Prevalence of biochemical osteomalacia in adults undergoing vitamin D testing

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Abstract

Objective

Prolonged severe vitamin D deficiency can cause osteomalacia, but the 25-hydroxyvitamin D (25OHD) concentration below which this occurs is unknown. We investigated the prevalence of biochemical osteomalacia in adults with a measurement of 25OHD.

Design, Measurement, and Patients

25OHD results between 1/1/2009 and 15/6/2020 were obtained from the regional laboratory database, together with measurements of serum calcium, parathyroid hormone (PTH) and alkaline phosphatase (ALP) within 6 months of the index 25OHD. We defined biochemical osteomalacia as all 3 of: albumin-adjusted serum calcium (aCa)<2.0 mmol/L, PTH>7.3 pmol/L, and ALP>150 IU/L. Possible osteomalacia was 2/3 criteria with the other test not done. 25OHD measurements associated with significant renal impairment, elevated hepatic transaminases or hypercalcaemia were excluded.

Results

110,046 25OHD measurements were identified over the 11.5y period. After removal of ineligible measurements, 42,171 25OHD measurements from 32,386 individuals with at least 2 of aCa, PTH, and ALP were included in analyses. Median 25OHD was 63 nmol/L; 8% were <25 nmol/L, and 33% <50 nmol/L. Five index 25OHD measurements met the definition of biochemical osteomalacia, and another 11 were possible osteomalacia. After reviewing available clinical records for these 16 episodes, we classified 9 cases as osteomalacia, and 7 as other diagnoses. Thus, the prevalence of biochemical osteomalacia was 0.02% (9/42,171) for 25OHD measurements, and 0.23% (8/3432) for 25OHD<25 nmol/L. All cases of osteomalacia with 25OHD measurements prior to supplementation had 25OHD≤18 nmol/L.

Conclusion

The prevalence of biochemical osteomalacia is very low, even in individuals with 25OHD<25 nmol/L.

Introduction

Vitamin D testing and supplementation consume considerable health care resources. The disorders of bone mineralization, rickets in children or osteomalacia in adults, are the only illnesses unequivocally caused by vitamin D deficiency,[1] and each is readily prevented with sunlight exposure or prevented or treated with vitamin D supplementation. However, the biochemical definition of vitamin D deficiency is controversial: while it is widely accepted that a 25-hydroxyvitamin D (25OHD) <25 nmol/L indicates vitamin D deficiency,[1,2] some authorities argue that much higher concentrations (50-100 nmol/L) should be considered as evidence of vitamin D deficiency.[3,4] Influential international bodies have adopted definitions of vitamin D sufficiency and deficiency based on these higher thresholds.[5]

Clinical experience and the physiology of bone remodelling suggests that osteomalacia only occurs when 25OHD concentrations are much lower than 25 nmol/L for a prolonged period of time and limited clinical research supports this view.[1] Investigating the prevalence of osteomalacia according to 25OHD concentrations might inform practice in managing skeletal health.

Osteomalacia is a histological diagnosis, but is associated with biochemical findings of hypocalcaemia, secondary hyperparathyroidism, and elevated alkaline phosphatase (ALP). Usually, the diagnosis is made on clinical grounds when typical symptoms (eg proximal muscle weakness and musculoskeletal pain) occur in a typical setting (eg lack of sunshine exposure) and are accompanied by the supportive biochemical changes. We set out to determine the prevalence of osteomalacia defined using biochemical tests, in individuals living in Auckland, New Zealand, who had a measurement of 25OHD, and to investigate

whether there is a threshold for 25OHD above which biochemical osteomalacia does not occur. To our knowledge, similar studies have not been previously published.

Methods

We obtained deidentified data from the Auckland regional biochemistry database for all measurements of 25OHD in adults (>18y) between 1/1/2009 and 15/6/2020. Over this time period, several different 25OHD assays were used in different laboratories, including the Diasorin radioimmunoassay, Diasorin Liaison, and immunoassays on the Roche, Siemens, and Abbot platforms. However, most tests were done at a single laboratory, mainly with the Roche assay. The limits of detection are listed in Figure 2. For each 25OHD measurement, we identified relevant biochemical test results within the 12-month index period: up to 6 months before or after the index 25OHD measurement. These tests were: serum calcium (reference range 2.1-2.55 mmol/L), serum phosphate (PO4, reference range 0.7 – 1.5 mmol/L), parathyroid hormone (PTH, reference range 1.7- 7.3 pmol/L), alkaline phosphatase (ALP, reference range 40-130 U/L), other liver function tests (GGT, ALT, AST), and serum creatinine. Serum calcium adjusted for albumin concentrations (aCa) was used if reported, otherwise aCa was calculated using the formula applied in the local laboratories: aCa = total calcium + 0.012 x (39.9 – [albumin]) if an albumin measurement was available within 7 days before or after the calcium measurement, and was between 20-50 g/L.

We excluded any 25OHD results associated with significant renal impairment, elevation of hepatic transaminases, or hypercalcaemia (aCa>2.60 mmol/L) during the index period. Significant renal impairment was defined as calculated eGFR<30 ml/min/1.73 m^2 using the MDRD formula: eGFR = $age^{-0.203}$ * $(Creatinine/88.4)^{-1.154}$ * 175 * 0.742 if female. Elevated

liver transaminases were defined as any of GGT>50 U/L in females, GGT>60 U/L in males, or AST or ALT>45 U/L.

We defined biochemical osteomalacia when all 3 of an elevated ALP (>150 IU/L), low aCa (<2.0 mmol/L), and elevated PTH (>7.3 pmol/L) occurred within the index period. Some individuals did not have all 3 measurements during an index period, therefore we defined possible biochemical osteomalacia as the presence of 2 of these 3 abnormalities during the index period and no measurement of the other test (that is ALP>150, aCa<2.0, PTH missing or ALP>150, PTH>7.3, aCa missing, or aCa<2.0, PTH>7.3, ALP missing.)

For each outcome, only the first occurrence of a 25OHD measurement associated with an episode of biochemical osteomalacia in an index period was considered in analyses. For example, a repeat 25OHD measurement 2 months after an 25OHD index measurement associated with an episode of osteomalacia would not be considered as a second episode of osteomalacia, but a measurement more than 6 months later also associated with osteomalacia would be considered a separate event. In the primary analyses, we included only index 25OHD measurements for which there were at least 2 out of 3 measurements of ALP, PTH or aCa.

The initial project was defined as an audit using de-identified data by the New Zealand ethics committees (HDEC) and therefore did not need ethical approval. After obtaining the initial results, we considered it would be valuable to obtain the hospital clinical records relevant to the potential episodes of biochemical osteomalacia we identified. We therefore sought ethics committee approval to link the de-identified data for the 16 identified cases with their

medical records. This record linkage was approved by the Northern B Health and Disability Ethics Committee (20/NTB/277).

Descriptive statistics (mean, SD, or proportions) are presented. All analyses were conducted with the R software package (R 3.5.1, 2019, R Foundation for Statistical Computing, Vienna, Austria).

Results:

110,046 index 25OHD measurements were identified from 81,441 individuals, 70% female, over the 11.5-year period. After removal of 25OHD measurements that did not meet the eligibility criteria because of other abnormal results in the index period, and 25OHD measurements that were not accompanied by at least 2 out of 3 of ALP, PTH and aCa measurements during the index period, 42,171 index 25OHD measurements from 32,386 individuals remained (Figure 1). Most excluded measurements were because of missing ALP, PTH, or aCa results during the index period. Of 88,842 otherwise eligible results, 46,671 (53%) had none (16%) or only 1 of these 3 measurements (37%; of which, 91% had ALP measurement, 8% aCa, and 0.9% PTH). Even when 25OHD was <25 nmol/L, these measurements were uncommon: 7317/88,842 (8%) of 25OHD were <25 nmol/L, but only 518 (7%) of these had all 3 measurements, 2914 (40%) had 2 of 3 measurements, 2671 (37%) had 1 of 3 measurements (93% ALP, 7% Ca, 0.5% PTH) and 1214 (17%) had none of the measurements during the index period.

Table 1 shows the characteristics and measurements associated with both all index 25OHD and only the eligible 25OHD measurements. Figure 2 shows the distribution of all 25OHD results and 25OHD results after ineligible results were excluded. Only eligible 25OHD

measurements and related index periods were included in the analyses. For these results, the median 25OHD was 63 nmol/L; 8% were <25 nmol/L, and 33% were <50 nmol/L. 7538 (18%) of 25OHD measurements had all 3 of aCa, ALP, and PTH measurements during the index period, while 34,633 (82%) had only 2/3 of these measurements. 402 (1%) index 25OHD measurements were accompanied by a low aCa, 1307 (3%) an elevated ALP, and 1509 (18%) an elevated PTH. 5 index 25OHD measurements were accompanied by all 3 abnormalities, and a further 11 25OHD measurements by 2 abnormalities and the other test was not done (5 low aCa and high PTH, and 6 low aCa and high ALP). All of these index 25OHD measurements were in different individuals. Thus, there were 5 individuals with biochemical osteomalacia, and 11 cases of possible biochemical osteomalacia.

To further categorise these events, we obtained the hospital medical records relevant to the episode of potential biochemical osteomalacia for these 16 individuals. Table 2 shows the details of the cases, and Figure 3 the temporal pattern of the key results. After considering the clinical details and temporal pattern of the results, we considered all 5 cases of biochemical osteomalacia to be confirmed cases, 4 of the possible cases to be confirmed cases of biochemical osteomalacia, and 7 possible cases to have alternate diagnoses. None of the cases had fractures, formal radiological or histological assessment or a diagnosis of osteoporosis documented.

Thus, 9/32,386 (0.03%) individuals with an eligible 25OHD measurement were found to have biochemical osteomalacia over the 11-year period. 8/9 individuals had 25OHD measurements before vitamin D supplementation started: all results were ≤18 nmol/L, with 4/9 results below the limit of detection. One individual had a 25OHD measurement (Table 2 ID3, 71 nmol/L) 11 days after the other tests results, and 10 days after receiving a

prescription for bolus vitamin D supplementation. By the time of the 25OHD measurement, the aCa was normal. We considered this was a case of treated biochemical osteomalacia. Thus ultimately, 0.02% (9/42171) of all eligible 25OHD measurements, 0.06% (8/13,981) of 25OHD<50 nmol/L, and 0.23% (8/3432) of 25OHD<25 nmol/L were associated with a final diagnosis of biochemical osteomalacia. Of these 9 cases, 6 were in women, 5 were in individuals with Indian ethnicity, 2 were in individuals with Māori ethnicity, 6 were identified in the community, 4 were diagnosed in winter months (June-August), and 3 were in individuals ≥80y. Of cases with available clinical details, 4/6 documented limited sun exposure as the cause of the osteomalacia, and 2/6 gastrointestinal malabsorption.

The relationships between 25OHD, and aCa, PTH, PO4, and ALP for the eligible index 25OHD measurements are shown in Figure 4. Changes in mean aCa, ALP, and PO4 with decreasing 25OHD were only very small, whereas mean PTH concentrations when 25OHD was undetectable or <12.5 nmol/L were approximately double those for 25OHD>25 nmol/L.

Discussion

Over a 11.5-year period, we identified 9 cases of biochemical osteomalacia, a prevalence of 0.02%/25OHD measurement, or 2 cases per 10,000 25OHD measurements. The distribution of 25OHD was similar for all measurements and eligible measurements meeting inclusion and exclusion criteria: median 25OHD was 62 nmol/L, and 63 nmol/L respectively, 8% were <25 nmol/L, and 33-35% were <50 nmol/L. Population-based data for New Zealand reported a mean 25OHD of 50 nmol/L in 1997[6] and 63 nmol/L in 2008/9.[7] The similar/higher results in the current study along with the low proportions of 25OHD<25 nmol/L suggests that clinicians are not targeting 25OHD measurements to individuals at risk of vitamin D deficiency. Furthermore, even when low 25OHD measurements occurred, more than 50% did

not have measurements of the relevant biochemical markers: 17% of 25OHD <25 nmol/L had no measurement of ALP, aCa, or PTH between 6 months before and after the 25OHD measurement; and 37% had only one of these measurements, almost always ALP, which may have been measured as part of the broader "liver function tests" panel. This suggests that clinicians do not consider these biochemical tests important when assessing low vitamin D status.

To our knowledge, the prevalence of osteomalacia in a New Zealand study has not previously been reported, and likewise there are few contemporary reports of the prevalence of osteomalacia in other countries.[1] The prevalence of 2 cases/10,000 25OHD measurements, or 3 cases/10,000 individuals with a 25OHD measurement is very low, consistent with clinical experience that osteomalacia is a rare condition in Auckland. Given this prevalence, it seems surprising that so many 25OHD measurements occur. The adult population of Auckland was approximately 1-1.1 million during the 11.5y time period of this study, meaning that about 1 in 13 adults had a 25OHD measurement during this period. This high rate is more surprising given the common local recommendation to give vitamin D supplements to individuals at high risk of vitamin D deficiency rather than measuring 25OHD.[8] Presumably, practitioners were measuring vitamin D for reasons other than clinical suspicion of osteomalacia.

The low prevalence of osteomalacia could be viewed as a success - perhaps clinicians are very aware that it can be prevented by vitamin D supplementation and were prescribing supplements liberally, thereby keeping the prevalence low. But if this was the explanation, it is not clear why 25OHD would be measured so commonly, given local guidance recommends against 25OHD measurement in asymptomatic individuals. More than half of 25OHD measurements did not have accompanying relevant biochemical tests within 6 months of the

test. ALP was available for 80% of 25OHD measurements, most likely as part of the liver function test panel, whereas aCa was available for <50% of 25OHD measurements and PTH in only 10%. Even when 25OHD was <25 nmol/L, the frequency of accompanying biochemical tests to investigate the possibility of osteomalacia remained low, with >50% not having aCa measured, and only 8% having a PTH measurement. This suggests that clinicians either are not sufficiently concerned about the risk of osteomalacia to request these tests when low 25OHD results are obtained, or do not feel that such tests are necessary. In our view, if 25OHD is <25 nmol/L and there is clinical suspicion of osteomalacia, or the patient is otherwise unwell, checking the serum calcium (and PTH if calcium is low) is appropriate. It is also possible that clinicians recognise the rarity of osteomalacia. Even in individuals with 25OHD<25 nmol/L, the prevalence of biochemical osteomalacia was only 0.23% (23 per 10,000), indicating that the overwhelming majority of individuals with these vitamin D concentrations will not develop osteomalacia. The definition of vitamin D deficiency as 25OHD<25nmol/L therefore seems inappropriate for adults because it does not indicate a high risk of associated disease.

Defining thresholds for vitamin D deficiency is clinically important. If higher thresholds are used (eg 50 or 75 nmol/L), then vastly more people are labelled as having vitamin D deficiency, and may be advised to take vitamin D supplementation. However, if such groups are not at higher risk of osteomalacia, this represents a misdiagnosis of vitamin D deficiency, raising needless anxiety and wasting resources. In support of this view, meta-analyses of randomised controlled trials of vitamin D supplements report that vitamin D supplementation does not improve musculoskeletal health, prevent falls or fractures, or treat/prevent other non-skeletal conditions in populations considered vitamin D insufficient (25OHD 25-50 nmol/L) or sufficient (25OHD≥50 nmol/L).[9-13]

It has often been argued that conducting placebo-controlled clinical trials in individuals or populations with low vitamin D status (eg 25OHD<25 nmol/L) is unethical, and that in such trials, all participants should receive vitamin D supplements.[14] Our results do not support that view. Given the very low prevalence of osteomalacia in individuals with 25OHD<25 nmol/L and the small changes in biochemistry associated with such levels, placebo-controlled trials of vitamin D supplements can be justified in such individuals. Few trials (12 up until December 2015) with clinically meaningful endpoints have been conducted in populations with 25OHD<25 nmol/L, and these reported mixed effects of vitamin D supplementation: about 1/3 of trials reported beneficial effects and 2/3 no effects.[15] This represents an evidence gap as there is insufficient evidence to decide whether vitamin D supplementation is beneficial for such populations, in clear contrast to the situation of populations with 25OHD>25 nmol/L for which there is strong evidence for lack of benefit of vitamin D supplementation.[9-13] We suggest that there is no need to conduct further randomised clinical trials with clinical outcomes except in groups with low vitamin D status (eg 25OHD <25 nmol/L) who we have shown here are at very low risk of osteomalacia. Before such trials were undertaken, it would be important to document clear evidence that 25OHD<25 nmol/L was unequivocally associated with the clinical outcome after confounding variables were accounted for.

Clinical details were only available for 6 of the 9 cases of biochemical osteomalacia identified, but these details were consistent with known risk factors for osteomalacia. Three cases occurred in individuals ≥80y with limited sun exposure, 7 in individuals with ethnicity associated with higher risk of osteomalacia, and 2 in individuals with gastrointestinal malabsorption. The majority of cases were diagnosed and treated in the community, with

only 1/3 being diagnosed during a hospital admission or requiring hospital treatment. Interestingly, serum PO4 was only low in 1/9 cases of biochemical osteomalacia, suggesting it may not be a useful test in making this diagnosis. Osteomalacia is readily preventable for populations in which limited sun exposure is the primary cause. Prevention of such cases in the frail elderly can occur through sunlight exposure[16] but adherence to a formal sunlight exposure programme was low in a later clinical trial.[17] Therefore, it seems likely that effective prevention strategies would include ongoing programmes of vitamin D supplementation that are community based and focus on individuals with limited sun exposure at higher risk of osteomalacia. Alternative options would include food fortification, ensuring that foods consumed by high risk groups are fortified, rather than simply fortifying milk and other dairy products that may not be consumed by these groups.

There are limitations to this research to consider. Seasonal variation in these analytes occurs. When individuals did not have all analytes measured on the same day, this could potentially affect results. For example, an individual may have had a 25OHD measurement at its nadir in spring, but the PTH measurement at its nadir in autumn. However, we think any impact from this is likely small for two reasons: more than 50% of individuals had each analyte measured on the same day as 25OHD, and the amount of seasonal variation of each analyte was very small compared to the departure from the normal range we were assessing (for PTH, ALP, and aCa, the maximum - minimum monthly mean differences were 0.9 pmo/L, 4.5 IU/L, and 0.02 mmol/L respectively. Thus, adjusting for seasonal variation is unlikely to change our findings. Low vitamin D status, if defined as 25OHD<25 nmol/L, was relatively uncommon (8%) in this population, and cases of biochemical osteomalacia might have been missed because they had a measurement of only 1 of aCa, PTH, or ALP, or because there was coexistent renal or liver disease and therefore were excluded. Thus, the findings might differ

in more vitamin D deficient populations, those with renal or liver disease, and those with higher proportions of measurements of these analytes. We do not know why individuals had blood tests performed, so there may be a testing indication bias in these results. The 25OHD assays in this analysis changed over time and all have inherent limitations. Thus, the absolute 25OHD results might have been different if other assays had been used.

Conclusions

The prevalence of biochemical osteomalacia is very low, even in individuals with 25OHD<25 nmol/L. This has important clinical implications for the diagnosis of vitamin D deficiency, recommendations for vitamin D supplementation for asymptomatic individuals, guidance about measurement of 25OHD and the conduct of clinical trials of vitamin D supplementation in populations with low vitamin D status. Such trials would address this remaining important evidence gap regarding vitamin D supplementation in individuals without musculoskeletal symptoms who are not at high risk of osteomalacia.

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Table 1: Characteristics and results associated with index 25OHD measurements

Variable	All index 25OHD measurements			Eligible index 25OHD measurements		
	n	Mean (SD)	Range	n	Mean (SD)	Range
Age	110446	50.0 (16.7)	18.0-101.7	42171	50.5 (17.4)	18.0-101.7
Gender (F) (n, %)	77517	70.2	-	30623	72.7	-
25OHD (nmol/L)	108267	66.5 (33.5)	8-388	41483	67.7 (33.4)	9-350
Total calcium (mmol/L)	59983	2.35 (0.16)	0.71-5.82	41809	2.33 (0.13)	0.71-3.11
Albumin-adjusted calcium (mmol/L)	59898	2.33 (0.17)	1.03-5.80	41819	2.31 (0.12)	1.03-2.60
Ionised Calcium (mmol/L)	8330	1.18 (0.15)	0.28-3.22	3644	1.17 (0.11)	0.41-2.19
Phosphate (mmol/L)	34852	1.1 (0.3)	0.1-5.78	22795	1.1 (0.2)	0.1-5.78
Parathyroid hormone (pmol/L)	14387	9.0 (14.8)	0.5-293	8484	5.6 (5.0)	0.5-124
Alkaline Phosphatase (U/L)	92492	82.8 (53.2)	10-2741	41552	79.0 (40.2)	16-1941
Creatinine (umol/L)	92317	83.2 (91.5)	18-1943	40797	71.6 (19.4)	20-220
eGFR (mL/min/1.73m2)	92317	86.9 (27.3)	2- >120	40797	88.7 (25.4)	30- >120
Albumin (g/L)	95634	41.7 (4.5)	10-57	42157	41.1 (4.4)	10-57
ALT (U/L)	91383	27.8 (38.1)	1-2522	40708	20.9 (8.3)	1-45
AST (U/L)	59414	27.1 (65.2)	4-7673	24058	21.2 (6.0)	5-45
GGT (U/L)	92354	38.5 (72.4)	1-3024	41047	22.2 (11.0)	2-60

25OHD- 25-hydroxyvitamin D. Some 25OHD measurements were not reported with a numeric value (eg <10 nmol/L, see Figure 2 legend for details) and are therefore excluded from the n, mean, and SD for 25OHD.

Table 2: Details of 16 potential cases of biochemical osteomalacia

ID	Age (y)	Gender	Index 25OHD (nmol/L)	Albumin-adjusted calcium (mmol/L)	PTH (pmol/L)	ALP (U/L)	PO4 (mmol/L)	eGFR (ml/min/ 1.73 m²)	Initial Category[a]	Location of diagnosis	Ethnicity	Month of diagnosis	Clinical details
Final diagnosis : biochemical osteomalacia													
1	95.9	F	11	1.61	61	391	1.17	30	OM	Hospital	NZE	March	Clinical osteomalacia. Limited sun exposure
2	72.2	F	<10	1.82	56	331	1.97	35	OM	Community	Indian	September	No clinical details
3	23.6	F	71	1.85	13.6	180	1.04	>120	OM	Community	Indian	January	No clinical details but temporal pattern of results consistent with treated osteomalacia.
4	89.5	F	<12.5	1.75	44	351	1.02	34	OM	Hospital	NZE	July	Clinical osteomalacia. Limited sun exposure.
5	81.9	M	15	1.95	56	599	0.93	66	OM	Community	Māori	June	Clinical osteomalacia Limited sun exposure.
6	48.5	M	<10	1.7	40			95	Possible OM	Community	Indian	October	No clinical details
7	51.7	M	18	1.55		184	0.24	112	Possible OM	Hospital	Māori	January	Clinical osteomalacia. Severe malnourishment and electrolyte derangement from chronic diarrhoea/ gut malabsorption from common variable immune deficiency
8	69.3	F	<11	1.89		317	1.12	93	Possible OM	Community	Indian	August	Clinical osteomalacia. Limited sun exposure
9	24	F	18	1.69	25		1.41		Possible OM	Community	Indian	August	Clinical osteomalacia with underlying coeliac disease.

Alternate diagnosis												
35.9	F	69	1.99	9.2		1.39	82	Possible OM	Community	NZE	August	Isolated biochemical abnormalities while participating in a clinical trial of a novel drug. Abnormalities resolved by time of 25OHD measurement (1 month post aCa and PTH)
73.9	M	45	1.85	7.6		1.22	51	Possible OM	Hospital	NZE	January	Omeprazole induced hypomagnesemia with secondary hypocalcaemia
72.9	M	73	1.58		624	1.03	48	Possible OM	Hospital	NZE	March	Paget's Disease with zoledronate induced hypocalcaemia
93.6	F	160	1.77	7.6			40	Possible OM	Community	European	March	Abnormalities attributed to renal failure
80.5	F	34	1.86		318		60	Possible OM	Hospital	NZE	June	Metastatic breast cancer and chemotherapy induced hypocalcaemia
86.2	F	<10	1.99		180	0.97	35	Possible OM	Hospital	NZE	November	Malignancy with asymptomatic electrolyte derangement that resolved spontaneously
38.5	F	85	1.99		197		>120	Possible OM	Community	NZE	December	Metastatic lung adenocarcinoma and chemotherapy induced hypocalcaemia

[a]Initial classification based on data from index period associated with index 25-hydroxyvitamin D (25OHD) measurement.

PTH- parathyroid hormone; ALP- alkaline phosphatase; PO4- serum phosphate; eGFR- estimated glomerular filtration rate; OM- osteomalacia; NZE- New Zealand European

Figure 1: Flow of index 25-hydroxyvitamin D (25OHD) measurements

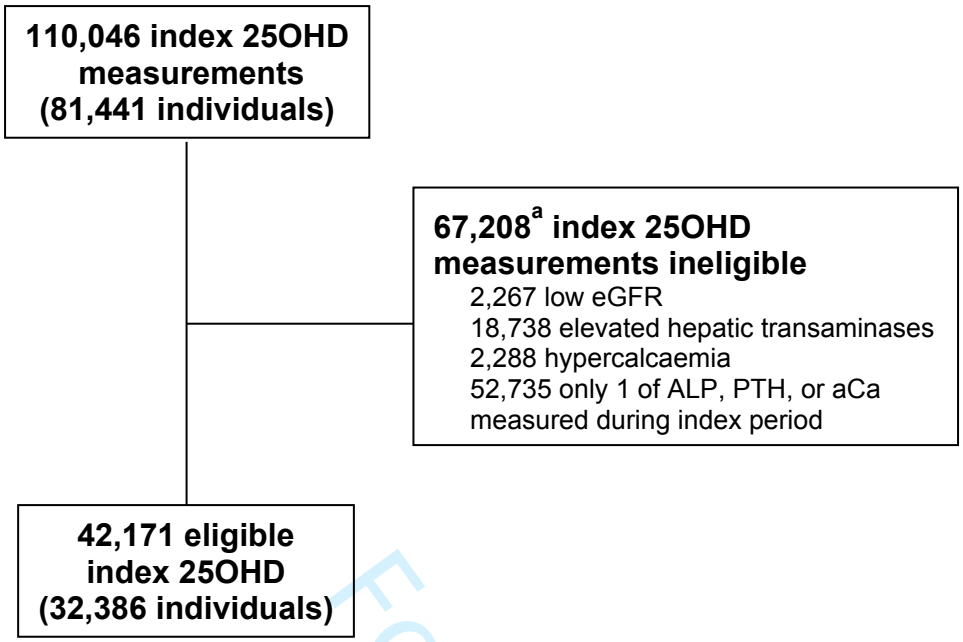
[a]The sum of the numbers of exclusions is greater than 67,208 because some measurements were associated with more than one exclusion criterion.

eGFR- estimated glomerular filtration rate; ALP- alkaline phosphatase; PTH- parathyroid hormone; aCa- albumin-adjusted serum calcium

Figure 2: the top panel shows the distribution of 25-hydroxyvitamin D (25OHD) for all index 25OHD measurements, and the bottom panel the distribution for the eligible 25OHD measurements. The black bars indicate results where the measurement was reported as "low" ie below the limit of detection, or "high" ie above the upper limit, or >250 nmol/L. (For the lower panel, 543 results were low: 23 reported as "<9 nmol/L", 393<10 nmol/L, 2<11 nmol/L, 18<12.5 nmol/L, 1<18 nmol/L, 39<20 nmol/L, 65 <24 nmol/L, 1<25 nmol/L and 1<69 nmol/L [threshold likely reported in error]; 176 were "high": 31 had a reported value (range 251-350 nmol/L), and 138 reported as ">175 nmol/L" , 5>250 nmol/L, 2>400 nmol/L)

Figure 3: temporal pattern of albumin-adjusted calcium (aCa), alkaline phosphatase (ALP), and parathyroid hormone (PTH) in 9 individuals with a final classification of biochemical osteomalacia (ID 1-9, Table 3. The text "Possible Osteomalcia" or "Osteomalacia" on the panels refers to the initial classification based on index period data only. The dotted vertical line is the time of 25OHD measurement.

Figure 4: the relationship between 25-hydroxyvitamin D (25OHD) and alkaline phosphatase (ALP), parathyroid hormone (PTH), albumin-adjusted serum calcium (aCa), and serum phosphate (PO4). Data are mean (SD). Low refers to a result reported as less than a numeric value (eg <10 nmol/L, see Figure 2 legend for details).



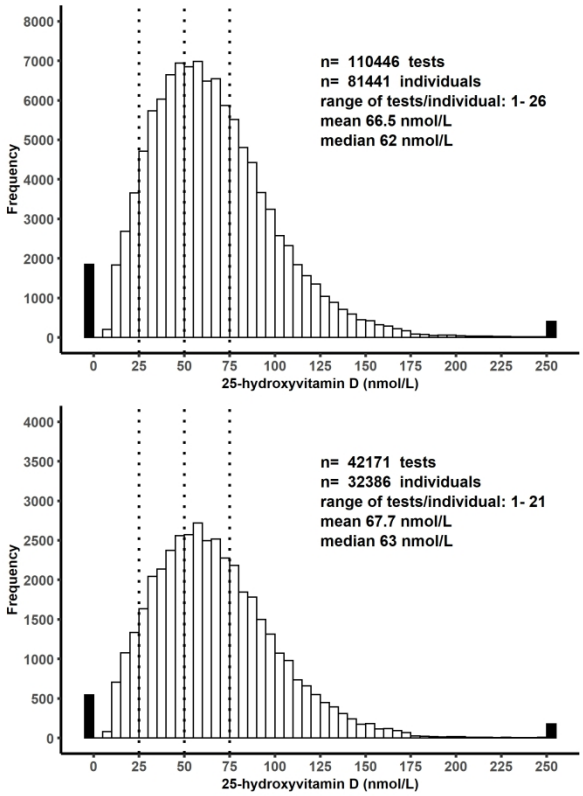


Figure 2: the top panel shows the distribution of 25-hydroxyvitamin D (25OHD) for all index 25OHD measurements, and the bottom panel the distribution for the eligible 25OHD measurements meeting the inclusion and exclusion criteria. The black bars indicate results where the measurement was reported as "low" ie below the limit of detection, or "high" ie above the upper limit, or >250 nmol/L. (For the lower panel, 543 results were low: 23 reported as "<9 nmol/L", 393<10 nmol/L, 2<11 nmol/L, 18<12.5 nmol/L, 1<18 nmol/L, 39<20 nmol/L, 65 <24 nmol/L, 1<25 nmol/L and 1<69 nmol/L [threshold likely reported in error]; 176 were "high": 31 had a reported value (range 251-350 nmol/L), and 138 reported as ">175 nmol/L" , 5>250 nmol/L, 2>400 nmol/L)

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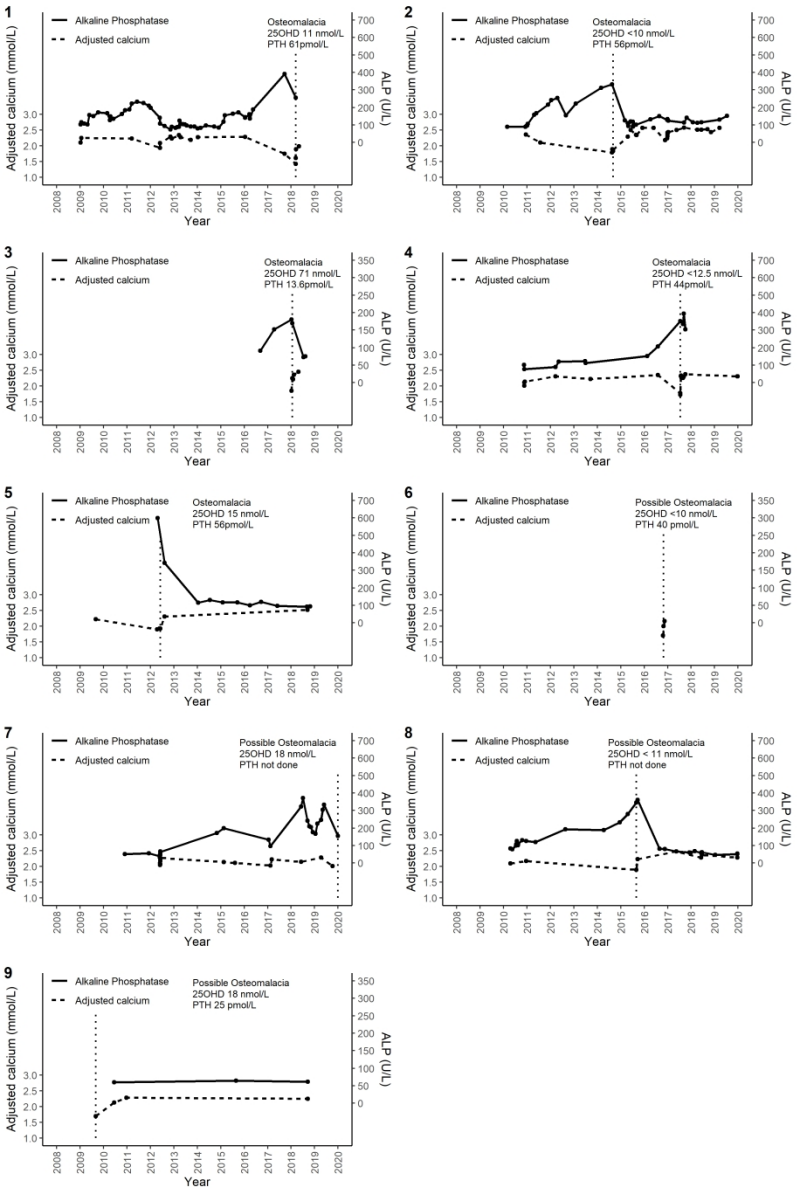

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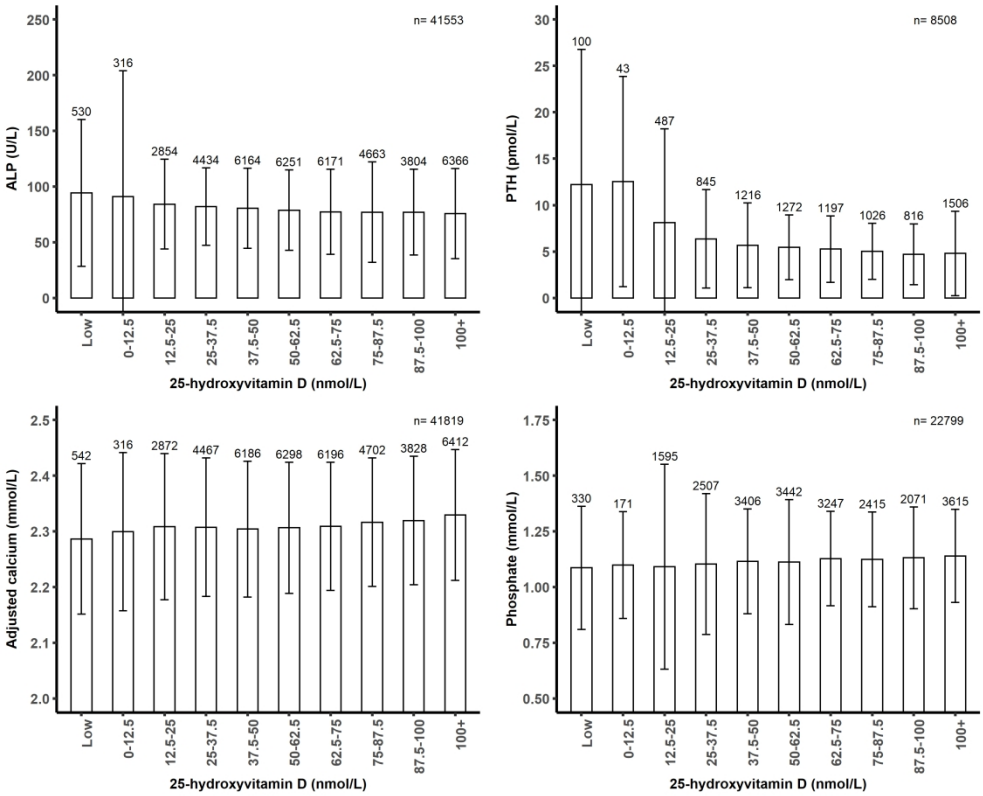

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254x203mm (300 x 300 DPI)