Chapter 3

Prediction of vitamin D deficiency by simple patient characteristics

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ABSTRACT

Background

Vitamin D status is currently diagnosed by measuring serum 25-hydroxyvitamin D (25(OH)D).

Objective

This study aimed to develop a risk profile that can be used to easily identify older individuals at high risk for vitamin D deficiency.

Design

This study was performed within the Longitudinal Aging Study Amsterdam, an ongoing cohort study of a representative sample of the Dutch older population (N = 1509 for the development sample and N = 1100 for the validation sample). Prediction models for serum 25(OH)D concentrations <50nmol/l and <30nmol/l were developed using backwards logistic regression. Risk scores were calculated by dividing the individual regression coefficients by the regression coefficient with the lowest B in order to create simple scores.

Results

Serum 25(OH)D concentrations <50 nmol/l and <30 nmol/l was present in 46.2% and 17.5%, respectively. The model for the prediction of concentrations <50 nmol/l consisted of 13 easily assessable predictors, whereas the model for concentrations <30 nmol/l contained ten predictors. The resulting areas under the curve were 0.78 and 0.80, respectively. The AUC in the external validation dataset was 0.71 for the <50 nmol/l model. At a cut-off point of 58 in total risk score (range: 8-97), the model predicted <50 nmol/l with a sensitivity of 61% and specificity of 82%, whereas these values were 61% and 84%, respectively, at a cut-off point of 110 in the total risk score (range: 6-204) in the model for <30 nmol/l.

Conclusion

Two total risk scores, including thirteen or ten predictors that can easily be assessed, were developed and are able to predict serum 25(OH)D below 50 nmol/l and 30 nmol/l accurately. These risk scores may be useful in clinical practice to identify persons at risk for vitamin D deficiency.

INTRODUCTION

Vitamin D deficiency is common in older individuals [1]. Depending on the used definition of deficiency, age, gender, lifestyle, season, and used method for determination, the percentage of individuals with deficiency ranges from 50 to 90% [1;2]. Vitamin D is essential for bone health; older individuals with vitamin D deficiency have a higher risk of falls and fractures [3]. In addition, vitamin D has been proposed to play a role in the development of many other disorders, such as cancer, diabetes, auto-immune disease and infections, and poorer cognitive function [4-7]. However, the effects on most outcomes remain to be proven by randomized controlled trials.

The Institute of Medicine (IOM) advises extra supplements in all older individuals to obtain the recommended serum 25-hydroxyvitamin D (25(OH)D) concentration of 30-50 nmol/l [8]. Recent advice from the Dutch Health Council also states that all men and women > 70 years of age should take a supplement of 20 µg vitamin D/d and all women aged > 50 years are advised to take 10 µg vitamin D/d to obtain the recommended 25(OH)D concentration of 50 nmol/l [9]. However, only about 50% of the individuals aged ≥65 years in the Netherlands has vitamin D deficiency (<50 nmol/l) according to the results of the Longitudinal Aging Study Amsterdam (LASA) [10] and those of the Hamlet study [11]. This suggests that 50% of the people aged ≥65 years take vitamin D supplements that may not be necessary.

The number of laboratory requests for the determination of serum 25(OH)D is increasing as a consequence of the proposed influence of vitamin D on many organs and diseases [12]. This contributes to an increase in healthcare costs [13]. It would be useful to have a model to predict vitamin D deficiency reliably and therefore identify individuals who could benefit from vitamin D supplementation without the need to determine 25(OH)D status.

Recently, a model for the prediction of vitamin D deficiency in Australian individuals was published [14]. However, an adequate model in European individuals does not exist. To decrease the costs of laboratory tests and the number of people who unnecessarily use vitamin D supplements, this study aimed to develop 2 prediction models for determining serum 25(OH)D <50 nmol/l as well as concentrations <30 nmol/l. To make these models easy to use in daily practice, only easily assessable predictors were included.

SUBJECTS AND METHODS

Study sample

Data for this study were derived from LASA. LASA is an ongoing cohort study of a representative sample of the Dutch older population. The sampling and data collection procedures have been described elsewhere in detail [15;16]. Briefly, a random sample of men and women, stratified by age, sex and expected five-year mortality rate, was drawn from population registers from eleven municipalities in the Netherlands. At baseline (1992/1993), 3107 subjects aged 55-85 years were interviewed. In 2002 a second cohort, consisting of 1002 individuals of 55-65 years, was recruited. After the 2002 measurement cycle, both cohorts were combined in subsequent measurement cycles. The study was approved by the Medical Ethics Committee of the VU University Medical Center and all participants gave informed consent.

For the present study, the second measurement cycle of the first cohort (1995/1996) was used. In the first cohort, persons who participated in the medical interview in addition to the main interview in 1995/1996 and were born in or before 1930 (aged 65 years and older as of January 1, 1996), were selected (N = 1509).

For the external validation of the model, we used the 2008/2009 measurement cycle of the first and second cohort combined (ages 61-95 years). Individuals of the first cohort, who were already in the sample for the development of our models, were excluded. For the validation sample, we included the participants of the medical interview (N = 1100).

Serum 25(OH)D

Morning blood samples were drawn in 1995/1996 and in 2008/2009. In 1995/1996, the participants were allowed to consume tea and toast but no dairy products, whereas participants in 2008/2009 fasted from midnight until after the morning blood collection. The samples were centrifuged and stored at -20˚C until determination in 1997/1998 or 2010/2011, respectively. A competitive binding protein assay was used in 1997/1998 (Nichols Diagnostics, San Juan Capistrano, CA, USA) and a radioimmunoassay in 2010/2011 (Diasorin, Stillwater, MN, USA). The inter-assay coefficients of variation were < 10% for both methods. Both methods were compared by measuring 117 samples (41 LASA participants and 76 samples measured for clinical purposes) with both methods. These analyses showed that levels of 25, 50, and 75 nmol/l measured with the Nichols assay equaled 31.1, 54.2, and 77.2 nmol/l, respectively, when measured with the Diasorin assay.

The correlation coefficient was r = 0.94. For this study, original values were used. All analyses were performed in the Endocrine Laboratory of the VU University Medical Center.

Potential predictors

The following potential predictors were included in the analyses: age, sex, Body Mass Index (BMI, in kg/m^2), smoking, alcohol use, level of education, season, vitamin use, physical activity (bicycling, walking, sports, gardening), degree of urbanization, medication use, self-rated-health, specific depressive symptoms, anxiety, functional limitations, partner status, pain during walking, memory complaints, and some specific items from the Mini Mental State Examination (MMSE). Predictors that were included in the final models (see table 2) are described in more detail below (detailed information on all potential predictors is available in a supplemental file at the end of this chapter).

Age and sex were derived from population registries. BMI was calculated as measured weight in kilograms divided by measured height in square meters. Self-reported smoking was divided into 2 categories: current smoker (including participants who stopped smoking within 5 years ago) and non-smoker. Alcohol use was assessed by self-report. Season was categorised into four categories, according to the meteorological seasons in the Netherlands (winter: December-February; spring: March-May; summer: June-August; and autumn: September-November). Over-the-counter vitamin use was based on self-report (yes or no). These multivitamin tablets typically contain 200-400 IU of vitamin D. Physical activity was assessed using the LASA Physical Activity Questionnaire, a validated interviewer-administered questionnaire [17]. For this study, only the first question on every domain (eg, “Do you walk outdoors?”) was used. For gardening, if participants did not have a garden, they were classified as “no gardening”. Medication use was assessed by asking the participants to show their medication containers to the interviewers; a dichotomisation was made (no medication use or the use of one or more medicines). Participants’ inability to use their own or public transportation was assessed by asking a question on the ability to perform this activity. Partner status was divided into two categories: having a partner or no partner. The Mini-Mental State Examination assessed whether a participant knew the current year [18]. The presence of appetite was assessed by using a question from the Center for Epidemiologic Studies-Depression Scale questionnaire (CES-D) [19].

Statistical analyses

Cutoffs for vitamin D concentrations were determined at serum 25(OH)D concentrations < 50 nmol/l and < 30 nmol/l, according to the guidelines from the IOM and those from the

Dutch Health Council [8;9]. The IOM states that concentrations < 30 nmol/l increase the risk of deficiency with regard to bone health and that concentrations between 30 and 50 nmol/l indicate an increased risk for inadequacy for some, but not all individuals [8]. The Dutch Health Council has set the required serum 25(OH)D concentrations at 30 nmol/l for adults and at 50 nmol/l for women aged >50 and men > 70 years [9].

All continuous variables were tested for linearity by using spline-curves and spline regression models [20]. When linearity was not present, the variable was categorised or dichotomised mainly according to known cut-off values derived from the literature. To determine the optimal cutoffs of continuous variables that needed to be categorized, different known cutoffs were tested and the cutoff with the highest predictive value was selected. Age was used as a continuous variable; all ages were centered at 65 years. BMI was dichotomised into non-obese (<30 kg/m^2) and obese (≥30 kg/m^2). Alcohol use was dichotomised as ≤12 or >12 drinks/week.

The univariable ORs of the observed data were calculated using logistic regression analyses. Multiple Imputation of missing values was performed for participants of the medical interview (n=1509). Multiple Imputations were generated by using the Multivariate Imputation by Chained Equations (MICE) procedure [21]. With this regression-based method, missing values are estimated using an imputation model including information from other complete variables in the data set. We created 27 different imputed data sets according to the percentage of missing cases in the multivariable models [22].

To select predictors that could be used in the model to identify individuals at risk for vitamin D deficiency, we used a backwards selection procedure within the logistic regression analyses and taking into account the imputed data sets [23]. The selection criterion was *P* <0.157, Akaike's Information Criterion [24]. First, all potential predictors were included in the model; second, the predictor with the highest *P*-value was excluded from the model. This was done until all remaining predictors had a P value lower than the predefined selection criterion. The probability of being vitamin D deficient was calculated by using the following formula:

$$P_{deficient} = \frac{e^{(\beta 0 + \beta 1 \cdot x1 + \beta 2 \cdot x2 + \ldots + \beta n \cdot xn)}}{1 + e^{(\beta 0 + \beta 1 \cdot x1 + \beta 2 \cdot x2 + \ldots + \beta n \cdot xn)}}$$

Where P is the probability of being vitamin D deficient, β_0 is the constant and β_1, β_2 and β_n represent the regression coefficient of the predictors x_1, x_2 and x_n after they were pooled using Rubin's rules. By using these predicted probabilities, a receiver operator characteristic (ROC) curve was made. The goodness-of-fit of the model was tested by the Hosmer-Lemeshow test [25]. Internal validation of the model was determined by using

bootstrapping techniques, which provide information about the performance of the model in comparable patient datasets. On the basis of this information, regression coefficients and performance measures were adjusted [26].

The regression coefficients of the predictors in the pooled model were divided by the regression coefficient of the predictor with the lowest B to calculate individual risk scores. These individual scores can be summed to calculate the total risk score. The sensitivity, specificity, positive and negative predictive value of the total risk score were calculated. Specificity is defined as the proportion of the individuals who are not deficient and who are correctly classified as non-deficient. Sensitivity is the proportion of individuals who are deficient and who are correctly classified as deficient.

External validation was performed by calculating the AUC in the validation sample by using the internally validated regression coefficients. Missing values in the external validation sample were imputed using the same procedure as was performed in the development sample. The analyses were performed by using SPSS for Windows version 20 (SPSS, Inc) and R, version 2.15.0 (R Project for Statistical Computing).

RESULTS

Among the 1509 participants in the medical interview, 1106 participants had complete data on all potential predictors. Mean (±SD) serum 25(OH)D was 53.2 (±24.0) nmol/l. Serum 25(OH)D<50 nmol/l was present in 46.2% of the participants and 25(OH)D<30 nmol/l was present in 17.5% of the participants. The prevalence and the univariable ORs for serum 25(OH)D concentrations <50 and <30 nmol/l for the observed non-imputed data (n=1106) are presented in Table 1.

By using a backwards selection procedure, the final prediction model for serum 25(OH)D <50 nmol/l consisted of the following variables: older age, sex (female), BMI (>30kg/m^2), smoking, alcohol consumption (<13 consumptions/week), season, no vitamin supplement use, no bicycling, no sporting, no gardening, medication use, poor appetite, and without a partner (Table 2). The probability of vitamin D deficiency for a 65-year-old participant ranged from 2% when none of the predictors was present to 85% when all predictors were present. For an 80-year-old participant, the respective probabilities of being vitamin D deficient ranged from 6 % to 94%. The Hosmer-Lemeshow goodness-of-fit test for the multiple logistic regression was not significant, which indicates that the model fit the data well. After internal validation, the resulting AUC was 0.78. Nagelkerke's R^2 was 0.31. When the model was validated in the external dataset, the resulting AUC was 0.71.

Table 1. Baseline characteristics and univariable ORs for low serum 25(OH)D status (observed data)

	N	Percen-tage	Odds Ratio[1] <50 nmol/L (95% CI)	*P*-value	Odds Ratio[1] < 30 nmol/L (95% CI)	*P*-value
Age (years (SD))	1509	76.0 (6.7)[2]	1.1 (1.1, 1.1)	<0.001	1.1 (1.1, 1.2)	<0.001
Gender (% women)	1509	51.8	2.0 (1.6, 2.5)	<0.001	1.7 (1.3, 2.2)	0.001
BMI (% > 30 kg/m^2)	1481	20.4	1.7 (1.3, 2.2)	<0.001	1.3 (0.9, 1.8)	0.137
Smoking (%yes)	1495	23.8	1.1 (0.9, 1.5)	0.311	1.2 (0.9, 1.7)	0.196
Alcohol use (%no)	1506	80.2	1.9 (1.4, 2.5)	<0.001	3.0 (1.9, 4.8)	<0.001
Level of Education	1507					
Low		62.0	1.0 (0.7, 1.5)	0.858	0.9 (0.6, 1.4)	0.569
Middle		26.0	0.7 (0.5, 1.0)	0.057	0.5 (0.3, 0.9)	0.017
High		12.0	1.0		1.0	
Season of blood	1328					
Winter		29.0	2.3 (1.7, 3.1)	<0.001	2.2 (1.5, 3.4)	<0.001
Spring		22.4	2.7 (1.9, 3.7)	<0.001	1.6 (1.0, 2.4)	0.056
Summer		23.0	1.0		1.0	
Autumn		25.6	1.2 (0.9, 1.6)	0.270	1.0 (0.6, 1.6)	0.953
Vitamin use (% no)		78.2	1.4 (1.1, 1.8)	0.009	1.6 (1.1, 2.5)	0.008
Physical activity						
- bicycling (% no)	1460	46.0	3.6 (2.8, 4.5)	<0.001	4.4 (3.2, 6.1)	<0.001
- walking outdoors (%	1460	16.1	1.8 (1.3, 2.5)	<0.001	1.7 (1.2, 2.5)	0.006
- sporting (% no)	1460	63.1	2.1 (1.6, 2.6)	<0.001	1.9 (1.4, 2.6)	<0.001
- gardening (% no)	1458	59.5	3.0 (2.4, 3.7)	<0.001	4.1 (2.8, 5.9)	<0.001
Urbanisation (% city)	1508	57.6	1.4 (1.1, 1.7)	0.006	1.5 (1.1, 1.9)	0.013
Medication (% ≥ 1)	1509	76.1	1.9 (1.5, 2.4)	<0.001	2.2 (1.5, 3.2)	<0.001
Self-rated Health (% poor health)	1508	39.1	1.6 (1.3, 2.0)	<0.001	1.8 (1.4, 2.4)	<0.001
Depressive symptoms						
- appetite (% no)	1462	14.1	2.2 (1.6, 3.0)	<0.001	2.1 (1.4, 3.0)	<0.001
- depressive mood (%	1458	22.2	1.6 (1.2, 2.1)	<0.001	1.5 (1.1, 2.1)	0.014
Anxiety (% yes)	1460	10.0	1.6 (1.1, 2.2)	0.018	1.2 (0.8, 2.0)	0.345
Functional limitations						
- walking outdoors (%	1504	21.3	2.5 (1.9, 3.4)	<0.001	2.5 (1.8, 3.4)	<0.001
- transportation (% no)	1505	22.7	3.3 (2.4, 4.4)	<0.001	3.8 (2.8, 5.2)	<0.001
- walking stairs (% no)	1493	35.7	2.7 (2.1, 3.4)	<0.001	3.0 (2.3, 4.0)	<0.001
Partner status (% no partner)	1509	42.9	2.7 (2.2, 3.4)	<0.001	2.5 (1.9, 3.3)	<0.001
Pain: walking (% yes)	1297	27.5	1.7 (1.3, 2.3)	<0.001	1.7 (1.2, 2.4)	0.001
Memory						
- year (% no)	1507	6.2	1.6 (0.9, 2.6)	0.091	2.6 (1.5, 4.5)	0.001
- date (% no)	1497	25.7	1.0 (0.8, 1.3)	0.830	1.2 (0.8, 1.6)	0.373
- memory complaints (% yes)	1507	26.7	1.1 (0.8, 1.4)	0.521	1.2 (0.9, 1.6)	0.212

[1]Odds Ratios were calculated by using logistic regression analysis, [2]mean (SD).

The final model for the prediction of vitamin D concentrations < 30 nmol/l consisted of ten predictors: older age, smoking, alcohol consumption (<13 consumptions/week), season, no vitamin supplement use, no bicycling, no gardening, medication use, limitations in the use of own or public transportation, and the inability to tell the present year. After internal validation, the resulting AUC was 0.80, Nagelkerke's R^2 was 0.28. The probability of vitamin D deficiency for a 65-year old individual ranged from 0.2% to 45% according to the number of predictors. For an 80-year old individual, these respective probabilities were 0.8% to 72%. The Hosmer-Lemeshow goodness-of-fit test was not significant in the majority of the imputed data sets. External validation in our validation sample was not possible; only 3.8% of the participants had vitamin D concentrations <30 nmol/l.

All regression coefficients were transformed into simple scores (see Table 2) to enable general practitioners or other health care professionals to easily use the models and to predict the risk of being vitamin D deficient. The total score of the model for <50 nmol/l ranged from 8 to 97, whereas the score ranged from 6 to 204 for the <30 nmol/l model. The probability of having serum 25(OH)D concentration <50 nmol/l per each 10-points increase in the total risk score and the prevalence of these scores in our sample are shown in Figure 1. A participant with a score between 71 and 80 has 87% chance of being vitamin D deficient (<50 nmol/l), whereas a person with a score between 11 and 20 has a 3% chance.

The diagnostic and predictive values of both developed models, according to different cut-offs (per 10 or 20 points) in the total risk score, are shown in Tables 3 and 4. In both models, the lower the cutoff, the lower the specificity and the higher was the sensitivity. The higher the cut-off, the higher the specificity and the lower the sensitivity.

Table 2. Risk profiles for low serum 25(OH)D status in older adults (65 years and older).

Predictors	Model < 50 nmol/L, N = 1509			Model < 30 nmol/L, N = 1509		
	B, after internal validation	Odds ratio (95% CI)	Score	B, after internal validation	Odds ratio (95% CI)	Score
Constant	-3.899			-6.032		
Age (years)	0.071	1.1 (1.1, 1.1)	1[1]	0.077	1.1 (1.1, 1.1)	2[1]
Gender: female	0.441	1.6 (1.1, 2.2)	6	-	-	-
BMI: >30 kg/m^2	0.261	1.3 (0.9, 1.8)	4	-	-	-
Smoking: yes	0.345	1.6 (1.1, 2.2)	5	0.494	1.7 (1.1, 2.5)	13
Alcohol use: <13 consumptions/week	0.400	1.6 (1.1, 2.3)	6	1.037	3.0 (1.8, 5.0)	28
Season						
Winter	0.820	2.2 (1.5, 3.3)	12	0.761	2.2 (1.4, 3.5)	21
Spring	1.092	3.1 (2.1, 4.7)	15	0.471	1.6 (1.0, 2.7)	13
Summer	-	-	0	-	-	0
Autumn	0.201	1.1 (0.8, 1.7)	3	0.037	1.0 (0.6, 1.7)	1
Vitamin use: no	0.616	1.8 (1.3, 2.5)	9	0.729	2.2 (1.4, 3.3)	20
Bicycling: no	0.583	1.8 (1.3, 2.4)	8	0.728	2.1 (1.5, 3.2)	20
Sporting: no	0.387	1.5 (1.1, 2.0)	5	-	-	-
Gardening: no	0.541	1.7 (1.3, 2.3)	8	0.787	2.3 (1.5, 3.5)	21
Medication use: yes	0.272	1.3 (1.0, 1.8)	4	0.324	1.4 (0.9, 2.2)	9
Presence of appetite: no	0.305	1.5 (1.0, 2.3)	4	-	-	-
Limitations in transport use: yes	-	-	-	0.425	1.6 (1.1, 2.3)	12
Partner status: no	0.380	1.4 (1.0, 1.9)	5	-	-	-
Remembering year: no	-	-	-	0.546	1.8 (0.9, 3.4)	15

Odds Ratios were calculated using multiple logistic regression analysis.
The score is the regression coefficient divided by the lowest regression coefficient, which is the coefficient of age for <50 nmol/L and summer for <30 nmol/L, and rounded to the nearest integer. A higher score indicates a higher risk for vitamin D deficiency.
[1] per year above 65 years of age

Table 3. Diagnostic values of the developed risk profile for serum 25(OH)D <50 nmol/l at different cut-off points in the total risk score. N = 1509

Cut-off in the total risk score	Percentage at high-risk group	Sensitivity (%)	Specificity (%)	Σ [1](%)	PV+[2] (%)	PV –[3] (%)
≤ 8	100	100	0	100	50.5	-
≤ 18	98.5	99.7	2.8	102.5	51.1	91.3
≤ 28	93.0	98.7	12.7	111.4	53.6	90.5
≤ 38	79.6	93.6	34.7	128.3	59.4	84.1
≤ 48	59.5	79.5	60.9	140.4	67.5	74.5
≤ 58	39.5	60.6	82.1	142.7	77.5	67.1
≤ 68	21.1	36.1	94.1	130.2	86.2	59.1
≤ 78	7.4	12.9	98.3	111.2	88.3	52.5
≤ 88	1.3	2.5	99.9	102.4	95.0	50.1

Scores higher than the cut-off scores mean that vitamin D deficiency is present according to the risk model. [1] Σ = sum of the sensitivity and specificity. [2] Positive predictive value. [3] Negative predictive value.

Table 4. Diagnostic values of the developed risk profile for serum 25(OH)D <30 nmol/l at different cut-off points in the total risk score. N = 1509

Cut-off in the total risk score	Percentage at high-risk group	Sensitivity (%)	Specificity (%)	Σ [1](%)	PV+[2] (%)	PV –[3] (%)
≤ 10	100	100	0.1	100.1	18.5	100
≤ 30	98.8	100	1.4	101.4	19.0	100
≤ 50	94.6	96.9	6.6	103.4	19.8	98.8
≤ 70	84.2	99.3	19.3	118.6	22.2	99.2
≤ 90	63.4	91.5	43.1	134.6	27.2	95.7
≤ 110	43.2	84.2	66.3	150.5	36.7	94.7
≤ 130	27.0	62.0	81.1	143.1	43.2	90.2
≤ 150	12.3	38.4	93.8	132.2	58.9	86.8
≤ 170	2.6	9.9	99.1	109.0	71.8	82.6

Scores higher than the cut-off scores mean that vitamin D deficiency is present according to the risk model. [1] Σ = sum of the sensitivity and specificity. [2] Positive predictive value. [3] Negative predictive value.

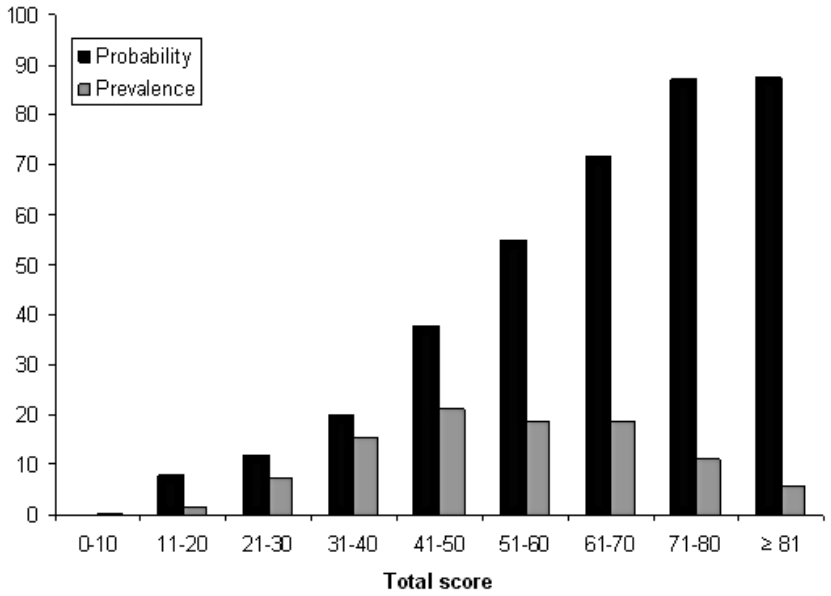

Figure 1. Probability and prevalence of vitamin D deficiency (< 50 nmol/L) per ten points increase in total risk score. For example, an individual with a score between 71 and 80, will have a risk of approximately 90% for being vitamin D deficient (probability). Approximately 10% of the participants had a score between 71-80 (prevalence). N = 1509

DISCUSSION

This study showed that serum 25(OH)D concentrations <50 and < 30 nmol/l could be predicted accurately by easily assessable predictors. We identified older age, sex (female), higher BMI, smoking, less alcohol use, season, no vitamin supplement use, no bicycling, no sporting, no gardening, medication use, poor appetite, and having no partner as predictors for concentrations <50 nmol/l. For vitamin D concentrations <30 nmol/l, older age,

smoking, less alcohol use, season, no vitamin supplement use, no bicycling, no gardening, medication use, limitations in the use of transportation and the inability to remember the present year were identified as the final predictors.

To the best of our knowledge our study is the first study that successfully developed models to predict vitamin D deficiency in the general older European population. Most previous studies describe a prediction model for concentrations of serum 25(OH)D instead of vitamin D deficiency [27-30]. Explained variance of serum 25(OH)D in these models ranged from 21% to 40% [27-30]. In our models Nagelkerke's R^2 were 0.31 and 0.28, which could be interpreted as pseudo-explained variance. In general, for logistic regression models, these values are lower and must be interpreted carefully; Nagelkerke's R^2 is not the same as explained variance based on linear models [31]. AUCs were 0.78 and 0.80 for our models, which means that 78 and 80% of the participants, respectively, were classified correctly. After external validation of the model <50 nmol/l the AUC was still 0.71. Three previous studies performed analyses on the prediction of vitamin D deficiency in non-European populations [14;32;33]. However, one study did not report the AUC at all [33]; the second reported AUCs of 0.79-0.80 for different models with different cutoffs for vitamin D deficiency and after external validation these remained 0.65-0.70 [32]. The performance of the latter models are comparable to ours, but the contents of the models are different. In our model, only easily accessible predictors were included. In the previous models also laboratory variables, such as serum calcium, albumin or parathyroid hormone were included. By including these variables, there will be a restriction in cost savings. In addition, these analyses were performed in dialysis patients and not in the general older population [32]. The third and most recent study was performed in Australia [14]. The performance of these reported models were comparable to ours, but the predictors included in the model are also more challenging to assess. For example, physical activity was measured in metabolic equivalent task hours/week (an indicator for energy expenditure during activities). In our model this was assessed by a simple question on whether a participant cycles or walks outdoors (yes/no). Another difference is that season was not included in the models as in Australia this may be of less influence on vitamin D status compared with the Netherlands. Season was one of the strongest predictors in our models. This means that the same individual will have a higher total score in the winter than in the summer or spring. With a higher total score, the probability of being vitamin D deficient or insufficient will be higher. This means that it may depend on the season whether this individual needs vitamin D supplements.

Our results may have practical implications with respect to identifying persons at risk for low vitamin D status, and thereby may influence the need for vitamin D supplementation or measurement of serum 25(OH)D concentration. At present, the Dutch

guidelines for supplementing vitamin D are quite general [9]. By using our model, the risk could be calculated and vitamin D supplements use (or serum vitamin D testing) may be more specifically advised based on the prediction of a low serum 25(OH)D. This will contribute to health care cost savings.

The developed models may be used in several ways. First, they can be used to identify individuals at such a high risk for low vitamin D status that vitamin D supplements can be recommended without further testing of serum 25(OH)D. Second, the risk models can be used to identify persons in whom low vitamin D status is very unlikely and who therefore may not require vitamin D supplementation. For individuals with an intermediate risk, serum 25(OH)D determination is required to make a definite decision on whether vitamin D supplements are needed or these individuals can simply be advised to take vitamin D supplements. This latter decision will depend on the preferences of the patient and physician. To determine the ideal cutoffs for the two mentioned aims, Table 3 can be used. For the first aim, ie, to predict individuals at high risk of vitamin D deficiency (<50 nmol/l), the cutoff om the total risk score may be 68. By choosing this cutoff, 21.1% will be classified as deficient. Eighty-six percent of the individuals, classified as deficient according to the model are actually deficient (positive predictive value). This means that only 14% of those with a positive score will be wrongly advised to take vitamin D supplements (44 of the 1509 participants). For the second aim, ie, to identify individuals in whom low vitamin D status is unlikely, the cutoff may be 38 points. By choosing this cutoff, 20.4% of the individuals will be classified as sufficient; 84.1% of participants who are classified as sufficient will actually have a more than sufficient vitamin D status, meaning that only 15.9% of these individuals classified as “sufficient” will not be advised to take vitamin D supplements when they actually should be taking them (49 of the 1509 participants). The same procedure to define cutoffs can be used within the other model. However, in the future, further discussion on the optimal cutoffs of the total risk score for high and low risk is necessary.

Although the prediction model for concentrations < 50 nmol/l in this study was successfully validated in an external population, it should be noted that it cannot be automatically used in every population. This study was performed in a relatively healthy older population and the external validation data set was based on a comparable kind of population. Whether the risk score can be used in, for example, institutionalised and frail older people still needs to be determined.

In addition to the mentioned uncertainty on the generalizability of this model to other populations, this study has some limitations. The LASA study does not have detailed information on the use of vitamin D supplements. Therefore, we used the use of over-the-counter multivitamin supplements in our model, but this may not necessarily reflect the

use of vitamin D supplements. Last, the validation sample consisted of somewhat younger individuals than the participants of the development sample and the measurements took place more than 13 years later. Therefore, the performance of the model in the external data set may be underestimated. The strengths of this study are the large sample sizes and the method of recruitment of the participants, which reflects the Dutch older population in general. Furthermore, by using a multiple imputation technique no participants were excluded from the analysis because of one or more missing values.

In conclusion, the results of this study showed that a risk model for vitamin D deficiency in older persons could be developed by using simple patient characteristics. Two risk scores, based on 13 or 10 easily measurable predictors, were developed and were able to predict vitamin D deficiency accurately. These risk scores may be useful in clinical practice to identify persons at risk for vitamin D deficiency. In the future, the models should be further simplified.

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SUPPLEMENTARY TEXT

Age and sex were derived from population registries. BMI was calculated as measured weight in kilograms divided by measured height in square meters. Smoking was assessed by self-report and divided into two categories: current smoker and non-smoker. Participants who had stopped smoking within 5 years ago, were classified as smoker. Alcohol use was also assessed by self-report. Level of education was converted into years of education, and subsequently divided into three categories: low (≤ 9 years), intermediate (10-12 years), and high level (> 12 years). Season was categorised into four categories, according to the meteorological seasons in the Netherlands (winter (December-February), spring (March-May), summer (June-August) and autumn (September-November)). Vitamin supplement use was assessed by asking a question on over-the-counter vitamin use (yes/no). Physical activity was assessed using the LASA Physical Activity Questionnaire, a validated interviewer-administered questionnaire about the duration and frequency of activities during the past two weeks [1]. For this study only the first question on every domain (for example: "Do you walk outdoors?") was used. For gardening, if participants did not have a garden, they were classified as no gardening. Degree of urbanisation was assessed using the classification of Statistics Netherlands, which recodes the postal codes of the Netherlands into five categories based on the number of addresses per squared kilometre [2]. According to the division of Statistics Netherlands, a dichotomisation was made in rural and city. Medication use was assessed by asking the participants to show their medication containers to the interviewers. For this study, a dichotomisation was made in no medication use versus the use of one or more medicines. Self-rated health was assessed by asking the question: "How is your health in general?" and the first two answer categories (excellent/good) and the last three answer categories (fair/sometimes good and sometimes bad/poor) were combined to create two categories. The specific depressive symptoms were asked by the Center for Epidemiologic Studies Depression Scale questionnaire [3]. Only the questions on appetite and depressive mood were used. Anxiety was assessed by the question: "Do you have sudden feelings of panic?", a question originally coming from the HADS-A questionnaire [4]. The functional limitations were assessed by asking the ability to perform six major activities of daily life. The ability to perform the following activities were examined in this study: walking five minutes outdoors without resting, using their own or public transportation and walking stairs. Partner status was divided into two categories (having a partner versus no partner). Any pain during walking was based on self-report (yes/no). Memory was assessed by some questions of the MMSE [5]. We used the questions on year and date. In addition, a question on the presence of memory complaints was used.

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