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Pregledni članak

VITAMIN D – CHALLENGES IN DIAGNOSING AND MONITORING OF HYPOVITAMINOSIS D

VITAMIN D – IZAZOVI U DIJAGNOZI I PRAĆENJU HIPOVITAMINOZE D

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Summary: During recent years, it has been recognized that sub-optimum vitamin D-status, often defined as decrease of PTH concentrations in r esponse to supplementation of vitamin D, is a ver y widespread finding with potential health effects on a population level. As a consequence, ther e is a continuously increasing interest in the laborator y-based assessment of the vitamin D status, with 25-hydr oxyvitamin D as the most widely used analyte. However, there is a number of challenges in the characterization of the individual vitamin D status that are addressed in this article; they include analytical issues of 25-hydr oxyvitamin D measur ement; interpretation of results; development of guidelines for rational indication for laborator y testing; and evaluation of the role of 25-hydroxyvitamin D in the context of complementary markers of the vitamin D status.

Keywords: vitamin D, hypovitaminosis D, r eference ranges, analytics

Kratak sadr`aj: Poslednjih godina je primećeno da je suboptimalni status vitamina D, koji se često definiše smanjenjem koncentracije PTH kao odgovor na suplementaciju vitaminom D, veoma raspr ostranjeno stanje sa potencijalnim efektima na zdravlje populacije. K ao posledica, inter es za laboratorijsko određivanje statusa vitamina D kontinuirano raste, gde se kao analit najčešće koristi 25-hidroksivitamin D. Međutim, postoji veliki br oj izazova u karakterizaciji statusa vitamina D o kojima se govori u ovom radu; oni uključuju analitičke probleme u odr eđivanju 25-hidroksivitamina D; interpretaciju rezultata; definisanje pr eporuka za racionalnu indikaciju za laboratorijsko određivanje; i procenu uloge 25-hidroksivitamina D u kontekstu komplementar nih markera statusa vitamina D.

Klju~ne re~i: vitamin D, hipovitaminoza D, r eferentne vrednosti, analitika

Introduction

During the past years, the inter est of the scientific community in vitamin D has incr eased continuously (1). In addition, deficiency in vitamin D – »the sunshine hormone« – has gained substantial attention in general population. As a consequence, a continuous and substantial increase in ordering of vitamin D related laboratory tests can be obser ved in many industrialized countries.

The identification of vitamin D deficiency as the cause of rickets, followed by the eradication of this disorder in the first half of the last centur y was a major and ver y impressive success of scientifically based medicine. However, following the eradication of severe rickets due to widespr ead supplementation of vitamin D during the first year of live, the vitamin D system drew little attention in medicine during the subsequent decades. This was changed in 1980s when the measur ement of 25-hydr oxyvitamin D and PTH as valid and convenient markers of the vitamin D status became r outinely available by ligand binding tests. It became evident that poor vitamin D status, described as lowering of PTH levels following the administration of vitamin D, is ver y common in many populations worldwide at least in the wintertime. Furthermore, there has been a growing evidence that vitamin D is of r elevance in many physiological and potentially pathophysiological systems beyond calcium and phosphate homeostasis, leading to the concept of noncalcemic or even pleiotr opic effects of

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vitamin D. High prevalence of hypovitaminosis D is in sharp contrast to good supply of virtually all other vitamins in most industrialized societies. However, it is well recognized that the vitamin D status is mainly determined by UV irradiation of the skin while food sources of vitamin D are mainly restricted to fatty fish, unless fortification of foods is given as in some countries. Consequently, the vitamin D status reflects lifestyle, particularly a sedentary lifestyle, and not the general situation of food supply in the society. Notably, the role of direct absorption of the metabolite 25-hydroxyvitamin D from meat may also be relevant (2).

However, the actual impact of low vitamin D status on health beyond the first year of life is still poorly characterized. There may be a significant but rather weak association of laboratory markers of the vitamin D status with the rate of fractures (3). Moreover, many of the intervention studies on vitamin D supplementation display an elusive (and rather disappointing) picture with relation to the risk of falls and fractures (4–9). This is even more the case for non-skeletal outcomes including the total mortality during study observation periods (10). However, in the extensive number of randomized controlled studies, there was very little if any evidence of a potential of doing harm by supplementing vitamin D.

For both potential skeletal and non-skeletal impact of the vitamin D status, the fundamental problem in the interpretation of study results is that there is probably an inter-correlation of the active lifestyle with the outdoor activity as a global and very prominent protective health determinant of UV-irradiation and thus vitamin D status on the other hand. This means that the association of a good vitamin D status with positive health outcome may not be a causal one. Thus, the vitamin D status may just represent a general indicator of health: a healthy person has more outdoor activity and thus better vitamin D status compared to chronically ill person. From the complex plethora of the epidemiological data and data on intervention studies, however, it becomes increasingly evident that it is probably useful to avoid at least profound hypovitaminosis D in all age classes, and that consequently positive effects of vitamin D supplementation can predominantly be expected in chronically vitamin D depleted individuals.

Recommendations for oral vitamin D intake, aiming at maintaining the sufficient vitamin D status despite the widespread condition of minimal sun light exposure, have been given by several medical institutions. The most widely recognized recommendations are from the US Institute of Medicine (11) and from the US Endocrine Society (12). The upper range of these recommended daily doses are quite high with 4000 IU per day which reflects the wide range of tolerability (which notably is in contrast to maximum calcium intake) (2, 13). However, there are no reliable available data concerning the actual extent of oral vitamin D supplementation on population level; probably it is very low in most countries, although the cost for vitamin D supplements is moderate, with approximately 3 € per month in Europe. Clear dose recommendations for supplementation, now generally assuming very low risk potential of vitamin D supplementation and availability of the inexpensive supplements, mean that, independently from sun exposure, vitamin D deficiency can efficiently be avoided in an individual. This clearly raises the question about the usefulness of assessing the vitamin D status individually by use of the expensive laboratory tests.

Available markers

There is a number of analytes available for the characterization of the individual vitamin D status. Measurement of cholecalciferol, vitamin D3 itself, is feasible using chromatographic methods; however, biological half-life of this compound is short and this analyte has not been found useful. On the other hand, it would seem logical to quantify serum concentrations of the active metabolite of the vitamin D system, 1,25-dihydroxyvitamin D3. However, this parameter can paradoxically remain within the normal or even high concentration range in hypovitaminosis D. This might be explained by secondary hyperparathyroidism. Consequently, there is general consensus that this analyte is not useful for the assessment of the individual vitamin D status. In case of renal diseases, low concentrations may be found due to reduced hydroxylation of 25-hydroxyvitamin in the kidney. Anyhow, 1,25-dihydroxyvitamin D is not recommended for monitoring of calcium and phosphorus homeostasis in patients with end stage renal disease. Measurement of this analyte can be necessary to differentiate very rare inborn disorders presenting with hypocalcemia in early childhood (i.e., vitamin D-resistant rickets due to vitamin D receptor deficiency or 1,25 hydroxylase deficiency) or in the unexplained hypercalcemia in adults, potentially attributable to chronic granulomatous diseases such as sarcoidosis.

The intermediate metabolite of vitamin D, 25-hydroxyvitamin D, is generally accepted as the most useful marker of individual's vitamin D status; however, it must be recognized that 25-hydroxyvitamin D is also a surrogate marker of the vitamin D -status. It has biological half-life of several weeks. The analyte is measured in its total serum concentration; quantification of the free fraction, not bound to vitamin D binding globulin or other proteins, is not established.

Decreased serum calcium is, without any doubt, an important indicator of severe hypovitaminosis D, as also applies to increased alkaline phosphatase as a marker of bone involvement and osteomalacia. Assessment of the urinary calcium excretion is attractive to monitor the functional vitamin D status, since calcium

absorption is, to an important degree, controlled by the vitamin D system. As an alternative to measurement in a 24-hour urinary collection, the urinary calcium-to-creatinine ratio can be assessed. However, there are few data available on the usefulness of this technically simple parameter.

Measurement of serum 25-hydroxyvitamin D in contrast is rather expensive marker and is often still categorized among »esoteric type tests«. Indeed, when comparing the costs of 25-hydroxyvitamin D measurement and the expenses for vitamin D supplementation, it becomes evident that global recommendations for laboratory diagnostics in the context of the vitamin D-status must be considered very carefully. In Germany, for example, measurement of serum 25-hydroxyvitamin D is charged with about 20 € while less than 8 € are needed for supplementation with 1000 IU of vitamin D per day from December through February as the most critical months.

Reference ranges and target concentration ranges for 25-hydroxyvitamin D

Similar to serum glucose or cholesterol concentrations, it is essential in the case of 25-hydroxyvitamin D to distinguish between population based »normal« concentrations and desired concentration range that is optimal for health. The most widely recognized recommendation statements are those from the US Institute of Medicine (11) and from the US Endocrine Society (12), as also applies to dose recommendations. While the first document declares serum 25-hydroxyvitamin D concentrations above 20 ng/mL as sufficient, the latter document describes the concentration range between 20 and 30 ng/mL as »vitamin D insufficient«. Concentrations below 10 ng/mL correspond to clear hypovitaminosis D in most patients, typically with clearly elevated concentrations of PTH and often with decreased serum calcium. Calcium absorption can be often demonstrated to be reduced in these cases. Affected individual may have bone pain and muscle weakness, but may as well be free of any symptoms. Indeed, it must be noted that 25-hydroxyvitamin D concentrations below 10 ng/mL are frequent findings in many populations in winter (14–15). The lower normal range cut-offs are tried to be based on the response of PTH concentrations to administration of vitamin D. Several reports describe that 25-hydroxyvitamin D concentration above 20 to 30 ng/mL, increasing the vitamin D supplementation, does not lower PTH any more. However, some recent data have also questioned such a »plateau effect« (7).

Bone histology of individuals which died suddenly in accidents or suicide had features of the impaired bone mineralization in some cases in which the serum concentrations of 25-hydroxyvitamin D between 20 and 30 ng/mL were found, what contributed to recommendation of the Endocrine Society for a desired level above 30 ng/mL (16).

It is suspected by several researchers that optimal concentrations of 25-hydroxyvitamin D may differ with respect to skeletal effects and extra-skeletal effects.

Intervention studies on vitamin D tend to show beneficial effects when serum 25-hydroxyvitamin D concentration above 30 ng/mL is achieved (5), what further contributes to observation of 30 ng/mL as the lower limit of desired concentrations. Probably beneficial health effects of vitamin D supplementation are most relevant in the concentration range below 10 ng/mL of a patient's 25-hydroxyvitamin D and will diminish in an asymptotic manner when serum concentrations above 20 ng/mL are obtained.

Data from studies on Massai shepherds, however, suggest 25-hydroxyvitamin D serum concentrations in the range above 50 ng/mL as »natural«. In contrast, epidemiological studies from a large number of countries and regions demonstrate very high prevalence rates of vitamin D deficiency with a substantial impact of season and age. In a large survey in Germany, for example, even from May to October, nearly 75% of 65- to 79-year-old women had serum 25-hydroxyvitamin D below 20 ng/mL in March (14). This means that deficiency may represent the »normal« situation in many settings. Hypovitaminosis D may also be highly prevalent in »sunny« countries such as the Middle East ones (17).

Substantial criticism concerning the formulation of target serum concentrations of 25-hydroxyvitamin D is related to heterogeneity of analytical methods used in respective epidemiological studies. This was an important motivation for organizations and researchers to improve the degree of standardization of 25-hydroxyvitamin D measurement (18–20).

A very fundamental issue in all attempts to identify lower limit of serum 25-hydroxyvitamin D concentration is much less discussed: since in most regions of the world the vitamin D serum shows pronounced seasonal variation, the comprehensive and conclusive assessment of the vitamin D status of an individual should be based on several observations throughout the year. A serum 25-hydroxyvitamin D concentration below 10 ng/mL has probably completely different relevance for health when given to one person for one or two months in winter or in contrast to another person throughout the year – as often observed in institutionalized elderly people. Assessment of the relevant long-term vitamin D status, which is probably most important for health condition, is poorly addressed challenge of laboratory medicine at present (*Figure 1*).

Furthermore, it must be critically assessed how strong the association of low 25-hydroxyvitamin D concentrations with different health outcomes is. With

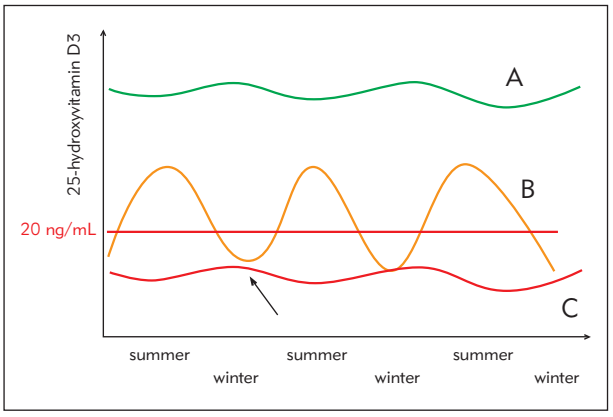

Figure 1 Typical long-term courses of serum 25-hydroxyvitamin D. Line A may represent a person with continuously high degree of sun exposure throughout the year as found in equatorial regions. Line C may represent a person in the Central Europe with substantial outdoor activity in summer and vitamin D deficiency during winter. Line C represents a person with the sustained vitamin D deficiency throughout the year, e.g. in a chronically disabled elderly person. Note that a single measurement of 25-hydroxyvitamin D in winter may not be appropriate to display the fundamental differences in the vitamin D status of the individuals B and C, respectively (arrow).

respect to bone, it is evident that the vitamin D status is one among the excess of genetic and environmental variables that have impact on bone quality. The attempt to deduce an optimum target concentration ranges of the surrogate marker 25-hydroxyvitamin D from intervention studies is extremely complex: these studies typically differ in fundamental variables such as base-line vitamin D status, dosage of vitamin D, duration, concomitant supplementation of calcium, age of subjects, and monitoring of compliance.

In many countries, vitamin D2 (ergocalciferol) is used for food fortification, supplementation and for the therapy of hypovitaminosis D. However, it can not be assumed that vitamin D2 (which is not part of the natural diet) and physiologically occurring vitamin D3 are bioequivalent. Consequently, when using the 25-hydroxyvitamin D assays, which quantify both vitamin D3 and vitamin D2, the result, for example, of 20 ng/mL of total 25-hydroxyvitamin D may be biologically of different meaning if this concentration is realized by vitamin D3 or D2. This is primarily independent of the percentage of reactivity of particular vitamin D2 assays and also an area of uncertainty in 100% »equimolar« assay.

Probably owing to enzyme 24-hydroxylase which inactivates 25-hydroxyvitamin D by alternative metabolization compared to activation by 1-hydroxylase, the toxicity of vitamin D is hardly to be detected. Hypercalcemia can be found in 25-hydroxyvitamin D concentrations above 100 ng/mL which are exclusively observed in individuals under seriously inadequate supplementation regimens (e.g., administration of month-adjusted supplementation doses in a daily pattern for longer time periods) (21).

Analytics of 25-hydroxyvitamin D

The quantification of 25-hydroxyvitamin D in serum is an analytical challenge for several issues.

The analyte is very tightly and to very high degree bound to vitamin D-binding protein and reliable measurement of total 25-hydroxyvitamin D concentrations requires complete dissociation of the analyte from its protein bonds. In radioimmune assays and in chromatographic methods, this can be achieved by application of the organic solvents such as acetonitrile. However, such solvents in high concentrations are incompatible with anti-bodies used in immunoassays. In manual immunoassays, solvents can be evaporated to dryness, but in automated ligand binding tests the complete release of the analyte from its bonds by using antibody-compatible reagents is the crucial technical challenge.

In regions where vitamin D2 is used for supplementation and therapy, differential reactivity of tests with vitamin D2 may contribute to between-method bias of results in individual samples.

With respect to ligand binding tests for 25-hydroxyvitamin D, the competitive assay principle has to be used since the assay offers not enough potential epitopes for development of sandwich-assays. Competitive ligand binding assays for small molecules, however, are notably prone to matrix effects which can be poorly identified and which can be variable between the samples without an option to normalize these sample-individual effects.

In ligand binding assays (involving antibodies or recombinant vitamin D-binding protein) lot-to-lot consistency of reagents is a crucial technical challenge for the entire manufacturing process but clearly a prerequisite for meaningful long-term epidemiological data.

Between-sample variability of matrix effects is also an issue in LC-MS/MS, however, it can be equalized and controlled for by the principle of isotope dilution. Yet, an application of LC-MS/MS in laboratory medicine is still based on individual instrument installation and method implementation with a very poor standardization of instrumentation. With such self-developed and not fully automated tests run on highly complex instruments, the risk of systematic bias is given for several reasons, also including many potential sources of gross errors (22, 23).

These technological challenges of routine assays were aggravated by the fact that mass spectrometric reference methods as a guideline for assay development were not available until 2004 – in con-

trast to many steroid analytes which were addressed by mass spectrometric reference methods much earlier. Similarly, in contrast to such analytes as testosterone or progesterone, the reference material preparations have become available only recently. The introduction of LC-MS/MS based methods has profoundly changed the situation of 25-hydroxyvitamin D quantification. It has become state-of-the-art within a short time that routine ligand binding tests are validated against LC-MS/MS; a process which is now based on large series of samples by the manufacturers. Two candidate reference method procedures based on LC-MS/MS have been described so far (19, 24), but LC-MS/MS is also applied now for 25-hydroxyvitamin D measurement in a substantial and growing number of routine laboratories,. Availability of reference methods has also allowed the introduction of the first reference material by the US National Institute of Standardization (NIST) in 2010. The largest vitamin D-proficiency testing scheme, the DEQAS program from the United Kingdom indeed demonstrates a trend to improved harmonization of serum 25-hydroxyvitamin D measurement. More than 1000 laboratories worldwide take part in this scheme; about 10% of these laboratories use LC-MS/MS at present.

In 2004, a landmark study by Binkley et al. (25) highlighted the poor agreement of 25-hydroxyvitamin D results from different routine laboratories. Since that time an important improvement in standardization of 25-hydroxyvitamin D measurement has been achieved, however, a recent survey (26) still demonstrates standardization problems for several automated routine tests.

The quality requirements for 25-hydroxyvitamin D-measurement have been addressed by very important theoretical work (27), which incorporates individual within-person biological variation of the analyte and addresses both diagnosing and monitoring settings as well as distinct performance goals for reference and routine methods.

25-hydroxyvitamin D – an exemplary analyte

For several aspects 25-hydroxyvitamin D can be looked upon as an exemplary analyte in the clinical chemistry:

- The analyte impressively demonstrates the substantial matrix dependency of ligand binding assays. Three of the four first NIST reference materials are based on horse serum; it was found that automated ligand binding tests give in part drastically biased results in these non-human materials.
- The analyte has, in line with the immunosuppressant monitoring, demonstrated the applicability of LC-MS/MS also for large scale application in routine laboratories.
- The analyte has demonstrated that LC-MS/MS may be also limited with respect to specificity toward structural isomers. The more recently identified 3-epi-isomer of 25-hydroxyvitamin D3 is co-quantified together with 25-hydroxyvitamin D in standard LC-MS/MS methods (as shown for the sample of DEQAS scheme), while most immunoassays do not cross-react with this analyte. The relevance of this finding is still elusive since probably in most individuals (except children) the concentrations of the 3-epi isomer is low and there is insufficient knowledge about the actual biological role of the compound; however, it is illustrated that the analytical specificity of LC-MS/MS is not absolute and must be questioned in a systematic way, as it is also the case with immunoassays. It further shows that delicate chromatographic separation – even including isomer separation – may be required for the accurate LC-MS/MS results (28).
- The analyte impressively demonstrated the potential that »home-brew« LC-MS/MS methods generate spurious results although mass spectrometry per se is a very powerful technology. In one of the biggest scandals of clinical chemistry in the US, the commercial Quest Laboratories had to call back thousands of LC-MS/MS results which were traced back to insufficient quality standards in the calibration and the quality management of LC-MS/MS analytics (29). It was also shown that the introduction of common calibration materials for LC-MS/MS can substantially improve the reliability and commutability of LC-MS/MS results for specific analytes.
- The analyte demonstrated that the automation of immunoassays (implemented consequent to increasing numbers of analysis requests) may compromise the quality of analytics. While classical radioimmunoassays for 25-hydroxyvitamin D were (and still are) rather reliable, a high degree of analytical bias was found in particular for the first automated tests. This shows that the commercial interests of *in vitro* diagnostics companies may be in evident competition with the goals of analytical reliability. It is in some assays evident that standards of analytical quality have been in part sacrificed to the potential earnings in a marker showing the substantial increase in request volumes. The rapid commercialization and the prominent marketing of 25-hydroxyvitamin D measurement by both in vitro diagnostics companies and by

other players in healthcare in many countries demonstrate the very high interest to introduce new sources of commercial earning – while the actual contribution of individual 25-hydroxyvitamin D measurement in a public health perspective is at least questionable. The analyte today exemplifies that quality goals for a laboratory test, with respect to agreement of the routine assay results with quasi-reference method results, are set more or less arbitrarily in clinical chemistry. Indeed, the community of laboratory medicine seems to tolerate rather limited correlation and agreement with reference methods in case of 25-hydroxyvitamin D. On the other hand, the analyte shows that the method comparison studies of the routine assays in relation to LC-MS/MS including hundreds of samples, in contrast to very few samples in the GC-MS era, can be recognized as a standard procedure in laboratory medicine.

– The extensive discussion about desired serum concentration ranges of the analyte (e.g., 20 vs. 30 ng/mL) demonstrates what high degree of standardization and commutability of results from different assays and long-term stability of measurement accuracy in a worldwide setting covering decades of observation is expected from the laboratory medicine. Consequently, the idea and the importance of the unbroken chain of traceability in clinical chemistry – from a reference preparation of a standard to individual routine method patient's results – are illustrated very clearly by this analyte.

Ordering of tests – when is measuring 25-hydroxyvitamin D useful?

Probably so far, the most important role of quantification of serum 25-hydroxyvitamin D in medicine has been epidemiological research in the context of huge number of studies. Based on this analyte, a high prevalence of vitamin D-responsive high PTH concentrations in most population studies has been demonstrated and recommendations for lifestyle optimization, individual supplementation but also food fortification have been made. It is intriguing for scientists and physicians that a well-defined and long known physiologically occurring compound is potentially involved in a huge number of health-related processes and chronic diseases, that, in contrast to other vitamins, sub-optimum concentrations have a high prevalence, and that intervention is evidently very simple to achieve. Based on and stimulated by these results, however, there is evidently continuously growing interest to characterize the vitamin D status for the individual person as well. Since this is potentially addressed in a population of wide dimension with substantial impact on healthcare economics, there is an urgent need to elaborate scientifically based recommendations for test ordering of 25-hydroxyvitamin D and/or other tests witch might be useful to characterize the individual vitamin D status. Assessing the indication for individual 25-hydroxyvitamin D quantification at present represents a substantial challenge to laboratory medicine in many regions and settings.

Measurement of serum 25-hydroxyvitamin D can have a useful role in diagnosing the severe and symptomatic vitamin D deficiency with osteomalacia. In such not infrequent cases with bone pain, potentially radiological signs of osteomalacia, low serum calcium concentrations and increase serum alkaline phosphatase activity together with anamnestic features (minimal sun exposure and no supplementation) a definite diagnosis can be made by demonstrating very low serum 25-hydroxyvitamin D. In these cases, a speedy correction of the hypovitaminosis D is warranted and can be achieved by high-dose schemes continued by maintenance doses. Monitoring of the efficiency of this therapy by repeated measurement of 25-hydroxyvitamin D might be considered.

With respect to non-symptomatic individuals, there seems to be widespread agreement that it is probably useful and relevant goal that individuals avoid to have serum 25-hydroxyvitamin D concentrations below 20 ng/mL, and in particular to be in this deficient concentration range for extended periods of time. In many countries the pre-test probability of being vitamin D deficient is very high in autumn, winter and spring on one hand, and on the other, 1000 to 2000 IU of vitamin D supplementation is rather inexpensive and has a very favourable risk profile. It might be concluded that an individual's decision to supplement vitamin D does not have to be guided by analyses performed in healthy people. Given the wide therapeutic range of vitamin D with maximally tolerated daily intake of up to 4000 IU according to the Endocrine Society, a »safety therapeutic drug monitoring« does not seem necessary.

The Endocrine Society (12) recommends no »population screening« for vitamin D deficiency but testing in individuals »at risk for deficiency«, which is a very imprecise statement. More specifically testing is further recommended in people in whom »a prompt response to optimization of vitamin D status could be expected«. This includes patients with the osteomalacia, hyperparathyroidism, older adults with the history of nontraumatic fractures, but also pregnant and lactating women, or Hispanic adults according to opinion of the Endocrine Society.

Monitoring of an individual vitamin D-steady-state supplementation regimen to optimize health effects might be reviewed for several considerations:

- Assessment of compliance
- To rule out, individually, diseases which are associated with high vitamin D requirements or leading to unfavourable pharmacokinetic conditions. Such conditions may lead to serum 25-hydroxyvitamin D concentrations within the deficiency range despite compliance with standard doses (in particular, impaired absorption due to gastrointestinal abnormalities like gluten sensitive enteropathy, pancreatic insufficiency, or situations after major surgery, accelerated metabolization (e.g., by antiepileptic drugs); renal loss of vitamin D binding protein and vitamin D in renal diseases; or overwhelming distribution in fat tissue in obesity).
- To adjust and reduce long term supplementation of vitamin D according to individual lifestyle patterns (sun exposure in specific seasons, clothing habits, use of sun protection, latitude of residence) and physiological variables (pigmentation and skin type, metabolization rate). During which months of the year the supplementation is not necessary? Is 600, 800, 1000, 2000 or even more IU/d, vitamin D required to achieve serum 25-hydroxyvitamin D concentrations above 20 or 30 ng/mL in winter? Obviously, this is a very ambitious approach with respect to logistics of sampling, financial resources and the potential to optimize health effects beyond standard regimens.

There is at present an inconsistent body of data available with respect to that latter issues of pharmacokinetics and individual activation of vitamin D in the skin (2, 30–39). While the recommendations of the Endocrine Society read that »to raise the blood level of 25OHD above 30 ng/mL may require at least 1500–2000 of supplemental vitamin D«, Gallagher et al. (40) report that »a vitamin D(3) dosage of 800 IU/d increased serum 25-(OH)D levels to greater than 50 nmol/L in 97.5% of women.« A fundamental flaw of dose-response studies on long-term oral application of compounds to outpatients is that the degree of compliance cannot be assessed in a conclusive manner.

Long term health effects of serum 25-hydroxyvitamin D in the range of 20–30 ng/mL or above is more or less speculative and probably rather limited. Any attempts to investigate these potential effects are extremely demanding and probably hardly to achieve: it must be assumed that differential health effect in not clearly deficient range but in »sub-optimum« range of 25-hydroxyvitamin D serum concentrations (20–30 ng/mL) may be long-term effects, requiring years or even decades to become detectable. Randomized, placebo controlled studies over such periods of time will hardly be conducted, particularly because serum concentrations would have to be titrated to the relevant concentration range. With the increasing 25-hydroxyvitamin D serum concentrations, most probably beneficial health effects approach »zero« in an asymptotic manner.

Habits of vitamin D supplementation on one hand and measurement of 25-hydroxyvitamin D on the other, and their respective impact on public health are not necessarily linked and should be discussed (more or less) separately. Clearly the first point, supplementation and sun exposure, has much more important impact compared to the latter in otherwise healthy persons. Probably severe hypovitaminosis D is avoided in the overwhelming majority of healthy individuals with 1000 IU vitamin D per day during months with practically absent vitamin D synthesis in the skin (this roughly means October through March in the Central Europe). According to present knowledge, the risk of side effects is minimal. Increased rates of nephrocalcinosis are to be observed only in combination with calcium supplementation, which indeed has a much smaller therapeutic range and should be recommended very carefully (13). Inborn deficiency of 24,25-hydroxylase potentially leading to hypervitaminosis D during supplementation is extremely rare (41) and may be detected by measuring the serum calcium in case of suggestive symptoms.

Further perspectives and challenges

Historically (including rather recent changes in the predominant lifestyle patterns in industrialized societies), the role of vitamin D supply may have changed. In regions with some distance to equatorial latitudes (but also including the Mediterranean region), human population coped with the absence of endogenous vitamin D production during several months of the year for about 100.000 years. Clothing and lifestyle habits of several last generations probably have led to decrease of the vitamin D stores which are acquired during the later summer and which are available for the darker months in the majority of individuals today. Moreover, today millions of people experience almost no exposure to sun light throughout the year. Anyhow, it is not clear at all to what extent vitamin D addressing in adults, being initiated during the past few years, will affect public health.

At present, the laboratory assessment of the vitamin D status is predominantly focused on measurement of 25-hydroxyvitamin D in serum, but it is not at all clear how reliable this marker actually is to this end. 25-hydroxyvitamin D represents a metabolic precursor pool for further conversion to active principle of the vitamin D system (i.e. 1,25-dihydroxyvitamin D3, also termed D-hormone). The actual individual availability of this precursor for activation may be determined by the degree and avidity of binding to vitamin D binding protein, a molecule with well

recognized genetic polymorphism (42). Furthermore, genetic polymorphism of the vitamin D receptor is recognized. This might be also the case for anabolic and catabolic enzymes of 25-hydroxyvitamin D. Based on these considerations, it is likely that the true »optimum« level of total 25-hydroxyvitamin D might indeed be highly variable between individuals. Consequently, it is useful to assess potential functional markers of the vitamin D status. Those, however, may be different with respect to calcemic effects of the vitamin D system and for non-calcemic/extra-skeletal effects, respectively.

Doubtlessly, PTH has an important role in this context; in an outpatient setting, however, the particular preanalytical requirements of this analyte can be a substantial problem. Serum calcium and phosphate are less sensitive markers of vitamin D deficiency, but an assessment of the urinary calcium excretion (in a 24 h-collection or determined as a calcium-to-creatinine ratio) might be also of interest.

The quantification of serum 24, 25-hydroxyvitamin D might have relevance as well (43). It can be assumed that the action of the 24-hydroxylase regulates the vitamin D homeostasis by active metabolization and inactivation of 25-hydroxyvitamin D in case of the excess vitamin D supply. Consequently, an increasing ratio of 25-hydroxyvitamin D3 to 24, 25-hydroxyvitamin D3 may potentially indicate a functional saturation of the vitamin D metabolic system.

Beyond single laboratory analyses related to vitamin D system to a distinct point of time, protocols should be elaborated to characterize the individual vitamin D supply throughout the year. Multi-point measuring procedures, aiming at displaying the 25-hydroxyvitamin D »area-under-the-year curve« (or for other markers, respectively), might be of particular relevance in epidemiological studies (36). It could be also studied if multi-marker approaches to characterize the vitamin D status were useful. Such scores or approaches using biomathematical pattern recognition might include all known markers of the vitamin D status in synopsis but potentially also new markers to be determined in studies with the metabolomic approach.

Dietary uptake of calcium and/or calcium supplementation must evidently be addressed within the vitamin D status context. Indeed, the tolerable range of calcium uptake is far smaller compared to »therapeutic range« of vitamin D, and high calcium load together with sufficient vitamin D status may induce the risk of nephrocalcinosis. However, there is no valid approach to characterize the uptake of calcium individually by laboratory tests at present. This would be very desirable since the prediction of the calcium consumption by food questionnaires is difficult and not very reliable. With 1000 mg per day as a common dosage of calcium supplementation, and an upper tolerable limit of 1200 mg, any significant uptake of calcium from the diet may already be a problem.

Widespread routine vitamin D testing in some industrialized countries has become an economic burden for health care systems. Consequently, for laboratory medicine one essential challenge in the monitoring of the vitamin D status is – despite all analytical issues – to avoid an over use of testing by utilization management. The community of laboratory medicine has to moderate between the legitimate interest in innovative approaches of preventive medicine, on one hand, and the reasonable use of resources on the other hand, by providing the scientifically sound recommendation for ordering of tests (44).

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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