Proposal for the reporting of vitamin D determination.

Intersociety Consensus Document from Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica-SIBioC, European Ligand Assay-ELAS-Italia, Società Italiana dell'Osteoporosi, del Metabolismo Minerale e delle Malattie dello Scheletro-SIOMMMS, Società Italiana di Endocrinologia-SIE, Gruppo Italiano Bone Interdisciplinary Specialist-GIBIS, Associazione Medici Endocrinologi-AME, Società Italiana di Reumatologia-SIR, Società Italiana di Medicine Interna-SIMI, Società Italiana di Ortopedia, Medicina e delle Malattie Rare dello Scheletro-ORTOMED.

Francesco Bertoldo[1], Luisella Cianferotti[2], Annamaria Colao[3], Ruggero Dittadi[4], Sandro Giannini[5], Salvatore Minisola[6], Maurizio Rossini[7], Fabio Vescini[8], Giovanni Lombardi[9,10]

[1]Medicina d'Urgenza, Dipartimento di Medicina, Università degli Studi di Verona, Italy
[2]Unità di Malattie del Metabolismo Minerale ed Osseo, Azienda Ospedaliero-Universitaria Careggi, Dipartimento di Scienze Biomediche, Sperimentali e Cliniche 'Mario Serio', Università degli Studi di Firenze, Italy
[3]Dipartimento di Medicina Clinica e Chirurgia, Unità di Endocrinologia, Scuola di Medicina, Università Federico II di Napoli, Italy
[4]UOC Medicina di Laboratorio, Ospedale dell'Angelo, ULSS3 Serenissima, Mestre, Italy
[5]Unità di Clinica Medica 1, Dipartimento di Medicina, Università degli Studi di Padova, Italy
[6]UOC Medicina Interna A, Malattie Metaboliche dell'Osso Ambulatorio Osteoporosi e Osteopatie Fragilizzanti, Università La Sapienza, Roma, Italy
[7]Unità di Reumatologia, Dipartimento di Medicina, Università degli Studi di Verona, Policlinico GB Rossi, Verona, Italy
[8]Unità di Endocrinologia e Metabolismo, Azienda Sanitaria Universitaria del Friuli Centrale Ospedale Santa Maria della Misericordia, Udine, Italy
[9]Laboratorio di Biochimica Sperimentale e Biologia Molecolare, IRCCS Istituto Ortopedico Galeazzi, Milano, Italy
[10]Department of Athletics, Strength and Conditioning, Poznań University of Physical Education, Poznań, Poland

[1]Società Italiana dell'Osteoporosi, del Metabolismo Minerale e delle Malattie dello Scheletrico (SIOMMMS)
[2]Società Italiana di Ortopedia, Medicina e Malattie Rare dello Scheletro (ORTOMED)
[3]Società Italiana di Endocrinologia (SIE)
[4]European Ligand Assay (ELAS-Italia)
[5]Gruppo Italiano Bone Interdisciplinary Specialist (GIBIS)
[6]Società Italiana Medicina Interna (SIMI)
[7]Società Italiana di Reumatologia (SIR)
[8]Associazione Medici Endocrinologi (AME)
[9]Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBioC)

ABSTRACT

Apart from the still-discussed extra-skeletal effects, vitamin D plays a key role in calcium and phosphate homeostasis and its deficiency is associated with bone disorders such as rickets, osteomalacia and the increased risk of osteoporotic fractures. 25-hydroxy vitamin D [25-(OH)D] is the circulating metabolite commonly used as a marker of vitamin D status.The aim of this document is to express, on the basis of the available guidelines, an Intersociety consensus on how to report the values of the main marker of vitamin D status: 25-hydroxy vitamin D [25-(OH)D]. The main points of this proposal are: to use nmol/L as the measurement units, to measure the analyte at the end of winter/beginning of summer, and to consider an analytical variability of ±10%. In addition, the decision levels to be reported in the report are agreed upon.

***Key words**: vitamin D, laboratory report, reference range*

Correspondig Author: Francesco Bertoldo, Medicina d'Urgenza, Dipartimento di Medicina, Università degli Studi di Verona, Verona, Italy Email: francesco.bertoldo@univr.it

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INTRODUCTION

D vitamers (generically referred to as vitamin D), represented by cholecalciferol, calcifediol, 1,25-dihydroxy vitamin D_2 and D_3 (calcitriol) and the 24,25-dihydroxy form of cholecalciferol and ergocalciferol, play a key role in calcium and phosphate homeostasis and are therefore essential for the proper metabolism of all body tissues. Classically, vitamin D is attributed an essential function in bone mineralisation processes, evidenced by the causal association between deficiency states and conditions such as rickets, osteomalacia and increased risk of osteoporotic fractures in the adult population (1). Moreover, possible extra-skeletal positive effects of vitamin D on, for example, the muscular, immune and cardiovascular systems and cancer mortality cannot be confirmed or ruled out on the basis of the available conflicting evidence (2,3).

The issue of vitamin D deficiency is of considerable relevance within the Italian population, in view of the aforementioned pathological implications and the high prevalence of this condition: in fact, it has been shown in several studies that at least half of the Italian population may have blood values of 25-(OH)D <50 nmol/L (4). In the light of this growing awareness, recent years have seen an often unjustified increase not only in the prescription of vitamin D supplementation but also in the measurement of serum levels of total 25-(OH)D (including the 25-hydroxy forms of vitamin D_2 and D_3). The latter is currently considered the most reliable biomarker for assessing vitamin D status, despite an important variability linked to the different methods available (with bias between methods that can exceed 20%) and the inherent imprecision of the measurement itself, which is often not taken into account when interpreting the laboratory report.

In order to make the prescription of 25-(OH)D measurements and supplementation with cholecalciferol and calcifediol more appropriate on the basis of the available evidence, the Italian Society of Osteoporosis, Mineral Metabolism and Skeletal Diseases (SIOMMMS) published recommendations on the management of vitamin D deficiency in 2022. For the general healthy population, values of 25-(OH)D <25 nmol/L were considered as deficient, values between 25-50 nmol/L as insufficient and values between 50 and 125 nmol/L as optimal. Population groups and conditions at risk of hypovitaminosis D are listed in Table 1; those in need of treatment for osteoporosis can be distinguished, on the basis of the values indicated above. In patients at risk of hypovitaminosis D, the baseline determination of 25-(OH)D values is not recommended, but they are required to be supplemented irrespective of the baseline level, in view of the high risk of hypovitaminosis or to ensure the effectiveness of anti-fracture therapy and to reduce complications such as hypocalcaemia (5).

The SIOMMMS Guidelines were followed, in February 2023, by the updating by the Italian Drug Agency (AIFA) Nota 96, concerning the refundability by the National Health System (SSN) of vitamin D supplements, and introduced for the first time in 2019, with the declared aim of improving prescriptive appropriateness and optimising public health expenditure in this area. A number of scientific societies (SIBioC, SIOMMMS, ELAS Italia and AME) had expressed their opinion about the Nota 96, in an inter-society document in 2020, underlining critical issues encountered in clinical practice concerning the indications for the determination of 25-(OH)D and the threshold values to be considered for the refundability of vitamin supplementation (6). The new version of Nota 96 maintains the distinction between patients for whom the refundability of vitamin D supplementation is guaranteed irrespective of the determination of 25-(OH)D levels (institutionalised persons, persons with severe motor deficits or those bedridden at home, pregnant or bedridden women, persons suffering from osteoporosis from any cause who are not candidates for remineralising therapy) and subjects who are only entitled to refundability in relation to blood levels of 25-(OH)D. In the latter category, different threshold values for therapeutic intervention are identified:

- <30 nmol/L for people who are symptomatic or asymptomatic due to vitamin D deficiency;
- <50 nmol/L in patients on long-term therapy with drugs interfering with vitamin D metabolism or adults suffering from malabsorption;
- <75 nmol/L in patients diagnosed with primary or secondary hyperparathyroidism and in patients with osteoporosis from any cause or other bone disorders who are candidates for remineralising therapy. 112 nmol/L is suggested as a threshold value not to be exceeded because of the risk of vitamin D toxicity (7).

Apart from maintaining important divergences with what is recommended in the SIOMMMS 2022 guidelines, Nota 96 rules, in many circumstances, the refundability of vitamin supplementation on the measurement of 25-(OH)D levels, prompting an increase in an already high number of determinations made today and to consider 'decision-making' levels questionable, in light of the available scientific evidence that led to the recent SIOMMMS recommendations on the topic.

LABORATORY DIAGNOSTICS

Technical considerations regarding the analytical methods

Both the SIOMMMS Guidelines 2022 and the AIFA Nota 96 indicate blood levels of total 25-(OH) vitamin D, including the 25-hydroxy forms of cholecalciferol (D_3) and ergocalciferol (D_2), as biomarkers for the assessment of vitamin D status. Immunometric methods, which are mainly automated and most widely used in laboratories, and mass spectrometry methods (LC-MS) are available for determination. Both are burdened by significant analytical variability that should be considered when interpreting the laboratory report. Analytical variability refers to both the intra- and inter-assay variability within a specific test, and especially between different tests. The latter point is of absolute importance for inter-test and inter-laboratory comparability of results. It should also be borne in mind that there is a physiological variation in vitamin D

concentrations over time, also related to seasonality. This variability can, over a period of several months, exceed 30% (8). To limit intra-individual variability, we reiterate the recommendation, also contained in the SIOMMMS 2022 guidelines, to carry out the determination in the late winter-early summer period (5,9).

In immunoassays, the coefficient of variation is around 4-7%. Sample pre-treatment, which is necessary to separate vitamin D from the main binding protein (Vitamin D Binding Protein, VDBP), is one of the limiting factors for the precision and accuracy of these assays, in view of both the different extraction methods of the different assays and the varying levels of VDBP (e.g., during pregnancy). Immunoassays may also have difficulty distinguishing equimolarly between vitamin D_2 and D_3 or the 24,25-hydroxylated form of vitamin D (10,11).

For mass spectrometry assays, the coefficient of variation is at least 3-4%. Among the main factors limiting the precision and accuracy of these methods is the existence of stereoisomers of 25-(OH) vitamin D, the main one being the C3 epimer, 3-epi-25-(OH)D, which is mainly present in paediatric age and has little biological activity. It should also be borne in mind that the separating gel inside the sample tubes may hinder the ionisation process of the molecules, which is essential for this type of assay (11,12).

In order to assess the degree of harmonisation of the different methods available and to certify those with specific levels of accuracy and precision, the Center for Disease Control and Prevention (CDC) established the Vitamin D Standardisation Program. The data for 2022 show that, although there has been a gradual improvement over the years, between 30 and 60 per cent of the measurements collected still show biases higher than those envisaged by the programme, with values averaging between 15 and 20 per cent, with mass spectrometry assays performing better. In contrast, inaccuracies, expressed as a coefficient of variation, range between 4 and 10%, with no significant differences between the various methods.

Notwithstanding the improvements, there are still significant differences between the two types of assays available for the 25-(OH)D assay. Mass spectrometry performs better from the point of view of measurement accuracy, while in terms of imprecision it does not appear to be better than the other methods. The ease of use, speed and high throughput of automated immunoassays is contrasted by the better measurement accuracy of LC-MS/MS methods, at least when not applied on a large scale. For routine measurements one can therefore opt for immunometry, while for specific diagnostic needs (e.g. rickets, osteomalacia, suspected toxicity) it would be preferable to opt for mass spectrometry.

The use of two distinct units of measurement, nmol/L, provided for by the International System, and ng/mL, historically widespread in Italy, further complicate the issue, increasing the level of inaccuracy. The two units of measurement often co-exist within the single report, making the interpretation of the data risky. The expression of the measurement in nmol/L appears preferable in any case, both for concordance with what is expressed internationally, and for greater measurement accuracy with immunometric methods, which can measure the D_2 and D_3 forms, which have different molecular weights, in a non-equimolar manner. However, the equation for conversion must be remembered:

nmol/L= ng/mL x 2.5

Considerations regarding the indications for the determination of 25-(OH) Vitamin D

Over the last 15 years, a generalised increase has been observed in Italy, as in the rest of the world, in the number of tests for the determination of 25-(OH) D, with often inappropriate prescriptions, mainly due to avoidable repetitions of the measurement, and disconnected from assessments of bone health status (13). Since the introduction of Nota 96, which is intended to regulate the refundability and, in fact, the prescription of vitamin supplementation but also containing indications regarding the appropriateness of the determination of vitamin D, there is no data on the trend of 25-(OH)D test prescriptions in Italy.

AIFA Nota 96 does not recommend screening for 25-(OH)D levels in the general population, but does provide guidance on supplementation in the case of occasional detection of vitamin D deficiency in asymptomatic individuals.

With the update of February 2023, AIFA has included subjects with severe motor deficits and bedridden at home in the category of patients at risk of hypovitaminosis and, therefore, with indication for supplementation regardless of blood levels of vitamin D; the remaining subjects belonging to this category are institutionalised persons, pregnant or lactating women, and patients suffering from osteoporosis from any cause who are not candidates for remineralising therapy. On the other hand, the refundability of vitamin supplementation is subject to the detection of 25-(OH)D values below a certain threshold in individuals undergoing therapy with drugs interfering with vitamin D metabolism or suffering from malabsorption [25-(OH)D <50 nmol/L], in individuals diagnosed with primary or secondary hyperparathyroidism, and in patients suffering from osteoporosis from any cause or other osteopathic diseases who are candidates for remineralising therapy [25-(OH)D <75 nmol/L].

The SIOMMMS 2022 guidelines agree with Nota 96 in not recommending screening for 25-(OH)D levels in the general population due to the lack of evidence of a favourable cost-benefit ratio (14,15).

In contrast to Nota 96, in the SIOMMMS guidelines obese individuals, elderly people, people with conditions associated with reduced sun exposure, individuals on a vegan diet, patients suffering from anorexia nervosa, chronic renal failure, diabetes mellitus type 2, cancer, and cystic fibrosis are also considered to be at risk for hypovitaminosis D. In these categories of subjects (Table 1), baseline measurement of 25-(OH)D levels is not recommended as vitamin D supplementation is considered mandatory.

Table 1
Populations/conditions at risk of hypovitaminosis D (SIOMMMS 2022 guidelines)

- Elderly (age ≥75 years)
- Institutionalised subjects or conditions associated with inadequate sun exposure
- Obesity
- Pregnancy and lactation
- Metabolic bone diseases and other skeletal disorders
- Vegan diet
- Anorexia nervosa
- Chronic renal failure
- Cancer (especially breast, prostate, colon)
- Type 2 diabetes mellitus
- Intestinal malabsorption and bariatric surgery
- Drugs that interfere with vitamin D absorption and hepatic metabolism (antiepileptics, glucocorticoids, antiretroviral AIDS drugs, antifungal

The guidelines suggest that blood levels of vitamin D should be measured when essential for the clinical management of the patient, e.g., for differential diagnosis or after starting supplementation to ascertain the attainment of optimal blood levels 3-6 months later. The recommendations expressed in the SIOMMMS guidelines are derived from systematic reviews showing that the determination of basal 25-(OH)D levels in subjects at risk of hypovitaminosis has not resulted in any benefit nor proved to be useful for choosing the dose to be supplemented or for reducing the risk of vitamin D toxicity, thus showing an uncertain cost/benefit ratio (16-18). Recent studies have also demonstrated the ability of certain algorithms based on clinical-anamnestic data to predict with good sensitivity (78%) the presence of severe hypovitaminosis D [25-(OH)D <25nmol/L], providing further evidence in support of the inappropriateness of baseline measurement of 25-(OH)D levels in individuals with risk factors for hypovitaminosis (19).

Baseline determination of 25-(OH)D levels is instead recommended by the SIOMMMS guidelines in patients with clinical suspicion of osteomalacia, for the purpose of diagnostic confirmation and differential diagnosis, and a subsequent repetition at least 3-6 months after initiation of supplementation is also recommended to assess the correction of the vitamin deficiency, stopping haematochemical monitoring once 25-(OH)D levels >75 nmol/L are reached. Assessment, at baseline and with subsequent periodic checks, of blood levels of vitamin D is also indicated in patients diagnosed with or suspected of primary hyperparathyroidism, both for differential diagnosis and to assess the appropriateness and adequacy of vitamin supplementation (20).

In subjects who are to start drug therapies for the primary or secondary prevention of osteoporotic fractures and in patients with other mineral and skeletal metabolic diseases, the SIOMMMS 2022 guidelines do not recommend baseline assessment of 25-(OH)D levels, vitamin D supplementation being mandatory in these cases as well. For any drug used for anti-fracture therapy, the efficacy in clinical trials has been evaluated by combining it with vitamin D supplementation; in clinical practice, in order to guarantee patients a therapeutic efficacy at least similar to that obtained in clinical trials, it is therefore indispensable to combine anti-fracture therapy with adequate vitamin D supplementation, irrespective of the basal 25-(OH)D values (21,22). Also in this case, consistent with what is suggested in patients with conditions at risk of hypovitaminosis D, the guidelines rather suggest a determination after the start of supplementation to verify the achievement of optimal 25-(OH)D levels.

As already expressed in the joint SIBioC-SIOMMMS-ELAS-AME paper published in 2020 (6), it must be considered that the non-specificity and high prevalence in the adult population of symptoms attributable to hypovitaminosis D reported in Nota 96 could lead, in line with the guidance provided by AIFA, to a disproportionate and inappropriate increase in 25-(OH)D measurements in the general population.

It is also evident that the AIFA Nota 96, apart from not yet including numerous categories of patients at risk of hypovitaminosis D, suggests the assessment of 25-(OH)D levels in subjects or in circumstances (e.g. basal levels in patients at risk of hypovitaminosis D) in which this is at least of doubtful clinical utility, since these are patients who should be indicated for vitamin supplementation irrespective of their blood vitamin D values. Paradigmatic in this sense is the case of patients who are candidates for anti-fracture therapy, in which Nota 96, by linking vitamin supplementation to the presence of 25-(OH)D levels <75 nmol/L, is at odds with the contents of AIFA Nota 79, which specifies that before starting any anti-fracture therapy an adequate intake of calcium and vitamin D is recommended, resorting to supplementation if diet and sun exposure are inadequate, without mentioning blood determination (23). The indications on the measurement of 25-(OH)D contained in the AIFA Nota 96 therefore translate on the one hand into the risk of an increase in inappropriate prescriptions of 25-(OH)D determinations both for the initial assessment and for the follow-up, with a consequent increase in public health expenditure, and on the other hand into a reduction of the appropriate and recommended prescription of vitamin supplementation in subjects in whom it should instead be guaranteed, exposing patients to the risk of inappropriate discontinuation of the drug (24).

Considerations regarding 25-(OH) Vitamin D decision levels

The February 2023 update of AIFA Nota 96 led to a change in the decision levels for the refundability of vitamin D supplementation compared to the first version in 2019, establishing 3 levels (<30 nmol/L, <50 nmol/L and <75 nmol/L, as described above) (7).

Analysing the contents of the vitamin D deficiency guidelines of the major international scientific societies, there is a general consensus that 25-(OH)D values <30 nmol/L are indicative of osteomalacia and thus in need of vitamin supplementation. There is also a consensus that 25-(OH)D values >75 nmol/L are optimal, while there is disagreement on the interpretation and management of patients with 25-(OH)D levels between 30 and 75 nmol/L, which in Italy includes a significant proportion of the adult (35%) and elderly (36%) population (4,25).

Evidence supporting the definition of blood levels of vitamin D below which vitamin supplementation should be initiated comes mainly from studies that have correlated 25-(OH)D values with various outcomes related to the presence of skeletal fragility or secondary hyperparathyroidism.

In a *post-mortem* cross-sectional study involving 675 subjects in whom causes of secondary osteoporosis had been ruled out, it was shown that only for 25-(OH)D values >75 nmol/L were there no patients with histological aspects compatible with osteomalacia on bone biopsy of the iliac crest. However, it is reported that for values of 25-(OH)D >50 nmol/L only 3% of patients present histological features of osteomalacia (26).

On the other hand, some studies have attempted to assess the trend in circulating PTH values in relation to 25-(OH)D levels, revealing the appearance of secondary hyperparathyroidism for 25-(OH)D values <75-87 nmol/L, suggesting this level as the reference limit (27,28). As shown by the studies of Adami et al. the curve of PTH levels related to 25-(OH)D is, however, dependent on the patient's calcium intake and age, which influences the efficiency of intestinal absorption processes and renal function, so it becomes difficult to establish a universal threshold value of 25-(OH)D based on PTH elevation (28,29).

From a decision-making point of view, it is considered more reliable to assess the association between vitamin D levels and skeletal outcomes such as increased risk of fractures.

Prospective studies have found a significantly increased risk of fractures for 25-(OH)D values <50 nmol/L in postmenopausal and elderly patients, respectively (30,31). Consistent with these findings, a 2017 meta-analysis including only prospective studies, demonstrated an increased risk of hip fracture for 25-(OH)D values <60 nmol/L (32). Also for values of 25-(OH)D <50-62 nmol/L, an increased risk of falling and a worsening in some lower limb function parameters were also found (33,34). Counterintuitively, several studies have not observed an improvement in skeletal fragility outcomes with increasing 25-(OH)D levels above 50-62 nmol/L (35-37).

There are also categories of patients in whom vitamin D supplementation has shown beneficial effects regardless of baseline 25-(OH)D values. A recent review of meta-analyses showed that calcium and vitamin D supplementation in institutionalised patients can reduce the overall risk of fractures and the risk for hip fractures, providing a rationale for supplementation irrespective of baseline values and thus of the laboratory determination of 25-(OH)D in this category of subjects (38).

In patients with osteoporosis from any cause undergoing anti-fracture therapy, vitamin D supplementation is necessary since all registered clinical trials have included the association with vitamin supplementation. In the majority of these clinical trials, basal laboratory assessment of 25-(OH)D was not performed (22). Furthermore, for those who are being treated with drugs to reduce the risk of fracture, some studies have found that 25-(OH)D values <75 nmol/L can be an important determinant of the failure of the therapy itself with the occurrence of fractures (21,39). Failure to supplement vitamin D and calcium in patients with fragility fractures undergoing remineralisation therapy has been associated not only with an increased risk of re-fracture but also with an increased mortality from all causes (40).

Considerations regarding safety levels for 25-(OH)D

In AIFA Nota 96 reference is made to two studies according to which there is a U-shaped trend in the curve relating 25-(OH)D levels and the incidence of adverse events (including mortality), with 25-(OH)D values >112 nmol/L being associated with an increased risk of such events (41). More recent studies have instead documented a progressive reduction in mortality as 25-(OH)D levels rise to around 50 nmol/L, with a subsequent flattening of the curve up to blood values of 125 nmol/L, without identifying within this range a threshold value associated with a progressive increase in the risk of death, even when assessing mortality from various causes (42-44). Most of the studies evaluating the adverse effects of vitamin D have been carried out by relating these results to the supplementation dose rather than to 25-(OH)D levels, so it is difficult to unambiguously establish a safe limit for 25-(OH)D. In a recent prospective study to evaluate the effects of different vitamin D supplementation regimens, no increase in the risk of falls, adverse events, hypercalcaemia or changes in bone turnover parameters was found for blood values of vitamin D up to 150 nmol/L (45). In a longitudinal study to evaluate the effects of different doses of vitamin D in hospitalised patients, 25-(OH)D levels between 100 and 200 nmol/L were reported in the majority of the study population without detecting any alterations in calcaemia (46).

AIFA Nota 96 also reports a presumed association between high vitamin D levels (>100 nmol/L) and increased risk of prostate and pancreatic cancer (7). In contrast, data from the recent VITAL study show a reduced risk of incidence of metastatic neoplasia or mortality from neoplasia of any origin for 25-(OH)D values

>100 nmol/L (47). Other studies have also reported that certain favourable extra-skeletal outcomes (including reduced cardiovascular risk, reduced all-cause mortality, reduced risk of auto-immune diseases) are associated with 25-(OH)D >100 nmol/L levels, although the evidence in this area is not conclusive [2]. It is therefore believed, based on the evidence, that the 'acceptable' and indeed in some conditions 'desirable' levels, are significantly higher than those suggested by the current version of Nota 96, and a 25-(OH)D value up to around 125 nmol/L, above which there is no definite evidence of clinical benefit, can be considered clinically safe as well as useful.

However, it is important to remember that vitamin D toxicity is associated with 1,25-(OH)2D levels, of which the measured concentration of 25-(OH)D is an index. Therefore, in the current state of knowledge, the upper limit of the reference range is not an indication of a direct risk of toxicity but, rather, a level of reasonable safety which, if exceeded, may be associated with a potential risk of toxicity in certain situations.

Definition of vitamin D status according to the SIOMMMS 2022 guidelines

In light of the evidence presented above, the SIOMMMS 2022 guidelines define vitamin D status in relation to 25-(OH)D levels by distinguishing between two categories of patients (19):

- in the general healthy population, 25-(OH)D levels < 30 nmol/L are considered deficient, values < 50 nmol/L insufficient, while the range between 50 and 125 nmol/L is defined as optimal;
- in the population at risk of hypovitaminosis D (Table 1) or requiring therapy for osteoporosis, 25-(OH)D levels <30 nmol/L are considered deficient, values <75 nmol/L are considered insufficient, while the range between 75 and 125 nmol/L is considered optimal;
- values of 25-(OH)D up to 125 nmol/L are conservatively considered safe.

Although AIFA's approximation to the SIOMMMS 2022 guidelines is evident and appreciable, making them in some respects more in line with these guidelines, the divergences that remain with regard to supplementation thresholds and toxicity values lead to problems of inappropriate discontinuation of vitamin supplementation in clinical practice, also in view of the current mode to report 25-(OH)D levels, which in most laboratories envisages the indication of values ≥ 75nmol/L as 'normal' without distinguishing between categories of patients. This is especially the case for patients undergoing remineralisation therapy according to Nota 79, in whom there is often the erroneous discontinuation of vitamin D supplementation in the presence of optimal 25-(OH)D values, which must be guaranteed for at least the duration of anti-fracture treatment. It should also be pointed out that AIFA attributes lower optimal 25-(OH)D levels than recommended by the guidelines to certain categories of patients who are clearly at risk of hypovitaminosis, such as those being treated with drugs interfering with vitamin D metabolism and patients suffering from malabsorption. As already reported in 2022, the decline observed by AIFA in the consumption of vitamin D supplementation after the introduction of Nota 96 may not coincide with an improvement in prescribing appropriateness but with a worsening of it, and the contents of the February 2023 update do not appear able to reverse this trend (24).

PROPOSALS REGARDING LABORATORY REPORTING

Based on the above considerations, the board discussed the possibility of adapting the laboratory reporting regarding 25-(OH)D levels, already defined in the joint paper of 2020 (6), also in order to harmonise it with the recent SIOMMMS 2022 guidelines (19). An attempt was also made to suggest a report that was both easy to interpret for the clinician and the patient.

- a first element of consensus is to unify the units with which the circulating vitamin D level is expressed. Often the expression in ng/mL and/or nmol/L (sometimes both present in the same report) exposes the data to misinterpretation. *The board unanimously suggests as the optimal reporting method the expression of the 25-(OH)D value in 'nmol/L'*, as this is considered the most metrologically correct unit of measurement and in agreement with what is expressed internationally. It might be kept in mind that the conversion from ng/mL to nmol/L is obtained by multiplying the value in ng/mL x 2.5;
- the board confirms that, in view of the physiological fluctuations in vitamin D values linked to seasonality, in a subject with a normal lifestyle *the best time to assess vitamin D status is the end of winter/beginning of summer;*
- the board points out in this respect that there is a fair amount of intra- and inter-assay variability linked to the methods used, especially for assays carried out in laboratories using different systems. From a practical point of view, *it is suggested to consider an analytical variation of at least 10% when intepreting 25-(OH)D determinations,* with the recommendation, however, to use the same method in any subsequent controls;
- with regard to the method of reporting, *we confirm the indication to express in the report not as normal (or reference) range, above which inappropriate therapeutic suspensions may occur, but decision values*. Modifying the indications of the Intersociety Document 2020, the agreed decision levels are as follows:
  - *a value of 25-(OH)D ≤30 nmol/L (12 ng/mL) is proposed as indicative of 'deficiency',* to allow identification of a class of subjects with a high probability of being affected by osteomalacia in the case of prolonged low values. This value is universally defined as corresponding to severe vitamin D deficiency and has a high predictive value for the diagnosis of osteomalacia;
  - for the general population, i.e. those not affected by the conditions listed in table 1, *a range of 25-(OH)D values between 30 and 50 nmol/L (12-20 ng/mL) is defined to identify those individuals who should be supplemented,* as values in this range are associated with an increased risk of fractures, incidence of

infectious diseases, neoplasms and cardiovascular mortality. These individuals should be supplemented to a level of at least 50 nmol/L (20 ng/mL);
- *an adequate condition is defined for the general population for values of 25-(OH)D ≥50 nmol/L (20 ng/mL),* which therefore does not give an indication for supplementation;
- *a range of 25-(OH)D values between 75 and 125 nmol/L (30-50 ng/mL) is defined as optimal* for individuals with osteoporosis or with conditions that are typically associated with hypovitaminosis D with possible impaired mineralisation (Table 1), since within this range the maximum level of effectiveness of therapy for reducing the risk of fragility fractures or impaired mineral metabolism is expected;
- the limit of 125 nmol/L (50 ng/mL) is reported not as a safety or toxicity value but because most of the skeletal and perhaps extra-skeletal beneficial effects do not appear to improve significantly beyond this value. On the other hand, *a value of 25-(OH)D >250 nmol/L (100 ng/mL) is considered to be in excess* and therefore not physiological (1,48,49).

In conclusion, it is suggested that the report about decision-making vitamin D levels be used according to following scheme:

25-(OH)D value (nmol/L)	Decision levels to be reported
<30	Deficiency
30-50	Insufficiency
>50	Adequacy (should not exceed 250 nmol/L)
75-125	Optimal value in patients with osteoporosis or conditions at risk of hypovitaminosis D

CONFLICT OF INTEREST

None

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