely depleted, serum retinol is homeostatically regulated, and vitamin A depletion can not be detected by these measurements. This homeostatic regulation depends on binding proteins released from the liver, which decrease under inflammation (Solomons 2012). Because of this, serum retinol may appear falsely low in cases of infection, hypoproteinemia, and with oral contraception or hormone replacement (Stephensen 2001). Similarly, the inverse applies in cases of hyperproteinemia or dehydration, where serum retinol measurements appear falsely high.

Despite these limitations, the most common measurement of vitamin A status at the population level is serum retinol. The current WHO recommendation includes a cut-off for low vitamin A status of <0.70 μmol/L for subclinical vitamin A deficiency, and <0.35 μmol/L for severe vitamin A deficiency (clinical deficiency) (WHO 1996). Some studies have suggested that a serum cutoff of <0.35 umol/L has high diagnostic accuracy when used on the individual level. (Underwood 1990). However, the most commonly used cutoff is <0.70 umol/L. This cutoff is recommended by WHO for use at the population level to estimate public health significance of vitamin A deficiency in a given region. However, it is recommended that risk factors for vitamin A deficiency, or other biochemical and clinical indicators are assessed concurrently with serum retinol before implementing widespread intervention (WHO 2011).

Serum/ Plasma Retinol Binding Protein

Retinol binding protein (RBP) is responsible for transporting fat-soluble retinol in circulation, therefore may be considered another indicator of vitamin A status. It is secreted from the liver

bound to retinol in a 1:1 ratio. Like serum retinol, plasma/serum RBP is also homeostatically regulated (Underwood 1979). Furthermore, RBP is also reduced during infection and inflammation, decreasing it's diagnostic ability in these circumstances (Noy 2013). However, RBP is less sensitive to damage from light and temperature, and therefore has been considered a possible method for vitamin A status assessment (de Pee 2002).

RBP can be measured in serum, plasma, or dried blood spots using western blot, enzyme immunoassay or radioimmunoassay (de Pee 2002, Graham 2007). Holo-RBP is the form of RBP that is secreted from the liver bound to retinol, however, enzyme immunoassay kits cannot distinguish between holo or apo forms of RBP. To address this, it is recommended that a subsample undergoes an additional measure to distinguish between holo and apo-RBP, from which values can be calibrated to reflect the true RBP:retinol ratio. The RBP-retinol ratio is an important factor to consider when using RBP as a diagnostic tool for vitamin A status (Zabetian-Targhi 2015).

Relative Dose Response and Modified Relative Dose Response

The Relative Dose Response (RDR) and Modified Relative Dose Response (MRDR) tests measure vitamin A status by assessing the change in serum retinol following an oral dose of vitamin A. If liver vitamin A stores are low, apo-RBP accumulates in the liver until a dose of vitamin A is given and retinol is available. Then, following the vitamin A dose, this RBP is rapidly secreted as holo-RBP (Amedee-Manesme 1987). This assessment works by comparing a baseline serum retinol with a measurement 5 hours after the vitamin A dose. The RDR test .

The MRDR test is a modified version of the RDR test which utilizes an oral dose of 3,4-didehydroretinyl acetate (DRA) which is metabolized to 3,4-didehydroretinol (DR), also called vitamin A2. After 4-7 hours, a serum sample is collected and vitamin "A2" is quantified as well

as serum retinol. Vitamin A2 or didehydroretinol is not typically found in human circulation except in rare cases of people who consume large amounts of freshwater fish, therefore, we can distinguish between vitamin A that was given via oral supplement vs. endogenous. The ratio of DR to retinol is taken, and is considered to indicate inadequate VA stores when >0.06 (Tanumihardjo 1990).

Reference Standards for Vitamin A Status

Liver Biopsy

Liver biopsy is considered the gold standard for measurement of vitamin A status because an estimated >90% of total body vitamin A is stored in the liver (Moore 1957, Raica 1972). Serum retinol and retinol binding protein are homeostatically regulated and do not reflect changes in vitamin A stores until the liver is significantly depleted. Liver biopsy allows measurement of the density of vitamin A storage and therefore provides a true representation of vitamin A status of the individual. Vitamin A concentration is significantly higher in the right lobe of the liver compared to the left, therefore biopsy samples must be retrieved from the right side (McLaren 1979).

Despite its accuracy, liver biopsy is not a practical method for measurement of vitamin A status for the general population. Although it is an invasive procedure, some studies have looked at liver biopsy measurements under appropriate circumstances such as hepatic surgery, autopsy, or other operations. Liver vitamin A concentrations are reported to remain unchanged in autopsy specimens for long periods, even after partial tissue autolysis (Olson 1978). This review will include these studies and assess the sensitivity and specificity of index tests compared with liver

biopsy. In addition, we will assess the vitamin A concentration cut-offs of <0.07 umol/g and 0.1 umol/g (IOM 2001; Solomons 2012; Tanumihardjo 2016).

Retinol Isotope Dilution

Retinol isotope dilution methods aim to assess more than just liver stores of vitamin A, but total body vitamin A stores. It has been estimated that when stores are high, 90% of total body vitamin A is present in the liver (Moore 1957, Raica 1972). However, animal studies show that with low vitamin A stores, a significant amount of total body vitamin A is present in extrahepatic tissues (Green 1994). Therefore, retinol isotope dilution aims to address this limitation of liver biopsy measurement by assessing total body vitamin A.

Isotope dilution methods work by administering an oral dose of stable or radioactive tracer labeled vitamin A. The assumption is that vitamin A in the body is metabolically active and will mix with the tracer throughout all possible vitamin A pools in the body. The following equation, commonly known as the Olson Equation, is used to calculate total body vitamin A stores from an isotope dilution test (Furr et al 1989).

$$TLR = F * dose * (S * a * [(H : D) – 1])$$

"Where TLR= total liver vitamin A, F= fraction of oral isotopic dose that is absorbed and retained, S = ratio of specific activity of retinol in plasma to that in liver after equilibration; a = correction for fraction of dose lost via catabolism; H :D = post equilibration hydrogen-to-deuterium ratio in plasma retinol after administration of the oral dose of deuterated vitamin A; and – 1 = correction for the contribution of the dose to vitamin A stores" (Green

2014). The theory behind the Olson Equation is that administering a known dose of vitamin A tracer and calculating the specific activity of plasma vitamin A allows estimation of the mass of vitamin A present in body storage pools. This depends on the assumption that the specific activity of vitamin A in plasma is equal to that in other exchangeable vitamin A pools once the dose has mixed with these pools. Thus, by determining plasma vitamin A specific activity and knowing the amount of isotope administered, the mass of vitamin A in body storage pools can be estimated.

Rationale

Vitamin A deficiency is a widespread public health problem, specifically in South Asia and Sub-Saharan Africa. Children are at risk for vitamin A deficiency-related diseases such as permanent blindness and increased susceptibility to infectious disease, as well as death. In order to identify and treat subclinical vitamin a deficiency before overt clinical symptoms develop, we need sensitive and specific vitamin A biomarkers. These biomarkers must also be non-invasive, and easy to apply as a routine screening. Identifying the most efficient way to assess vitamin A deficiency on the population level as well as the individual level is key for streamlining treatment.

Biomarkers with low sensitivity make it difficult to allocate high-dose vitamin A supplementation to those who need it. Biomarkers with low specificity may overestimate the prevalence of deficiency, which can create issues when using the information to plan large public-health interventions. However, a WHO report concluded that adverse events related to vitamin A supplementation are not caused by public health programs, but typically from abuse or misuse of supplements (WHO 2011).

This review will focus on assessing the sensitivity and specificity of vitamin A biomarkers compared with reference standards in children aged 0-5 years, as they are the most at-risk population for vitamin A deficiency disease. Validating sensitivity and specificity of common vitamin A biomarkers in this population is a priority to address child morbidity and mortality due to vitamin A deficiency.

Objective

This review will assess the diagnostic test accuracy of each of these biomarkers for use in infants and children younger than 5 years of age, in comparison to reference standards (retinol isotope dilution and liver biopsy).

Secondary Objectives

This review will compare different cut-off thresholds for liver vitamin A stores (<0.07 ug/g and <0.1 ug/g liver) and their differing sensitivity and specificity. In addition, we will investigate sources of heterogeneity for the sensitivity and specificity of each index test.

Methods

Inclusion criteria

Types of studies

Studies measuring vitamin A status by at least one reference-standard (liver biopsy or retinol isotope dilution) AND at least one index test. Study designs may be cross-sectional or cohort studies, randomized controlled trials, longitudinal studies, direct, indirect and random comparison studies. Case-control studies will be excluded. For studies that only report data using

a reference standard, the authors will be contacted to determine whether an index test was performed concurrently.

Participants

For this specific review, only studies addressing children and infants under the age of 5 years will be included.

Index Tests

The following index tests will be compared to at least one reference standard:

1. Serum/plasma retinol
2. Serum/plasma retinol binding protein
3. Relative dose response
4. Modified relative dose response

Target Conditions

We will assess the ability of index tests to identify subclinical vitamin A deficiency as compared to the reference standard.

Reference Standards

Liver Biopsy

Liver biopsy is the most direct way to assess vitamin A status, as >90% of total body vitamin A is stored in the liver (Morton, 1957 ; Raica et al. 1972). While liver biopsy is an inappropriate method to measure vitamin A status on a population level, there is research done under appropriate circumstances such as hepatic surgery or autopsy. We will include studies that

measured liver vitamin A in addition to an index test. We will calculate the sensitivity and specificity separately based on liver vitamin A concentration cut-offs of <0.07 and <0.1 μmol/g (IOM 2001; Solomons 2012; Tanumihardjo 2016)

Isotope dilution

Retinol isotope dilution is considered an optimal way to estimate total body vitamin A stores. It has been estimated that when stores are high, 90% of total body vitamin A is present in the liver (Moore 1957, Raica 1972). However, animal studies show that with low vitamin A stores, a significant amount of total body vitamin A is present in extrahepatic tissues (Green 1994). Isotope dilution aims to more accurately assess total body vitamin A. However, due to the lengthy process (3-7 days), and potentially high costs, isotope dilution is not a realistic way to assess vitamin A status on the population level.

Search Methods

Electronic searches

We will use search keywords and subject headings including vitamin A and variations of retinoid compound names, index tests, and reference standards. Search strategy was translated for use in different databases and included with the review.

Databases searched

1. Cochrane Central Register of Controlled Trials, included in the Cochrane Library
2. MEDLINE (PubMed), from 1966
3. Embase (OVID), from 1974
4. Web of Science Core Collection (Clarivate Analytics), from 1900
5. CINAHL (EBSCO), from 1982
6. SCIELO (Clarivate Analytics), from 2002
7. BIOSIS (Clarivate Analytics), from 1926

8. Global Index Medicus (AIM, from 1980; IMEMR, from 1980; IMSEAR, from 1989;LILACS, from 1982; WPRIM, from 1953)
9. Native Health Research Database, from 1970

Searching other resources

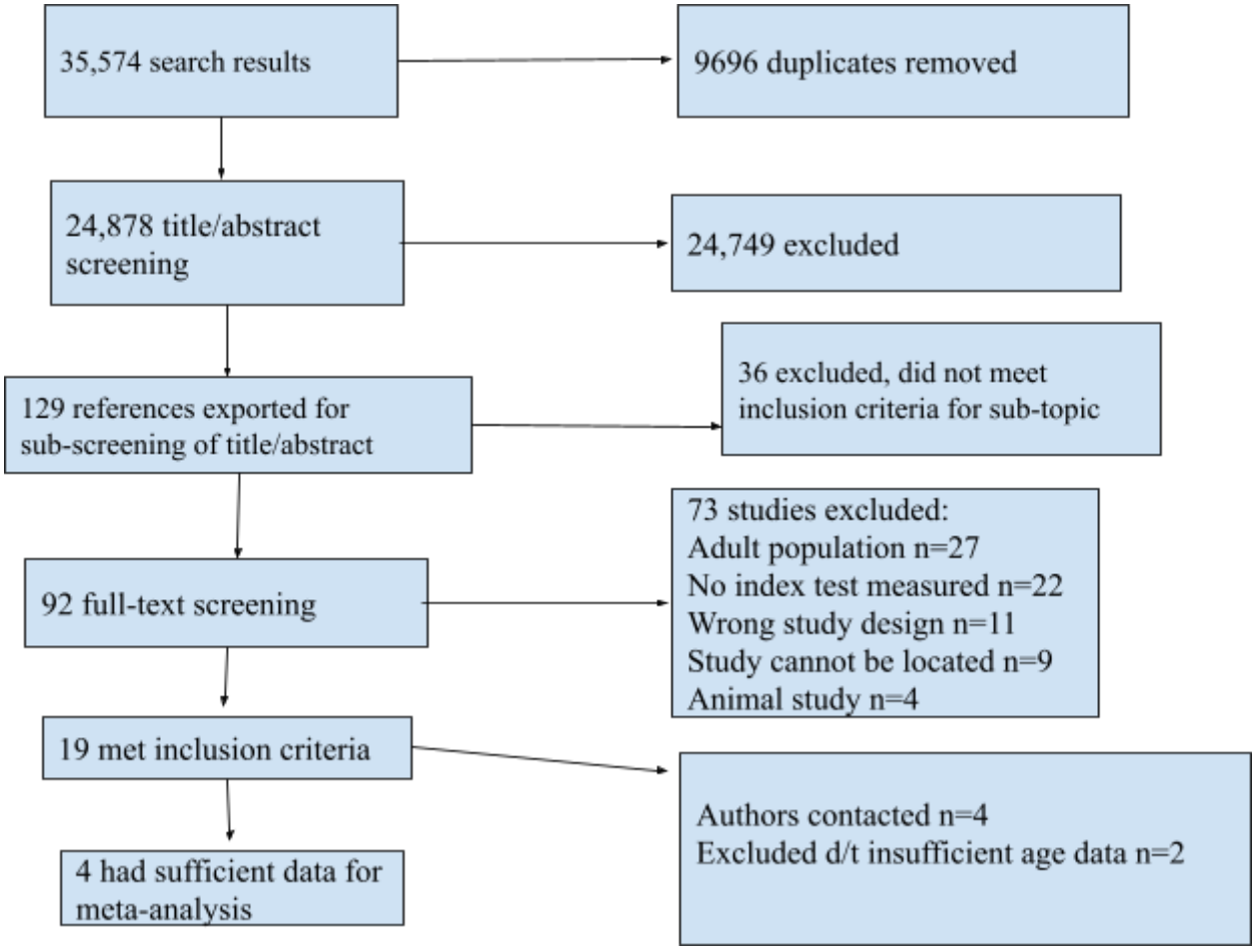
We will include conference proceedings and meeting reports. We will also access studies available from WHO regional offices, the nutrition section of the Centers for Disease Control and Prevention (CDC),the Micronutrient Initiative, National Institutes of Health (NIH), and International Life Sciences Institute Europe and collaborating centres.

Data Collection and Analysis

Selection of Studies

Search results were screened for inclusion and exclusion criteria in Covidence software. Each article was screened by at least one review author. For studies in which inclusion or exclusion criteria could not be determined from title/abstract screening, full text was obtained and reviewed. All full-text articles were reviewed for inclusion and exclusion criteria. For articles in which the full-text could not be found, Cornell Library was consulted for further assistance, and articles still unable to be located were excluded.

Figure 1: Study Flow Diagram



Data Extraction and Management

A data extraction form was drafted and reviewed by two other researchers. The data extraction template was created in Jotform, since the Covidence account will be utilized for the overall Cochrane review. Relevant information was extracted from each study, including number of true positives, false positives, false negatives, and true negatives for each available comparison between a reference standard and an index test. If not already calculated, sensitivity and specificity were calculated using these values. Additionally, positive and negative predictive values and likelihood ratios were extracted or calculated. If data in a paper was insufficient to calculate the necessary outcomes, the authors were contacted to obtain raw data.

Assessment of Methodological Quality

Methodological quality was assessed using QUADAS-II guidelines (Whiting 2011). The domains assessed were: "participant selection (method of participant selection and included participants), index test (description of test and how the test was conducted and interpreted), reference standard (description of reference standard and how it was conducted and interpreted), and flow and timing (any exclusion of participants from the study or analysis and intervals between index test(s) and reference standard assessment)".

For participant selection, signalling questions included:

- "Was a consecutive or random sample of patients enrolled?"
- "Was a case-control design avoided?"
- "Are there concerns that the included patients and setting do not match the review question?"

For index test and reference standard, the following signalling questions were used:

- "Were the index test results interpreted without knowledge of the results of the reference standard?"
- "If a threshold was used, was it pre-specified?"
- "Is the reference standards likely to correctly classify the target condition?"

Lastly, the following signalling questions were used to assess the flow and timing:

- "Was there an appropriate interval between index test and reference standard?"
- "Did all patients receive the same reference standard?"
- "Were all patients included in the analysis?"

Applicability concerns were assessed for patient characteristics and setting, index test, and reference standard. Both risk of bias and applicability were assessed with one form for each signalling question.

Statistical analysis and Data Synthesis

For studies measuring vitamin A by liver biopsy, a true positive case was defined as liver content <0.07 umol/g of liver. Sensitivity and specificity were also assessed with an alternative cutoff value of <0.1 umol/g of liver. Measurement via plasma retinol was considered a "true positive" or indicative of vitamin A deficiency, at levels <0.07 umol/L. For each included study, 2x2 tables were drawn and sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood ratios were calculated. Sensitivity and specificity of the serum vitamin A value was then calculated for each liver cutoff separately. These values were entered into Review Manager 5 (Revman 2020).

Four studies had sufficient information for meta-analysis. Forest plots were created for each study included in the meta-analysis. Additionally, sROC curves were created to assess differences in area-under-curve for the varying liver vitamin A cutoffs. Sources of heterogeneity were assessed if information was present to do so.

Investigations of Heterogeneity

The following sources of heterogeneity were assessed as applicable:

1. Age
2. Sex
3. Vitamin A status by biomarkers, clinical signs, vitamin A supplementation, or diet

4. Physiological condition: healthy, mixed, and ill by hospitalization status or clinical symptoms, or both, as defined by study authors
5. Inflammation and infection as defined by study authors as the prevalence of infection or inflammation by elevated C-reactive protein or alpha-1-acid glycoprotein
6. Study setting
7. Publication year
8. Study risk of bias or applicability
9. Thresholds used

Due to the constraints of only having 4 studies with sufficient data for meta-analysis, and restricting the population to children under 5 years of age, many of these sources of heterogeneity could not be investigated. None of the studies included in meta-analysis had reported inflammatory markers. One study stratified patients by physiological condition, and we explored intra-study heterogeneity between different types of protein-calorie malnutrition.

Sensitivity Analyses

The initial goals of this review included conducting a sensitivity analysis to assess the impact of including papers with high risk of bias, however, only four studies were eligible for inclusion in meta-analysis, two of which were rated low risk of bias, and two of which were rated unclear.

Results

Results of the search

We searched the literature available up to December 2020, which returned a total of 34,574 references. 9696 duplicates were removed, and 24,878 references were imported for title/abstract screening. After single-screening each reference, 129 studies were moved into a new covidence

account specific to this sub-population, and screened for eligibility criteria. Of these, 92 studies were assessed in full-text for eligibility. 19 studies met the inclusion criteria and were initially included in the review. 5 of these studies were excluded due to various reasons, mainly insufficient details regarding the age of participants. Some studies mentioned an age range that included the target population, however, the individual data necessary to construct a 2x2 table was not broken down by age. Finally, 14 studies were included for narrative review and meta analysis. Four studies had sufficient data to construct 2x2 tables and were included in the meta-analysis. Included studies are described in Figure 2, below.

Figure 2. Summary of Included Studies

First Author	Year of Publication	Country	Study Design	N	Age Range	Units	Disease status	Reference Standard	Index Test
Aklamati	2010	Zambia	RCT	8	3-4	years	Free from chronic disease, symptoms of malaria or diarrhea within the past 2 wk, and signs or symptoms of VA deficiency	Isotope Dilution	Serum/plasma retinol
Amédée-Manesme	1985	Europe, Iran	Cross-sectional	45	0-13	years	Liver disease (n=24), without liver disease (n=21), Wilson's disease n=3, hereditary fructose intolerance (n=2), hepatoblastoma n=3, portal vein obstruction (n=3)	Liver Biopsy	Serum/plasma retinol
Amedee-Manesme	1988	France	Cross-sectional	16	3-13	years	Liver disease	Liver Biopsy	Serum/plasma retinol
Amédée-Manesme	1985	France	Cross-sectional	79		"children"	Liver disease (n=71/79)	Liver Biopsy	Serum/plasma retinol
Fukui	1993	Japan	Cross-sectional	19	1-10	years	Biliary atresia	Liver Biopsy	Serum/plasma retinol, Relative dose response
Lopez-Teros	2013	Mexico	RCT	25	3-6	years	healthy (any conditions that could modify VA status were considered exclusion criteria)	Isotope Dilution	Serum/plasma retinol

Ong	1987		Cross-sectional	10	1.5-7	months	Biliary atresia with or without treatment	Liver Biopsy	Serum/plasma retinol
Tanumihardjo	1990	France	Cross sectional	3	3-5	years	Biliary atresia, Byler's disease, Tyrosinosis	Liver Biopsy	Serum/plasma retinol
Zaklama	1972		Cross-sectional	26	6-36	months	protein-calorie malnutrition	Liver Biopsy	Serum/plasma retinol

Methodological Quality of Included Studies

Below is a summary of the risk of bias ratings for each domain of the QUADAS-2 assessment for each study (Whiting 2011). As shown in Figure 3, most studies were rated as low or unclear risk of bias. The full breakdown and documentation of risk of bias ratings and rationale is available in Appendix 1.

Figure 3. Risk of Bias for Included Studies

Study	Patient Selection	Index Test	Reference Standard	Flow and Timing	Overall Rating
Aklamati 2010	high	low	low	unclear	unclear
Manesm 1985	unclear	low	low	unclear	unclear
Manesm 1988	low	low	low	unclear	low
Manesm and Therasse 1985	low	low	low	low	low
Fukui 1993	low	low	low	low	low
Ganguly 1988	low	low	low	low	Low
Lopez-Teros 2013	low	low	low	low	low
Ong 1987	low	unclear	unclear	low	unclear
Shenai 1985	low	low	low	unclear	low
Tanumihardjo 1990	high	low	low	unclear	unclear
Zaklama 1972	low	unclear	low	low	low

Figure 4. Applicability Concerns for Included Studies

Study	Patient Characteristics and Setting	Index Test	Reference Standard
Aklamati 2010	unclear	low	low
Manesm 1985	unclear	low	low
Manesm 1988	high	low	low
Manesme and Therasse 1985	unclear	low	low
Fukui 1993	low	low	low
Ganguly 1988	low	low	low
Lopez-Teros 2013	low	low	low
Ong 1987	low	low	low
Shenai 1985	low	low	low
Tanumihardjo 1990	low	Low	low
Zaklama 1972	low	Low	low

For the most part, there were low concerns around applicability for studies. Aklamati 2010 was downgraded for patient characteristics and setting because the population studied was 3-4 year old boys who were free from chronic disease, symptoms of malaria or diarrhoea within the past 2 wk, and signs or symptoms of VA deficiency. Because the target population for vitamin A diagnostic monitoring will include both boys and girls, often residing in areas where malaria and/or diarrhoea are endemic, the assessment of sensitivity and specificity of serum retinol in this population may not be consistent with what would be seen in the field.

Manesme 1985 was downgraded for applicability concerns due to the fact that the study provides an age range but does not specify individual ages of participants. In patients with severe

cholestasis, the age range was 1 month to 6 years, and in patients with liver disease but without cholestasis, the age range was 3 months to 13 years, both of which extend beyond the scope of this review. Ideally, each patient's age would have been available, and individual data would have been used accordingly to construct 2x2 tables. Manesme 1988 was rated "high" for applicability concerns under the patient characteristics and setting domain for the same reasons. There was a difference in rating between these two studies because the age range included for Manesme 1988 was ages 4-11, and therefore is less likely to encompass the target population of 0-5 years of age, compared to Manesme 1985 which specifically included patients with cholestasis "*early in life*". A full breakdown of RoB and applicability ratings can be found in Appendix 1.

Figure 5. Summary of Studies included in Meta-Analysis

Study	N	Disease status	Reference	Index Test	Sensitivity (%)	Specificity (%)	True +	True Negatives	False +	False -	Positive predictive value	Negative predictive value	+ Likelihood ratio	- Likelihood ratio
Amedee-Manesme 1988	16	Liver disease	Liver Biopsy	Serum retinol	100	77.7778	2	7	2	0	0.5	1	4.484304933	0
Tanumihardjo 1990	3	Biliary atresia, Byler's disease	Liver Biopsy	Serum retinol	0	100	0	3	0	0	0	0	0	0
Zaklama 1972	26	Protein-calorie malnutrition	Liver Biopsy	Serum retinol	70	25	7	4	12	3	0.37	0.57	0.93333	1.2
Ong 1987	10	Biliary atresia	Liver Biopsy	Serum retinol	20	100	1	6	0	4	1	0.6	-	0.8

Findings

Figure 6. Forest Plot for Serum Retinol Versus Liver Biopsy at Cut-off <0.07 umol/g of Liver

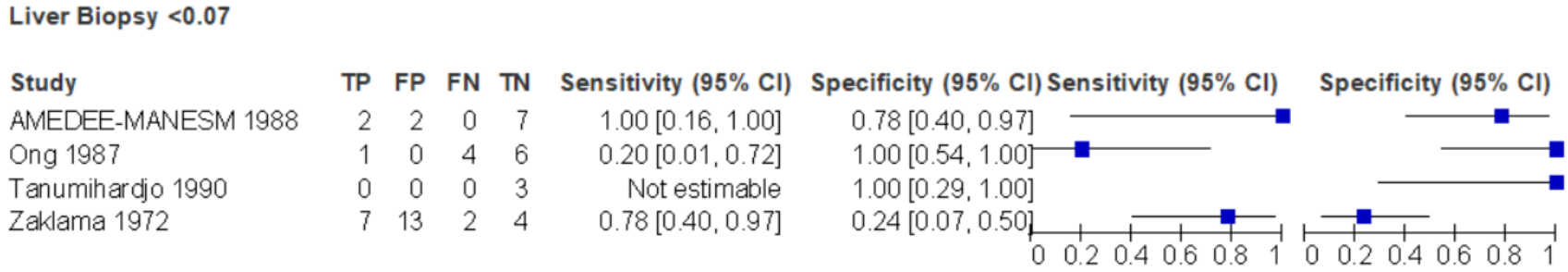
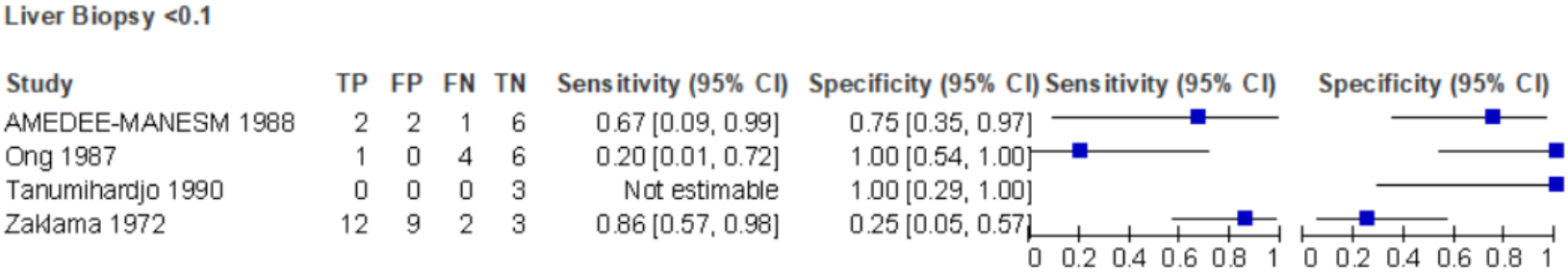


Figure 7. Forest Plot for Serum Retinol Versus Liver Biopsy at Cut-off <0.1 umol/g of Liver



Results Cont.

All four studies with sufficient data for meta-analysis compared the use of serum retinol and liver biopsy. A total of 55 participants were included in the analysis. At the <0.07 umol/g of liver cut-off value, there was significant heterogeneity in sensitivity between studies (Figure 6). The lowest sensitivity was observed in Ong 1987, which looked at 10 children with biliary atresia. This same study recorded 100% specificity, 60% negative predictive value, and 80% negative likelihood ratio. Heterogeneity was less apparent for specificity at the <0.07 umol/g cutoff, however, it is important to note that one of the included studies (Tanumihardjo 1990) exclusively reported true negatives, and therefore had 100% specificity.

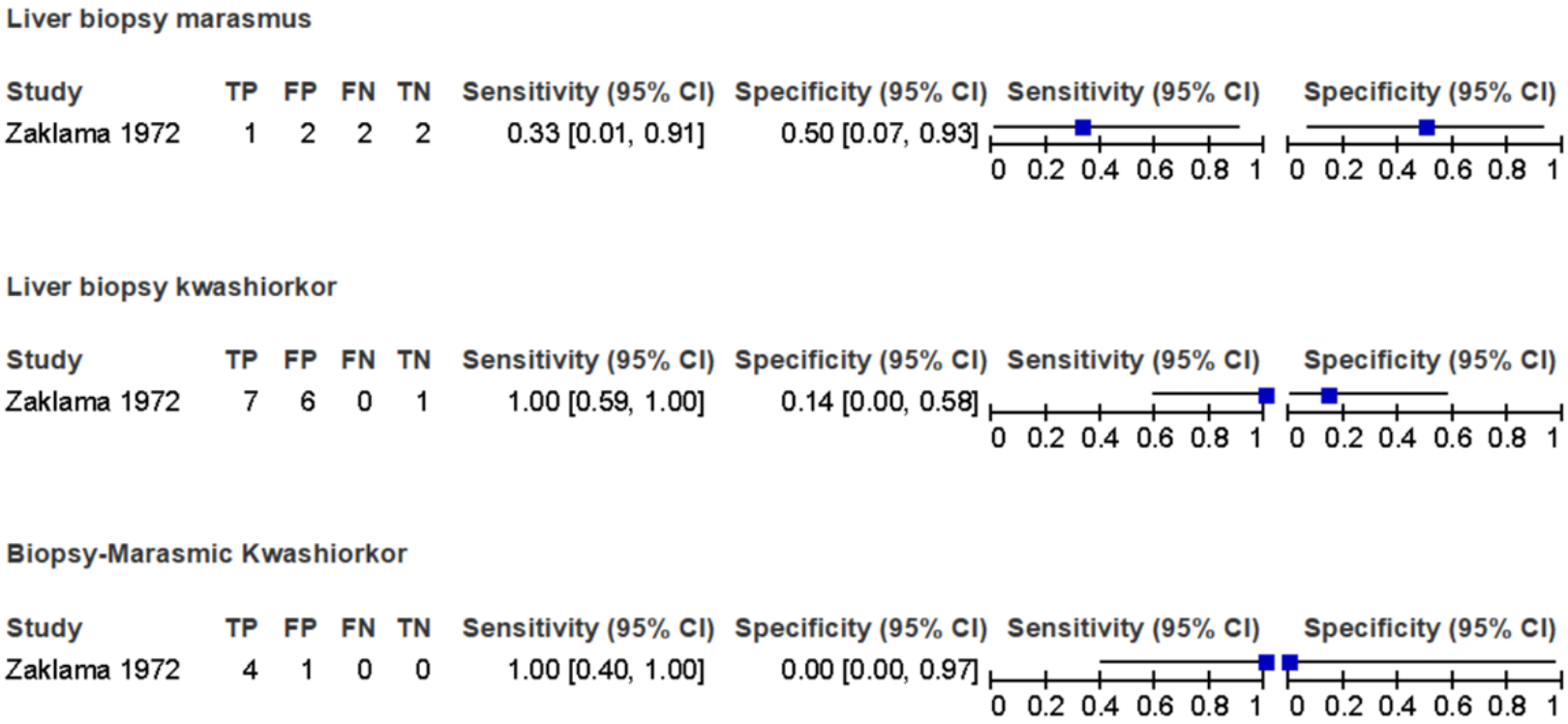
At the <0.1 umol/g liver cut-off, there was an 8% increase in sensitivity for the Zaklama 1972 study, and a 33% decrease for Amedee-Manesme 1988 (Figure 7). At the same time, this cutoff decreased specificity by 3% for Amedee-Manesme and increased specificity by 1% in Zaklama 1972. The most apparent difference between the two studies was the clinical condition of the population. Amedee-Manesme focused on children with liver disease, while Zaklama studied various types of protein-calorie malnutrition. The <0.1 cutoff shifted one participant in Amedee-Manesme from a true negative to a false negative, which created a significant change in specificity as there previously were no cases reported as false negatives. Therefore, these changes may not be as reflective of differences between these clinical populations, but also of the shifting of participants within each study.

Zaklama 1972 had the highest number of participants and reported a sensitivity of 78% and a specificity of 24% using the traditional liver cut-off of <0.07 umol/g. Positive predictive value was 37% and negative predictive value was 57%. Positive likelihood ratio was 0.9333, while the negative likelihood ratio was 1.2. This study included participants with protein-calorie

malnutrition of the following types: kwashiorkor, marasmus, and marasmic kwashiorkor. To investigate any differences in diagnostic accuracy between these conditions, separate 2x2 tables were constructed for each condition, and a forest plot was created (Figure 7).

Overall, sample sizes of the included studies were low, which created imprecision with sensitivity and specificity estimates. Confidence intervals were very wide, some having 90% differences between upper and lower ends. This makes it difficult to conclude that the observed sensitivity and specificity would remain when applied to a larger population. The ends of the confidence intervals for the sensitivity and specificity values in figures 6 and 7 encompass values that would create drastic conclusions for public health utilization of these biomarkers. For example, if the true sensitivity of serum retinol as compared to the liver cutoff value of <0.07 umol/g was 0.09 as included in the confidence interval for Amedee-Manesme 1988, serum retinol would be wildly inappropriate as a method for determining population prevalence of vitamin A insufficiency.

Figure 7. Forest Plot of Serum Retinol Versus Liver Biopsy in Various States of Protein-Calorie Malnutrition. Data from Zaklama 1972.



As reported in Figure 7, a forest plot breakdown of sensitivity and specificity by clinical condition from Zaklama 1972, there was 100% sensitivity in the kwashiorkor and marasmic-kwashiorkor groups, while the marasmus group's sensitivity was only 33%. For specificity, the marasmus group was the most efficient with 50% sensitivity, while the kwashiorkor and marasmic kwashiorkor subgroups had very low sensitivity (14% and 0, respectively). However, the kwashiorkor and marasmic kwashiorkor groups both had nearly zero true and false negative cases. Figure 10 depicts an ROC curve for the sensitivity and specificity of serum retinol as compared to liver biopsy at the <0.07 umol/g cutoff for these varying types of protein-calorie malnutrition. The marasmic-kwashiorkor condition seems to have the greatest AUC upon visual analysis, however, this assessment is inappropriate due to the small number of children included in the category.

Figure 8. Summary ROC Curve for Serum Retinol Compared to Liver Biopsy @ <0.07 umol/g liver cutoff

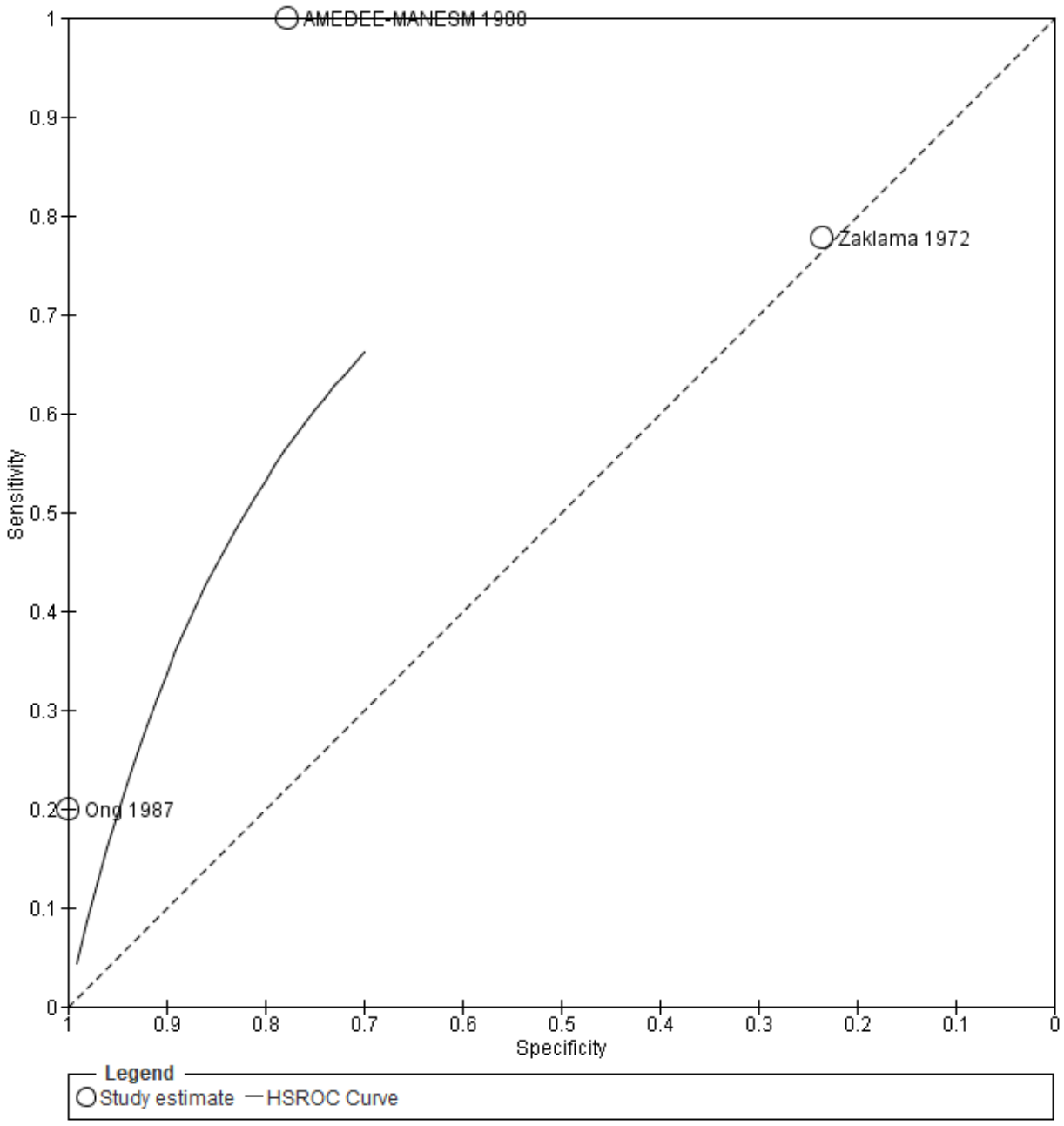


Figure 9. Summary ROC for Serum Retinol vs. Liver Biopsy at <0.1 umol/.g liver cutoff

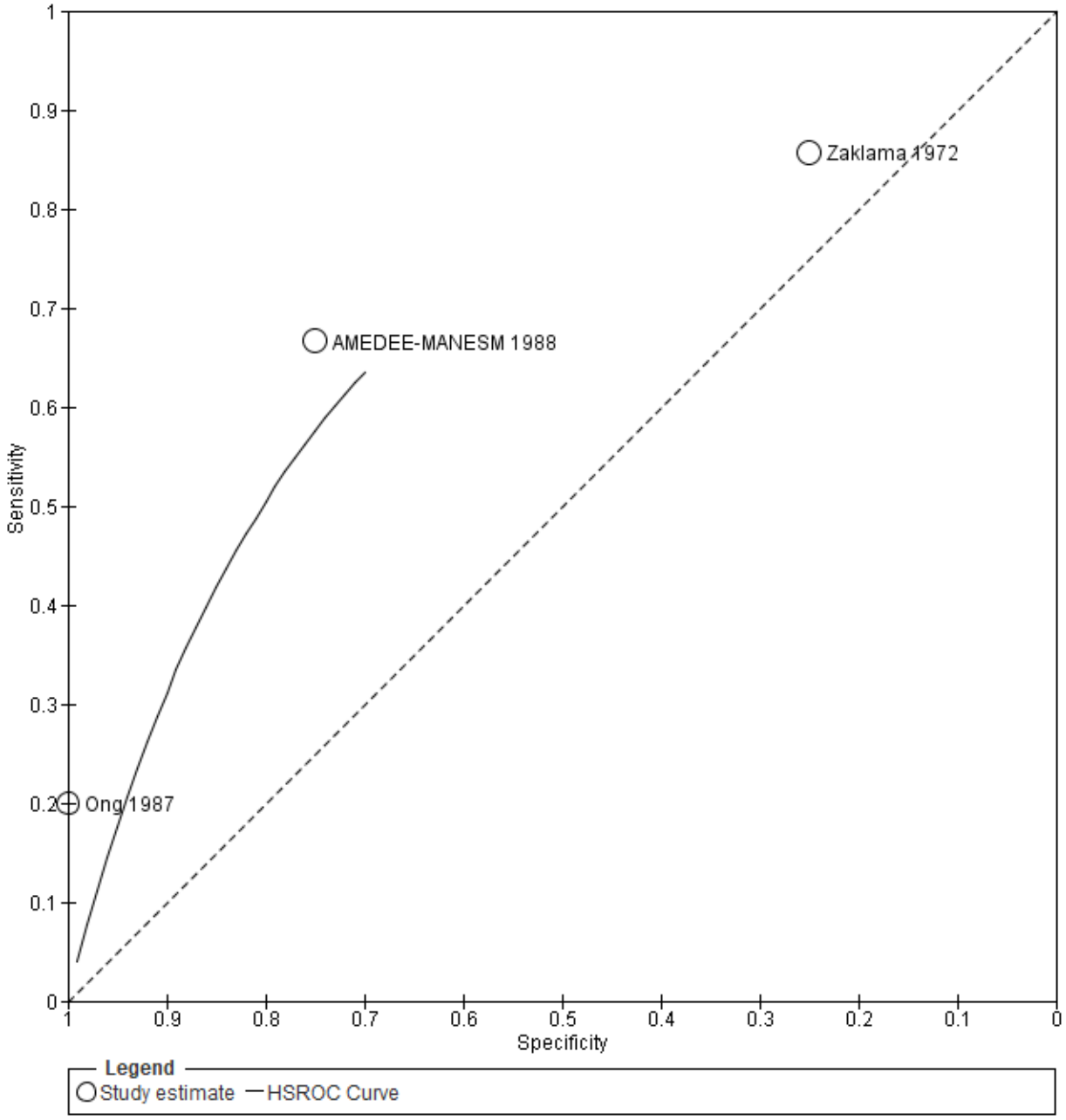
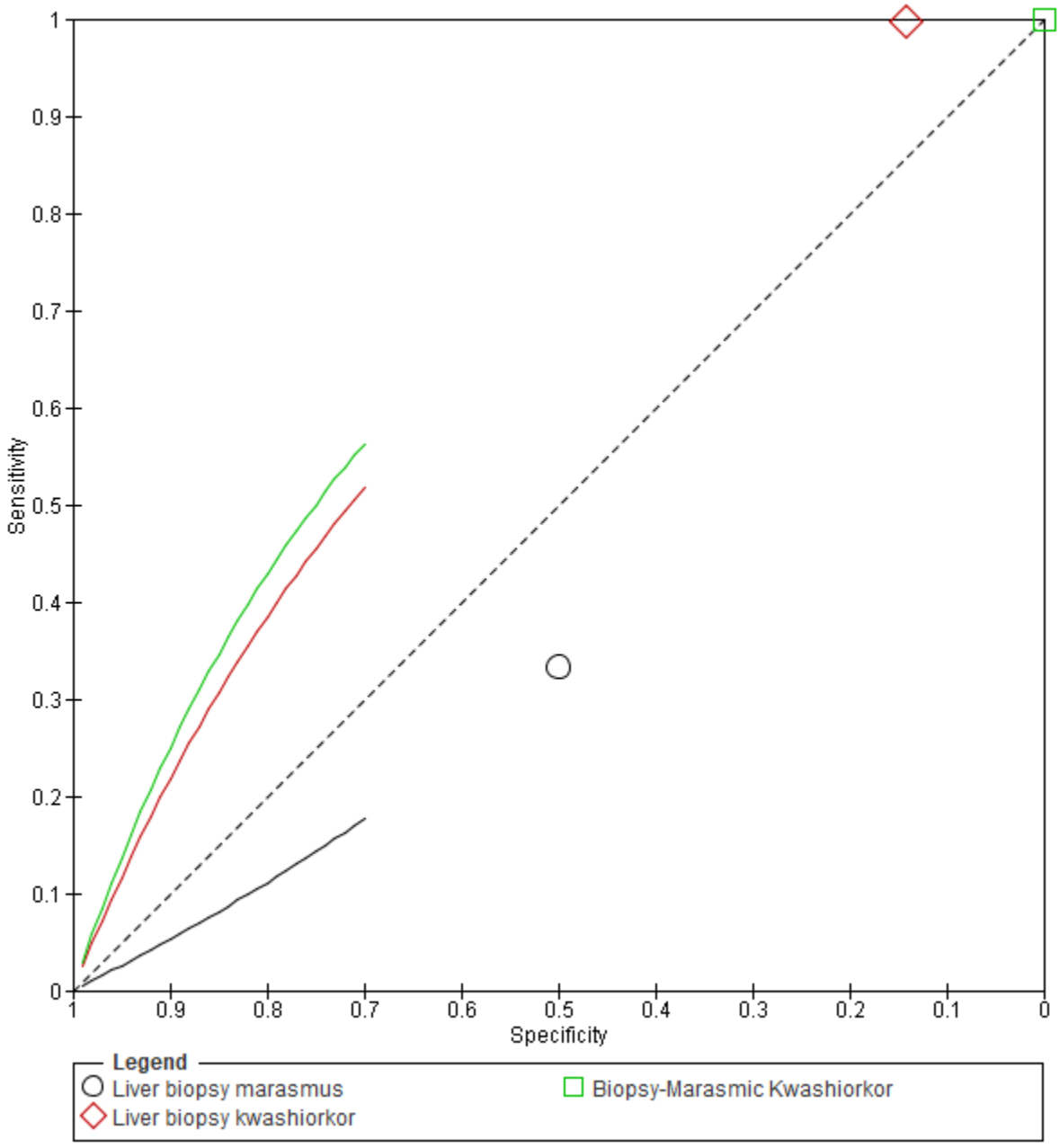


Figure 10. Summary ROC for Serum Retinol vs Liver Biopsy at Varying States of Protein-Calorie Malnutrition (Zaklama 1972)



Narrative Review of Other Included Studies

Aklamati 2010

A national survey conducted in Zambia found that 54% of children aged 6 months to 5 years were marginally deficient in vitamin A as defined by the serum retinol cutoff of <0.07 umol/L. This occurred despite the administration of a high-dose vitamin A supplement via the national vitamin A supplementation program, which reaches an estimated 80% of children in the qualifying age range (Clewes 2003). This study aimed to determine the reasons for the lack of effect of the vitamin A supplementation program. 20 boys aged 3-4 years old were enrolled, and were randomized to one of two groups--one received a tracer dose of retinyl acetate with either a high-dose VA supplement (intended to mimic supplementation program) or with a stable-isotope labeled VA intended to assess total body vitamin A stores. The vitamin A supplement group received 209.8 mmol VA in corn oil, and no high-fat foods were given concurrently with it to ensure comparability with the national supplementation program. Additionally, dietary intake was assessed via food frequency questionnaire, and plasma retinol was measured via HPLC. CRP and AGP were measured to assess inflammation.

This study was included in the review because it measured both serum retinol and used isotope dilution methods to estimate total body vitamin A stores, while also measuring inflammatory markers. Unfortunately, the data was not reported for each individual in a format easily extracted to a 2x2 table, and the authors were not responsive to email. However, the authors reported that plasma retinol levels were not associated with absorption, retention, or urinary elimination of vitamin A. Although plasma retinol levels were not associated with vitamin A tracer absorption or retention, none of the children included had serum retinol levels less than the traditional cutoff for marginal VA deficiency, which is our focus population.

Amedee-Manesme et al 1985

This study surveyed vitamin A status of 79 children, 71 of whom had liver disease, focusing on liver concentrations as a gold standard biomarker. The authors measured both serum levels of retinol as well as liver retinol, and retinyl esters. Similar to other studies in the review, this paper did not report each individual's measurements of vitamin A separately, but reported averages based on each subgroup of clinical condition. The authors were not responsive to email, and therefore we could not construct a 2x2 table. However, the authors did report the association between liver and plasma vitamin A level, as seen in the chart below.

Chart from Amedee-Manesme et al 1985

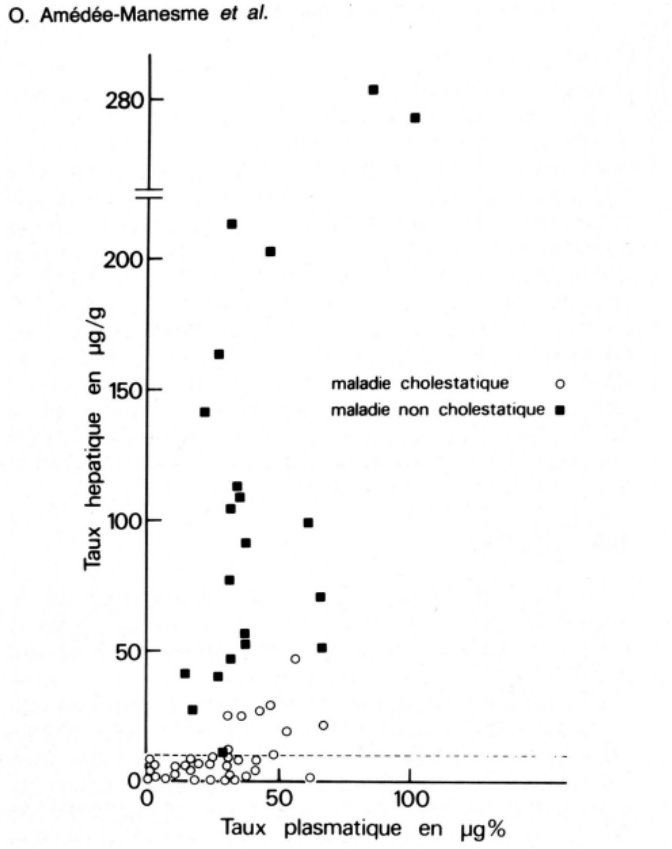


FIG. 2. — *Relation entre taux plasmatique en rétinol et taux hépatique en vitamine A.* La ligne brisée correspond à un taux hépatique de 10 µg de vitamine A par g de foie, limite inférieure au-dessous de laquelle on ne trouve que des patients cholestatiques.

Upon visual inspection, as expected, those without liver disease tend to have higher liver vitamin A stores at roughly the same plasma values. Those with liver disease cluster mostly under the 10 ug/g vitamin A plot line, with plasma levels mostly less than 50 ug%. These findings are consistent with the accepted knowledge that serum retinol does not fluctuate significantly unless liver stores are significantly depleted.

Fukui 1993

This study assessed vitamin A status of 19 children with resolved biliary atresia who had been receiving 5000 IU of vitamin A per day. Vitamin A status was measured via plasma, liver samples, and oral vitamin A tolerance tests. Children aged 1-10 years were included. Children were separated into groups based on level of serum bilirubin at time of examination. Below, you can see bar charts of average serum and liver vitamin A concentrations by increasing levels of total bilirubin.

Plasma and Liver Vitamin A Levels by Serum Total Bilirubin (Fukui 1993)

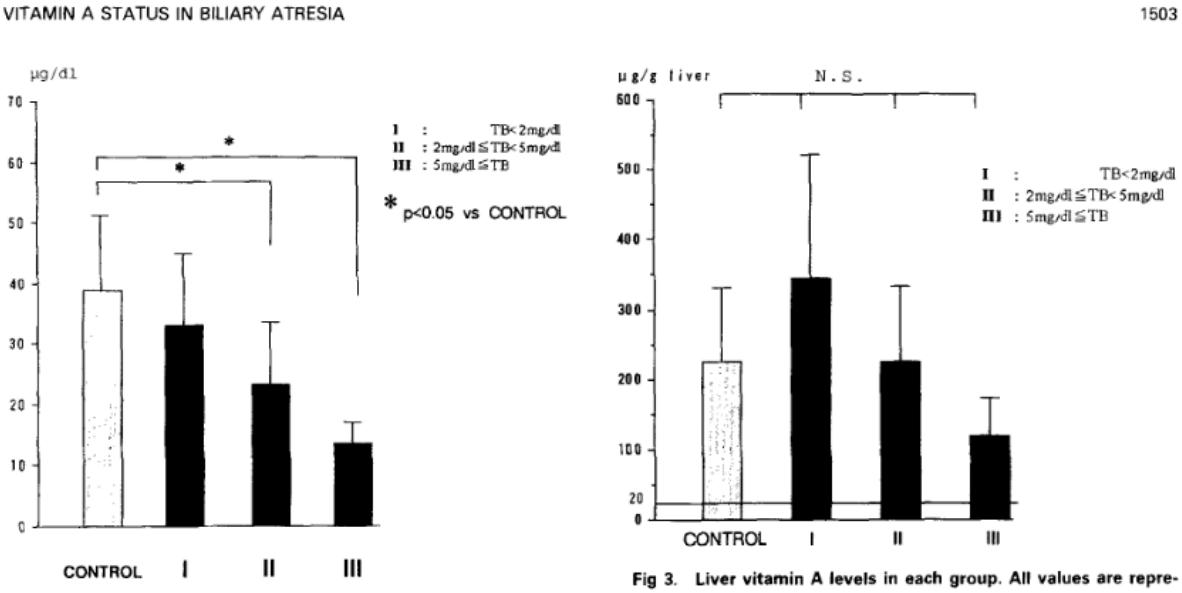


Fig 1. Plasma vitamin A levels in each group. All values are represented as mean ± SD.

Fig 3. Liver vitamin A levels in each group. All values are represented as mean ± SD.

Although individual values were not reported and did not allow for construction of 2x2 tables and exclusion of participants out of the age range, we can see based on these graphs interesting differences between control (healthy) children and those with varying levels of jaundice based on bilirubin levels. For plasma, there was a significant difference between healthy children and groups II and III (higher levels of jaundice). Interestingly, the control group has the highest plasma vitamin A, but this is not the case with liver stores. This may be due to the difference in recent vitamin A supplementation between healthy children and those who had operations for biliary atresia and are currently supplementing daily. We still see a stepwise decrease in vitamin A storage by level of jaundice, displaying some consistency between trends for the two different biomarkers.

Lopez-Teros 2013

This was a randomized controlled trial assessing the efficacy of vitamin A fortified milk for increasing vitamin A stores in moderately deficient preschoolers in Mexico. In assessment of the effectiveness of the fortified milk, the researchers measured total body vitamin A via retinol isotope dilution, as well as serum retinol, which qualified the study for inclusion in this review. Similar to previous studies, this paper did not report individual measurements of serum retinol and total body vitamin A as the research question was not sensitivity and specificity. However, this paper serves as an example that retinol isotope dilution may be a more efficient method for assessing the effectiveness of interventions aiming to change vitamin A status as it captures total vitamin A stores rather than transient changes in serum concentration. The population studied was considered moderately deficient in VA as confirmed by serum retinol concentration 0.35–0.7 mmol/L, which is likely consistent with children targeted by vitamin A supplementation programs in areas of public health concern.

Discussion

Summary of Main Results

This systematic review of the diagnostic accuracy of vitamin A biomarkers in children aged 0-5 years summarizes the current literature and provides a meta-analysis of four applicable studies. Four studies assessed the diagnostic accuracy of serum retinol (<0.07 umol/L) as compared to liver biopsy. None of the studies' intentions were to assess diagnostic accuracy, but each measured and reported the necessary index test and reference standard of interest to this review. These four studies had a small sample size, encompassing a total of 55 participants altogether. Due to the small number of studies and sample size within them, sensitivity and specificity were calculated with large confidence intervals. We have low confidence in the sensitivity and specificity reported in the forest plots in figures 6, 7, 8. In addition to large confidence intervals, significant heterogeneity was present between studies.

Strengths and weaknesses of the review

The main weakness of this review is the small number of studies with disaggregated data, greatly limiting the meta-analysis. Only four studies had sufficient data to construct 2x2 tables, which limited the ability to conduct the intended heterogeneity analyses. This also created large confidence intervals for sensitivity and specificity, since the individual studies also had small sample sizes. In some situations, the 95% confidence interval for sensitivity was (0.09, 0.99), which effectively includes almost the entire range of possible sensitivity values. In some studies, there were no true positives OR true negatives, making sensitivity and specificity impossible to calculate or biased.

One strength of this review was the extensive literature search, which was conducted with a strategy previously outlined in a Cochrane review protocol (Gannon 2020). Additionally, the risk of bias and applicability concerns of included studies were relatively low, with only three total domains being rated “high”.

Applicability of the findings to review question

The studies included in this review were all applicable to the review question such that they included the correct age groups, however, the majority of studies included in this review consisted of children with biliary atresia or liver disease, or healthy children. The objective of this review was to identify and assess non-invasive indicators of subclinical vitamin A deficiency, which is prevalent in low and middle income countries with high prevalence of diarrhea and infectious disease. Applying these tests to children with severe liver disease or who are healthy may not provide the assessment we need to apply these tests in the field. However, it is expected that studies obtaining liver biopsy samples from children are only conducted concurrently with a necessary procedure, leading to the overflow of liver disease population in this review.

Author’s Conclusions

Implications for practice

Due to the small number of studies addressing the research question, the small sample size of those included in meta-analysis, and the large confidence intervals for those included, no significant implications for practice were produced in this review. More research is needed in

this area to confirm the accuracy of serum biomarkers for vitamin A status assessment in children, or to calibrate serum retinol cutoffs for subclinical deficiency.

Implications for research

As exhibited in this review, research addressing the sensitivity and specificity of non-invasive, affordable, vitamin A biomarkers is lacking. Many of the studies included in this paper were conducted before 2000. While it may be difficult to ethically study this research question in children, it is still important to assess the diagnostic accuracy of vitamin A biomarkers in children under age 5. In the future, researchers should focus on assessing diagnostic accuracy of serum and plasma retinol, retinol binding protein, and RDR/MRDR as compared with isotope dilution. This will allow for ethical approval for research in this age group, more accurate assessment of total body vitamin A stores, and for assessment of children with other clinical conditions beyond liver disease.

Although isotope dilution equations themselves are based on limited research, we can hope to triangulate vitamin A status measurements through further diagnostic test accuracy research--both comparing liver biopsy AND isotope dilution with serum and plasma biomarkers. Isotope dilution provides the benefit of being non-invasive, however, it does require more than one measurement of participants, which may create logistic challenges both in research and in public health settings. Extensive research is needed both to assess and confirm the accuracy of current isotope dilution equations, as well as to compare its measurement accuracy with that of other vitamin A biomarkers.

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Appendix

Aklamati 2010

Patient Selection

A. Risk of Bias	
Patient Sampling	Enrolled 3-4 year olds who had not received a VA supplement in the past 2 months. In addition, this study sample consisted of only boys d/t ease of urine collection. However, a case-control sample was avoided by selecting children who were free from chronic disease, symptoms of malaria or diarrhoea within the past 2 wk, and signs or symptoms of VA deficiency.
Was a consecutive or random sample of patients enrolled?	No
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	High risk

B. Concerns regarding applicability

Patient characteristics and setting	This study only address boys who were free from chronic disease, signs and symptoms of malaria or diarrhoea, and free from symptoms and signs of VA deficiency. This could threaten the applicability of this study to the research question as the highest prevalence of VAD is in Sub-Saharan Africa and Southeast Asia, where malaria and diarrhoea are prevalent.
Are there concerns that the included patients and setting do not match the review question?	Unclear concern

Index Test

Index tests	Serum Retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Isotope dilution for the diagnosis of vitamin A deficiency
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	"As part of the larger study on the longer term impact of a HD-VA supplement on VA pool size, blood samples were collected before and 45 d after administration of the HD-VA supplement or placebo (corn oil) for measurement of the hemoglobin concentration and plasma concentrations of retinol, CRP, and AGP."
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	

AMEDEE-MANESM 1985

Patient Selection

A. Risk of Bias	
Patient Sampling	"Sixteen children presenting with Liver disease at the pediatric department at Bicetre Hospital (Paris, France) were the study subjects."
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Unclear
Could the selection of patients have introduced bias?	Unclear risk

B. Concerns regarding applicability	
Patient characteristics and setting	There is concern because we don't know which patients were under age 5, and the age ranges were well above 5 years, as high as 13.
Are there concerns that the included patients and setting do not match the review question?	High concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes
If a threshold was used, was it pre-specified?	Yes
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Liver biopsy for Vitamin A deficiency

Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	Does not specify whether retinol measurements were taken at the same time as the liver biopsy, however, the liver samples were obtained during surgery or when needed for diagnosis or patient management. Due to the clinical setting of this study, it is unlikely that blood samples would be drawn at a time innapropriate for comparison to liver biopsy.

Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	No
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	

AMEDEE-MANESM 1988

Patient Selection

A. Risk of Bias	
Patient Sampling	Not all patients enrolled were used in meta-analysis d/t not meeting age criteria. All patients had some sort of disease, several of which cholestasis
Was a consecutive or random sample of patients enrolled?	Unclear

Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	Paper gives age range of 4-11 but doesn't specify mean age or which patients are what age. There is certainly questions of applicability because of this.
Are there concerns that the included patients and setting do not match the review question?	High concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes
If a threshold was used, was it pre-specified?	Yes

Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias		
Target condition and reference standard(s)	Liver Biopsy for Vitamin A deficiency	
Is the reference standards likely to correctly classify the target condition?		Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?		Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk

B. Concerns regarding applicability

Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	It is unclear whether biopsy and serum were taken at the same time, however it is likely that they were due to liver biopsy collection being performed in the inpatient setting when patients were in for surgery or procedures.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	This study provided likely concurrent measurements of liver biopsy and serum retinol in children, however it is unclear which children should have been excluded from meta-analysis due to age restriction.

Amedee-Manesm and Therasse 1985

Patient Selection

A. Risk of Bias	
Patient Sampling	79 children were studied, 71 of which had liver disease. There is no further documentation specifically about patient sampling methods.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability

Patient characteristics and setting	This study looked at children, most of which had liver disease. The only concern may be that our target population may not have high prevalence of liver disease.
Are there concerns that the included patients and setting do not match the review question?	Unclear concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability

Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias		
Target condition and reference standard(s)	Vitamin A deficiency as detected by liver biopsy	
Is the reference standards likely to correctly classify the target condition?		Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?		Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	Liver vitamin A levels were obtained either during surgery or needle biopsy, and it is likely that plasma samples were taken during the same visit due to the clinical nature of the study setting.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Low risk

Notes

Notes	

Fukui 1993

Patient Selection

A. Risk of Bias

Patient Sampling	19 patients with biliary atresia were selected, but it is not specified whether selection was randomized or consecutive.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	The only concern is that the age range included in this study was 1-10 years, while the research question is addressing 0-5 years.
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias		
Target condition and reference standard(s)	Vitamin A deficiency detected by liver biopsy	
Is the reference standards likely to correctly classify the target condition?		Yes

Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	Plasma retinol was taken at baseline, 4 hours and 6 hours following an oral vitamin A tolerance test.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes

Could the patient flow have introduced bias?	Low risk

Notes

Notes	

Ganguly 1988

Patient Selection

A. Risk of Bias	
Patient Sampling	Subjects were selected from the Medical Termination of Pregnancy Clinics in Calcutta. "The authors had no role in the selection of cases which was carried out by the Professor of Obstetrics."
Was a consecutive or random sample of patients enrolled?	No
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability

Patient characteristics and setting	Aborted fetuses were used, which fits the target population of children under the age of 5.
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Serum retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Liver vitamin A as a measure of vitamin A deficiency
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias

Flow and timing	"The fetuses were received on ice at the operation theatre and dissected within half an hour after their removal from the uterus. Fetal blood was drawn by cardiac puncture."
Was there an appropriate interval between index test and reference standard?	Yes
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Low risk

Notes

Notes	

Lopez-Teros 2013

Patient Selection

A. Risk of Bias	
Patient Sampling	Preschool children were screened for eligibility in low socioeconomic areas of northwest Mexico, and parental consent was obtained from those who were deemed eligible.

Was a consecutive or random sample of patients enrolled?	No
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	"The inclusion criteria were mild to moderate VAD (moderate VAD serum retinol concentrations, 0.35–0.7 mmol/L and mild VAD, 0.7– 1.05 mmol/L) (1,26,27) and absence of subclinical inflammation (28). The presence of anemia (hemoglobin <110 g/L for children 0.5–4.99 y and <115 g/L for children 5–11.99 y) (29), malnutrition (any Z-score <22 SD), signs and symptoms of xerophthalmia, and any clinical or dietary condition that could compromise VA metabolism were considered exclusion criteria"
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Serum retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Vitamin A status by retinol isotope dilution

Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	"TBVA stores were determined using the deuterated retinol isotope dilution technique (see below) before and after the intervention period in both groups." Flow and timing does not pose an issue when comparing the two baseline measurements of serum retinol and TBVA by isotope dilution.

Was there an appropriate interval between index test and reference standard?	Yes
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Low risk

Notes

Notes	

Ong 1987

Patient Selection

A. Risk of Bias	
Patient Sampling	Notes that study used patients who were scheduled for gastroplasty or cholecystectomy, doesn't note randomization. 5 patients had VA treatment while the other 6 did not.
Was a consecutive or random sample of patients enrolled?	Unclear

Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	11 children with biliary atresia and no signs of vitamin A deficiency clinically
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	No

Could the conduct or interpretation of the index test have introduced bias?	Unclear risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias		
Target condition and reference standard(s)	Liver biopsy for subclinical vitamin A deficiency	
Is the reference standards likely to correctly classify the target condition?		Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?		Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?		Unclear risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	"Liver biopsies of sufficient size to allow both proper histopathological and biochemical studies were obtained for diagnosis during surgery. Concomitantly, blood (1 ml) was collected in the presence of sodium EDTA."
Was there an appropriate interval between index test and reference standard?	Yes
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	Low risk

Notes

Notes	

Saha 1988

Patient Selection

A. Risk of Bias	
Patient Sampling	Patients were enrolled that were undergoing autopsy after either an accident or coronary heart disese.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Unclear
Could the selection of patients have introduced bias?	Unclear risk

B. Concerns regarding applicability	
Patient characteristics and setting	There were 32 subjects studied between the ages of 0-19, but we their individual ages and measurements are not reported, limiting the applicability of this study to the review.
Are there concerns that the included patients and setting do not match the review question?	High concern

Index Test

Index tests	None reported

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	
If a threshold was used, was it pre-specified?	
Could the conduct or interpretation of the index test have introduced bias?	

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	High concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Vitamin A status as defined by liver vitamin A content (liver biopsy)

Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Yes
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	Since we don't know if any index measurements were taken, we cannot conclude anything about the flow and timing.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes

Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	

Shenai 1985

Patient Selection

A. Risk of Bias	
Patient Sampling	Infants were selected that died within 24 hours from birth. 18 infants were born within the hospital, and 7 were transferred from other hospitals within 24h of birth.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Serum vitamin A, serum RBP

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability

Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Liver biopsy for VAD
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias

Flow and timing	Blood and liver sample were both obtained during autopsy. Only 16 of the infants had blood samples taken. It is unclear whether only obtaining blood samples from 16 of 25 infants poses a risk of bias as we don't know if the infants that were excluded were in some way different from those who had blood samples.
Was there an appropriate interval between index test and reference standard?	Yes
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	

Tanumihardjo 1990

Patient Selection

A. Risk of Bias

Patient Sampling	Surgical liver biopsy samples from 7 diseased and 5 healthy subjects (aged 3-33--had to exclude those over age 5 for this paper). For the overall study, a case-control design was not avoided because they enrolled 5 healthy subjects, however, this doesn't necessarily present an issue because the sub-sample used for this paper does not contain the same ratio of healthy/diseased subjects. Additionally, the goal of the study was not necessarily to assess sensitivity and specificity, therefore the risk of bias may be low.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	No
Could the selection of patients have introduced bias?	High risk

B. Concerns regarding applicability	
Patient characteristics and setting	Only 3 of the subjects fit our inclusion criteria

Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Plasma retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	No
Could the conduct or interpretation of the index test have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias	
Target condition and reference standard(s)	Subclinical vitamin A deficiency as seen by liver biopsy
Is the reference standards likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?	Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias

Flow and timing	Does not specify when plasma retinol measurements were taken, but it is likely due to the inpatient setting that they were taken concurrently.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	Unclear risk

Notes

Notes	

Zaklama 1972

Patient Selection

A. Risk of Bias	
Patient Sampling	26 children suffering from protein-calorie malnutrition

Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Could the selection of patients have introduced bias?	Low risk

B. Concerns regarding applicability	
Patient characteristics and setting	Children with protein-calorie malnutrition--very applicable
Are there concerns that the included patients and setting do not match the review question?	Low concern

Index Test

Index tests	Serum retinol

All tests

A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear

If a threshold was used, was it pre-specified?	Unclear
Could the conduct or interpretation of the index test have introduced bias?	Unclear risk

B. Concerns regarding applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern

Reference Standard

A. Risk of Bias		
Target condition and reference standard(s)	Liver biopsy for subclinical vitamin A deficiency	
Is the reference standards likely to correctly classify the target condition?		Yes
Were the reference standard results interpreted without knowledge of the results of the index tests?		Unclear
Could the reference standard, its conduct, or its interpretation have introduced bias?		Low risk

B. Concerns regarding applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern

Flow and Timing

A. Risk of Bias	
Flow and timing	Blood sample was collected upon admission, liver biopsy samples were collected 2-4 days after admission
Was there an appropriate interval between index test and reference standard?	Yes
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Low risk

Notes