(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 January 2007 (04.01.2007)

PCT

(10) International Publication Number
WO 2007/001969 A1

(51) International Patent Classification:
A01N 45/00 (2006.01)

(21) International Application Number:
PCT/US2006/023774

(22) International Filing Date: 19 June 2006 (19.06.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/694,360 27 June 2005 (27.06.2005) US

(71) Applicant *(for all designated States except US)*: **THE GENERAL HOSPITAL CORPORATION** [US/US]; 55 Fruit Street, Boston, Massachusetts 02114 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **THADHANI, Ravi** [US/US]; 11 Byron Street, Boston, Massachusetts 02108 (US). **WOLF, Myles** [US/US]; 122 Davis Avenue, Brookline, Massachusetts 02445 (US).

(74) Agent: **FASSE, J., Peter**; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/001969 A1

(54) Title: VITAMIN D DEFICIENCY AND DIALYSIS

(57) Abstract: The invention includes methods and kits for reducing the risk of mortality of dialysis by assaying endogenous vitamin D levels in the serum or plasma of a dialysis patient or a patient in need of dialysis, comparing the endogenous vitamin D levels to a threshold level; and providing exogenous vitamin D (e.g., activated vitamin D) to patients with endogenous vitamin D levels below the threshold level.

VITAMIN D DEFICIENCY AND DIALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 60/694,360, filed on June 27, 2005, the entire contents of which are incorporated herein by reference.

BACKGROUND

Dialysis patients suffer from a 20% annual mortality — a prognosis worse than many cancers. Mortality rates in the first 90 days after starting dialysis are even higher. Currently, the typical practice with dialysis patients is to measure levels of parathyroid hormone (PTH), and to administer active vitamin D to patients with high PTH levels to attempt to reduce the levels of this hormone.

Vitamin D therapy can, in some cases, improve the bone and cardiovascular health, and perhaps the longevity, of dialysis patients (Teng et al., *J. Am. Soc. Nephrol.*, 16:1115-25 (2005)). In some patients, however, vitamin D therapy is linked to the development of adverse effects such as hypercalcemia and hyperphosphatemia.

SUMMARY

The invention is based, at least in part, on the discovery that low serum vitamin D levels correlate with higher rates of mortality in hemodialysis patients, and that administration of vitamin D therapy to patients with low vitamin D levels can reduce mortality rates. The invention includes methods of treating dialysis patients by measuring the patients' vitamin D levels, and administering vitamin D therapy to those with low levels to improve their survival on dialysis.

The invention includes methods for reducing the risk of mortality associated with dialysis (e.g., cardiovascular- or infectious-related mortality) by: (a) assaying an amount of endogenous vitamin D, e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D, in a sample e.g., a body fluid (e.g., blood, plasma, serum, or lymph), of a dialysis patient or a patient in need of dialysis; (b) comparing the endogenous vitamin D levels to a threshold level; and (c) if the endogenous vitamin D levels are below the threshold level, administering to the patient vitamin D, e.g., active or activated vitamin D (e.g., calcitriol, doxercalciferol, paricalcitol, or 1,25-dihydroxyvitamin D), in an amount effective to

reduce the patient's risk of mortality while on dialysis. In some embodiments, the decision to administer active vitamin D is made irrespective of an amount of parathyroid hormone (PTH) in a sample from the patient. The threshold level of 25-hydroxyvitamin D is typically a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g., about 10 ng/ml of 25-hydroxyvitamin D in the sample, e.g., the patient's serum or plasma. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D the sample, e.g., the patient's serum or plasma. The threshold level of 1,25-dihydroxyvitamin D is typically a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-dihydroxyvitamin D in the sample, e.g., the patient's serum or plasma. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D in the sample, e.g., the patient's serum or plasma.

The invention also includes methods of monitoring and treating a dialysis patient by: (a) identifying a patient who is undergoing dialysis, will undergo dialysis, or is suspected to require dialysis; and (b) assaying an amount of endogenous vitamin D, e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D, in a sample, e.g., a body fluid (e.g., blood, plasma, serum, or lymph), of the patient; (c) comparing the amount of vitamin D to a threshold level; and (d) if the amount of vitamin D is below the threshold level, providing exogenous vitamin D, e.g., active or activated vitamin D (e.g., calcitriol, doxercalciferol, paricalcitol, or 1,25-dihydroxyvitamin D),to the patient irrespective of serum levels of parathyroid hormone, calcium, or phosphorous. The threshold level of 25-hydroxyvitamin D is typically a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g., about 10 ng/ml of 25-hydroxyvitamin D in the sample, e.g., the patient's serum or plasma. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D the sample, e.g., the patient's serum or plasma. The threshold level of 1,25-dihydroxyvitamin D is typically a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-

dihydroxyvitamin D in the sample, e.g., the patient's serum or plasma. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D in the sample, e.g., the patient's serum or plasma. In some embodiments, the patient has been diagnosed with end-stage renal disease.

The invention also includes methods to estimate risk of mortality (e.g., cardiovascular-related mortality or infectious-related mortality) following initiation of hemodialysis for a given vitamin D level by assaying an endogenous amount of vitamin D, e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D, in samples, e.g., samples of a body fluid (e.g., blood, plasma, serum, or lymph), of a population of dialysis patients; and associating the vitamin D levels with risk of mortality (e.g., a differential risk of mortality among or between groups with differing vitamin D levels). The invention can further include methods of estimating mortality (e.g., cardiovascular-related or infectious-related mortality) in patients following initiation of dialysis by performing the above method, and further assaying vitamin D levels in a patient who is undergoing dialysis, will undergo dialysis, or is suspected to require dialysis, and comparing the vitamin D levels to the levels of the population, thereby estimating a risk of mortality. The patient population can be analyzed to determine threshold levels for a population, e.g., a subset of the patient population, based on patient risk factors included in the individuals within the population, including, e.g., age, sex, race, or ethnicity, and/or whether the patients have been diagnosed with diabetes.

The invention further includes methods for treating a patient undergoing dialysis, will undergo dialysis, or is diagnosed to require dialysis to reduce the risk of mortality (e.g., cardiovascular- or infectious-related mortality) associated with dialysis. The methods include first selecting the patient on the basis that the patient is undergoing dialysis, will undergo dialysis, or is diagnosed to require dialysis, and on the basis that the patient has or is known to have a level of endogenous vitamin D (e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D) below a threshold level, and then administering to the patient (e.g., because the patient has a level of endogenous vitamin D below the threshold level) vitamin D, e.g., active or activated vitamin D (e.g., calcitriol, doxercalciferol, paricalcitol, or 1,25-dihydroxyvitamin D), or a vitamin D composition in

an amount effective to lower the risk of mortality associated with dialysis. The threshold level of 25-hydroxyvitamin D is typically a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g. about 10 ng/ml of 25-hydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D. The threshold level of 1,25-dihydroxyvitamin D is typically a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-dihydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D.

The invention also includes methods for characterizing a patient's risk of mortality (e.g., cardiovascular- or infectious-related mortality) associated with dialysis that include obtaining a level of endogenous vitamin D (e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D) in the patient, comparing the level of endogenous vitamin D to a threshold value, and characterizing the patient's risk of mortality associated with dialysis (e.g., based upon the level of endogenous vitamin D in comparison to the threshold value). The threshold level of 25-hydroxyvitamin D is typically a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g., about 10 ng/ml of 25-hydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D. The threshold level of 1,25-dihydroxyvitamin D is typically a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-dihydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D.

The invention further includes methods for evaluating the likelihood that an patient will benefit from treatment with an agent for reducing the risk of mortality (e.g., cardiovascular- or infectious-related mortality) associated with dialysis, the agent selected from the group consisting of calcitriol, doxercalciferol, paricalcitol, a compound

that activates the vitamin D receptor, and 1,25-dihydroxyvitamin D. The invention includes obtaining a level of endogenous vitamin D (e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D) in the patient; and comparing the level of endogenous vitamin D to a threshold value, wherein a level of endogenous vitamin D below threshold value indicates that the patient will benefit from treatment with the agents. The threshold level of 25-hydroxyvitamin D is typically a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g. about 10 ng/ml of 25-hydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D. The threshold level of 1,25-dihydroxyvitamin D is typically a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-dihydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D.

A risk of mortality described herein can be a risk of mortality within a certain period of time following initiation of hemodialysis. For example, the period of time can be within 360 days of initiating dialysis (e.g., within 360, 330, 300, 270, 240, 210, 180, 150, 120, 90, 60, 30, or 15 days). In some embodiments, the period of time is within 5 years of initiating dialysis, e.g., 4, 3, 2, or 1 year. In some embodiments, a treatment described herein can reduce the risk of mortality of a patient undergoing dialysis by about 1.2-fold to 10-fold, e.g., 9-fold, 8-fold, 7-fold, 6-fold, 5-fold, 4-fold, 3-fold, 2-fold, 1.5-fold, or 1.2-fold, compared to the risk of mortality the patient would have without the treatment.

The invention also includes vitamin D assays (e.g., assay kits) for measuring vitamin D levels, e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D levels, in a body fluid, e.g., blood, plasma, serum, or lymph, that include a standard corresponding to a threshold level of vitamin D. Typically, the threshold level of 25-hydroxyvitamin D is a level between 1 and 30 ng/ml, e.g., between 5 and 15 ng/ml, e.g., between 8 and 12 ng/ml, e.g., between 9 and 11 ng/ml, e.g., about 10 ng/ml of 25-hydroxyvitamin. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 9, 10, 11,

12, 15, 20, 25, or 30 ng/ml of 25-hydroxyvitamin D. Typically, the threshold level of 1,25-dihydroxyvitamin D is a level between about 1 and 25 pg/ml, e.g., between 5 and 15 pg/ml or between 10 and 20 pg/ml, e.g., between 12 and 18 pg/ml, e.g., between 14 and 16 pg/ml, e.g., about 15 pg/ml of 1,25-dihydroxyvitamin D. In some embodiments, the threshold level of 25-hydroxyvitamin D is about 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D. In some embodiments, the assay kits include instructions to use the result of the assay to determine whether to administer vitamin D therapy to the patient if the vitamin D levels are below the threshold. In some embodiments, the assay kits include instructions for performing a method described herein.

The invention also includes kits for measuring an amount of endogenous vitamin D (e.g., 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D) in a sample from a dialysis patient or a patient in need of dialysis and methods of using the kits for determining whether the amount is below a threshold level. The kits include a reference standard corresponding to a threshold level of vitamin D (e.g., a threshold level described herein) and/or a protein that binds specifically to vitamin D (e.g., vitamin D binding protein (VDBP) or an antibody), and instructions to use the kit to compare an amount of endogenous vitamin D in a sample from the patient to the threshold level. In some embodiments, the kit can further include instructions to administer vitamin D therapy to the patient if the measured amount is below the threshold level.

An antibody or protein that "binds specifically to" an antigen, binds preferentially to the antigen in a sample containing other components (e.g., proteins).

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is a histogram showing the distribution of 25-hydroxyvitamin levels among hemodialysis patients in this study. The vertical dashed line indicates the lower limit of the normal range for 25D of 30 ng/ml.

FIG. 2 is a histogram showing the distribution of 1,25-dihydroxyvitamin levels among hemodialysis patients in this study.

FIG. 3 is a chart showing the distribution of 25D levels according to base-line parathyroid hormone (PTH) levels. PTH levels are divided into groups according to the ascending 10th percentiles of the distribution in the entire study population. Mean 25D levels are represented by the central point and bars represent the interquartile ranges (25th and 75th percentiles).

FIG. 4 is a chart showing the distribution of 1,25D levels according to base-line PTH levels. PTH levels are divided into groups according to the ascending 10th percentiles of the distribution in the entire study population. Mean 1,25D levels are represented by the central point and the bars represent the interquartile ranges (25th and 75th percentiles).

FIGs. 5A and 5B are charts showing the multivariable-adjusted risk of 90-day all-cause and cardiovascular-related mortality on hemodialysis according to 25D levels and whether or not patients received treatment with injectable active vitamin D. The reference groups (R) included subjects who were treated with injectable active vitamin D and had 25D levels > 10 ng/ml. * = $P < 0.05$. FIG. 5A shows mortality by all causes. FIG. 5B shows cardiovascular-related mortality.

FIGs. 6A and 6B are charts showing the multivariable-adjusted risk of 90-day all-cause and cardiovascular-related mortality on hemodialysis according to 1,25D levels and whether or not patients received treatment with injectable active vitamin D. The reference groups (R) included subjects who were treated with injectable active vitamin D

and had 1,25D levels > 15 pg/ml. * = P < 0.05. FIG. 6A shows mortality by all causes. FIG. 6B shows cardiovascular-related mortality.

DETAILED DESCRIPTION

Low levels of vitamin D, e.g., 25-hydroxyvitamin D (25-OH D) levels and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$ D), have been discovered to be associated with increased risk of mortality in dialysis patients, and this risk can be reduced by administration of vitamin D therapy. Accordingly, this application describes methods of monitoring and treating dialysis patients to reduce mortality by assaying vitamin D levels in body fluids and administering vitamin D therapy to patients whose levels are below a threshold level.

There are at least six forms of endogenous vitamin D: vitamin D_3 (cholecalciferol), which is the form of vitamin D produced by the body in response to sunlight; vitamin D_2 (ergocalciferol), which is produced in fungi and is used in many vitamin D supplements; and the 25-hydroxy (25-OH) and 1,25-dihydroxy (1,25-$(OH)_2$) forms of each of D_3 and D_2. Synthesis of 25-OH D (25-OH D_2 and 25-OH D_3) occurs primarily in the liver, whereas synthesis of 1,25-$(OH)_2$ D (1,25-$(OH)_2$ D_2 and 1,25-$(OH)_2$ D_3) occurs in the kidneys and, to a smaller extent, in other tissues. As used herein, vitamin D (D) can refer to either 25-OH or 1,25-OH_2, in either the D_3 or D_2 form, or both, or any compound that activates the vitamin D receptor.

Vitamin D levels, e.g., levels of 25-OH D and/or 1,25-$(OH)_2$ D, can be measured in any body fluid. Typically, a sample to be assayed includes blood, plasma, serum, or lymph. Samples of body fluids can be obtained from patients by standard means and assayed before, after, or both before and after commencement of dialysis treatment. Vitamin D levels can be assayed regularly following commencement of dialysis, e.g., monthly, biweekly, weekly, daily, or more often. If at any point the patient's vitamin D levels fall below the threshold, vitamin D therapy can be administered to the patient.

Typically, levels of 25-OH D and/or 1,25-$(OH)_2$ D are measured using any means known in the art, e.g., HPLC, competitive binding assays, and/or assays that utilize proteins that bind to vitamin D, e.g., vitamin D binding protein (VDBP) or antibodies against 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D. For example, the assays

can be enzyme-linked immunosorbent assays (ELISA), enzyme immunoassays (EIA), or radioimmunoassays (RIA).

Assays using high performance liquid chromatography (HPLC) and competitive protein binding assays are described in, e.g., Eisman et al., *Anal. Biochem.*, 80: 298-305 (1977); and Haddad et al., *J. Clin. Endocr.*, 33: 992-995 (1971). The vitamin D transport protein DBP, which has a strong preference for binding 25-OH D over 1,25-$(OH)_2$ D or vitamin D_2 or D_3, itself, can be used in a competitive binding assay (Bouillion et al., *J. Steroid Biochem.*, 13: 1029-1034 (1980)). Assay kits to measure 25-OH D and 1,25-$(OH)_2$ D that utilize ELISA and RIA methods are commercially available, e.g., from DiaSorin (Stillwater, MN), Alpco (Wyndham, NH), and IDS Ltd (Tyne and Wear, United Kingdom).

In general, any vitamin D assay can be used as long as it has good sensitivity and reproducibility in the range of the threshold level selected, such that an accurate assessment can be made as to whether the patient's levels are above or below the threshold. The assays can be performed simultaneously with known quantities of vitamin D to create a standard curve. One of the known quantities can correspond to a threshold level of vitamin D.

The threshold level of 25-hydroxyvitamin D is typically about 10 ng/ml, e.g., between 8 and 12 ng/ml of 25-hydroxyvitamin D, but can be 1, 2, 5, 8, 9, 10, 11, 12, 15, 20, or 30 ng/ml of 25-hydroxyvitamin D in the patient's serum or plasma. The threshold level of 1,25-dihydroxyvitamin D is typically about 15 pg/ml, e.g., between 5 and 15 pg/ml or 10 and 20 pg/ml of 1,25-dihydroxyvitamin D, but can be 1, 2, 5, 8, 10, 12, 14, 15, 16, 18, 20, or 25 pg/ml of 1,25-dihydroxyvitamin D in the patient's serum or plasma. The threshold levels are chosen based on data from a population of patients indicating that administering vitamin D therapy to patients with vitamin D levels below the threshold significantly improves their chances of survival relative to patients with vitamin D levels above the threshold. Vitamin D therapy may or may not significantly improve the survival of patients with vitamin D levels above the threshold. The threshold can be adjusted based on other patient risk factors included in the individuals within the patient population, including, e.g., age, sex, race or ethnicity, and whether the patient has been diagnosed with diabetes. The results described herein indicate that vitamin D deficiency

cannot be reasonably predicted by parameters currently used as a surrogate for vitamin D levels (e.g., PTH, calcium, and phosphorus).

Since vitamin D administration has been associated with adverse consequences in dialysis patients, including alterations in serum minerals and the potential for arterial calcification (Mallick and Berylne, *Lancet*, 2:1316-20 (1968); Cunningham, *Kidney Int. Suppl.*, 73:S59-S64 (1999); and Quinibi et al., *Kidney Int. Suppl.*, 83:73-80 (2002)), it is advantageous to administer vitamin D therapy only to patients who can receive the greatest benefit from the therapy, i.e., patients with low levels of 25-OH D and/or 1,25-$(OH)_2$ D. Typically, the threshold is chosen to improve the outcome of patients with levels below the threshold and to avoid potential adverse consequences of unnecessary vitamin D therapy for patients with levels above the threshold.

If the patient's vitamin D levels, e.g., 25-OH D and/or 1,25-$(OH)_2$ D levels, are below a threshold level, the patient can be administered vitamin D therapy. Non-limiting examples of forms of vitamin D that can be administered include vitamin D_3 (cholecalciferol), vitamin D_2 (ergocalciferol), calcitriol (1,25-$(OH)_2$ D_3, CALCIJEX™, Abbott Laboratories, Abbott Park, IL), doxercalciferol (1α-OH D_2, HECTOROL™, Bone Care International Inc., Middleton, WI), paricalcitol (19-nor-1,25-$(OH)_2$ D_2, ZEMPLAR™, Abbot Laboratories), 1,25-$(OH)_2$ D, or 25-hydroxyvitamin D. Typically, activated forms of vitamin D, e.g., forms that mimic the effect of 1,25-$(OH)_2$ D, e.g., calcitriol, doxercalciferol, or paricalcitol, or any other compound that activates the vitamin D receptor, are administered. Exemplary compounds useful in the methods described herein can be found in WO 2005/051893, WO 2005/051940, WO 2005/051938, WO 2005/051936, WO 2005/051898, WO 2005/037755, WO 2004/048309, US 6,410,523, US 6,603,031, WO 01/07405, WO 00/10958, US 5,380,720, and WO 98/39292. Some forms of vitamin D, e.g., paricalcitol, may provide a greater benefit than other forms, e.g., calcitriol, for a given patient.

The route of administration and dosage can be tailored to the type of therapy administered to provide a pharmaceutically effective amount. Typically, vitamin D therapy is administered orally or intravenously. In a comparison of survival rates of dialysis patients administered paricalcitol or calcitriol, paricalcitol was reported to

provide a significant long-term survival advantage over calcitriol (Teng et al., *New Engl. J. Med.*, 349:446-456 (2003)).

The biologically active form of vitamin D is the calcitropic hormone 1,25-dihydroxyvitamin D. This form is produced by hydroxylation of 25-hydroxyvitamin D in the kidney, after which the active form circulates in the blood in about picogram quantities. Measures of circulating 1,25-dihydroxyvitamin D primarily reflect kidney conversion of 25-OH D to 1,25-$(OH)_2$ D. In patients with end-stage renal disease (ESRD), the kidneys are no longer capable of converting 25-OH D to 1,25-$(OH)_2$ D, although a small amount of 1,25-$(OH)_2$ D is produced in other tissues of the body, where it is utilized locally (Reviewed in Dusso et al., *Am. J. Physiol. Renal Physiol.*, 289:F8-F28 (2005)). In patients with ESRD, it is predicted from the data presented herein that adequate levels of circulating 25-OH D are required to provide the substrate for local production of 1,25-$(OH)_2$ D by the tissues where it is required. Administration of vitamin D therapy to patients with low 25-OH D levels can increase the levels of the 25-OH D substrate to produce 1,25-$(OH)_2$ D locally in tissues where it is necessary. Cells throughout the body, including in the kidney, can convert 25-OH D to 1,25-$(OH)_2$ D, but this conversion is impaired in states of elevated phosphorus, acidosis, elevated levels of FGF-23, and uremia, all conditions prevalent in patients with kidney failure. Therefore, when serum levels of 25-OH D are low, therapy can include activated vitamin D (e.g., calcitriol, doxercalciferol, paricalcitol, or 1,25-$(OH)_2$ D) to overcome this impaired conversion and to provide cells throughout the body with the activated hormone form of vitamin D.

Also contemplated are assay kits used to measure vitamin D levels and determine whether to administer vitamin D therapy to a hemodialysis patient. In some embodiments, the kits include directions to determine whether an amount of vitamin D in a patient sample is above or below a threshold level described herein. In some embodiments, the kits include instructions to compare a measured level of vitamin D in a patient sample to a threshold level described herein. The kits can include a reference standard that corresponds directly to a threshold level of vitamin D described herein, or instructions to prepare a reference standard (e.g., by dilution of a stock solution) that corresponds to a threshold level described herein. In some embodiments, the kits include

a protein that binds to a vitamin D (e.g., an antibody specific for a vitamin D or vitamin D binding protein (VDBP), which can be conjugated to a detectable entity such as a radioisotope or enzyme. The kits can utilize a radioimmunoassay (RIA) or enzyme immunoassay (EIA) method (e.g., an enzyme immunosorbent assay (ELISA) method). The amount of vitamin D in a sample can be measured, e.g., by assaying binding to the protein that binds vitamin D and/or by measuring competition for binding to the protein that binds vitamin D between vitamin D in the patient sample and a labeled (e.g., radiolabeled) or immobilized vitamin D. In some embodiments, the kits can include reagents for extracting vitamin D from other components of the patient sample.

EXAMPLES

The invention is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the invention in any way.

Example 1. Vitamin D therapy alleviates an increased risk of mortality in patients with low levels of 25-OH D

To determine the correlation between vitamin D levels and mortality, a nested case-control study of incident hemodialysis patients was performed.

Patient data included information from 140 patients who initiated hemodialysis but died within the first 90 days of starting dialysis, and approximately 300 patients who initiated dialysis and survived the first 90 days dialysis. For all patients, serum samples were collected within the first 14 days of starting hemodialysis. Serum levels of 25-hydroxyvitamin D (25-OH D) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2$ D) were measured in these samples. Information on therapy with active vitamin D was also included in the patient data.

The results, as shown below, can be summarized as follows:

1. Low levels of 25-OH D are independently associated with increased risk of 90 day mortality in dialysis patients.

2. The risk of mortality is mitigated at all levels of 25-OH D by therapy with activated vitamin D.

3. The threshold level of 25-OH D that determines whether a given patient should receive vitamin D therapy is about 10 ng/ml.

Therefore, we show for the first time that low levels of 25-OH vitamin D are associated with increased risk of mortality after starting dialysis, and that the risk can be reduced if a patient also receives vitamin D (replacement for 25-OH or $1,25\text{-}OH_2$). Measured levels of $1,25\text{-}(OH)_2$ D were not associated significantly with risk of mortality in this study.

For each of the tables below, the Odds Ratio indicates the ratio of the estimated risk of death, as compared to a reference value. The 95% confidence interval (95% CI) gives an estimate of the range in which a given odds ratio is 95% likely to fall. The P value (P) indicates the probability that the measured odds ratio is not different from the measured reference value. A lower P value indicates higher statistical significance.

Table 1 presents a univariate analysis of 90-day survival according to 25-OH D levels at the start of hemodialysis expressed as a continuous variable. This analysis shows that risk of death in the first 90 days of dialysis decreases as 25-OH D levels increase. The measured odds ratio of 0.98 indicates that there is on average a 2% reduction in risk of mortality for each increase of 1 ng/ml of 25-OH D at the start of hemodialysis.

Table 1. 90-day survival according to baseline 25-OH D levels at the start of hemodialysis expressed as a continuous variable

	Odds Ratio	95% CI	P
Per 1 ng/ml increase in 25-OH D	0.98	0.97, 0.99	0.04

Table 2 presents a bivariate analysis of 90-day survival according to baseline 25-OH D levels at the start of dialysis. The odds ratio of 2.37 indicates that patients with 25-OH D levels less than 10 ng/ml at the start of dialysis had more than two times (2.37 times) the risk of death within the first 90 days of dialysis. This result was statistically significant (P = 0.008).

Table 2. 90-day survival according to baseline 25-OH D levels (≥ 10 or <10 ng/ml) at the start of hemodialysis

	Odds Ratio	95% CI	P
25-D ≥ 10 ng/ml	1	-	-
25-D < 10 ng/ml	2.37	1.25, 4.47	0.008

Table 3 presents a bivariate analysis comparing 90-day survival of patients who did not receive intravenous vitamin D therapy with patients who received some form of intravenous vitamin D. The odds ratio of 0.46 for IV vitamin D indicates that patients who received intravenous vitamin D therapy had less than half the risk of mortality compared to those who did not receive intravenous vitamin D. This result was statistically significant (P < 0.001).

Table 3. 90-day survival comparing IV vitamin D treatment versus no IV vitamin D

	Odds Ratio	95% CI	P
No IV vitamin D	1	-	-
IV vitamin D	0.46	0.30, 0.68	< 0.001

Table 4 presents a bivariate analysis comparing 90-day survival of patients who received no intravenous vitamin D, with patients who received paricalcitol or either calcitriol or doxercalciferol. The intravenous vitamin D agents decreased the risk of mortality by similar levels. The odds ratio for patients treated with calcitriol or doxercalciferol compared to no treatment was 0.54, whereas the odds ratio for patients treated with paricalcitol compared to no treatment was 0.41. Both results were statistically significant. The apparent difference between the sets of vitamin D agents is not seen when the data are analyzed by multivariate analysis.

Table 4. 90-day survival according to type of IV vitamin D versus no IV vitamin D

	Odds Ratio	95% CI	P
No IV vitamin D	1	-	-
Calcitriol/Doxercalciferol	0.54	0.31, 0.92	0.02
Paricalcitol	0.41	0.26, 0.66	< 0.001

Table 5 presents a bivariate analysis comparing 90-day survival among patients with high initial vitamin D levels ($\geq$ 10 ng/ml) who received intravenous vitamin D therapy (High D – IV D+) with each of: patients with high initial vitamin D levels who did not receive intravenous vitamin D therapy (High D – IV D-), patients with low initial vitamin D levels (< 10 ng/ml) who received intravenous vitamin D therapy (Low D – IV D+), and patients with low initial vitamin D levels who did not receive intravenous vitamin D therapy (Low D – IV D-). All of the groups had a higher risk of mortality than the High D – IV D+ group (odds ratio of 2.11 for patients with high initial levels of 25-OH D who did not receive intravenous D, 2.02 for patients with low initial levels of 25-OH D who did receive intravenous D, and 5.74 for patients with low initial levels of 25-OH D who did not receive intravenous D, all compared to patients with high initial levels of 25-OH D who did receive intravenous D). The difference in survival for patients with high and low initial levels of 25-OH D who received intravenous vitamin D was not statistically significant (P = 0.14). The greatest risk of mortality was seen in those patients with low initial vitamin D levels who did not receive intravenous vitamin D therapy. Those patients had almost six times the risk of death in the 90 days following dialysis as patients with high initial vitamin D levels who received vitamin D therapy (Odds Ratio of 5.74 compared to the reference group, P < 0.001).

Table 5. 90-day survival according to baseline 25-OH D levels (≥ 10 or < 10 ng/ml) at the start of hemodialysis and any IV vitamin D treatment versus none

	Odds Ratio	95% CI	P
High D – IV D+	1	-	-
High D – IV D-	2.11	1.36, 3.29	0.001
Low D – IV D+	2.02	0.80, 5.09	0.14
Low D – IV D-	5.74	2.25, 14.63	< 0.001

Table 6 presents a bivariate analysis comparing 90-day survival of patients with high (≥ 20 ng/ml), intermediate (10-20 ng/ml), or low (< 10 ng/ml) initial 25-OH D levels who received intravenous vitamin D treatment or none. The least risk of mortality was seen in patients with high initial 25-OH D levels who received some form of intravenous vitamin D therapy. The highest risk of mortality was seen in patients with low initial 25-OH D levels who did not receive intravenous vitamin D therapy (odds ratio of 6.11 compared to patients with high initial 25-OH D levels who did receive intravenous vitamin D therapy, $P < 0.001$). The risk of mortality was similar for patients with high and intermediate initial 25-OH D levels in both the treated and untreated groups.

Table 6. 90-day survival according to baseline 25-OH D levels (< 10, 10 – 20 or ≥ 20 ng/ml) at the start of hemodialysis and any IV vitamin D treatment versus none

	Odds Ratio	95% CI	P
High D – IV D+	1	-	-
Intermediate D – IV D+	1.17	0.61, 2.25	0.63
Low D – IV D+	2.15	0.82, 5.61	0.12
High D – IV D-	2.38	1.37, 4.15	0.002
Intermediate D – IV D-	1.98	0.99, 3.96	0.05
Low D – IV D-	6.11	2.31, 16.13	< 0.001

Table 7 presents multivariate analyses of the contribution of multiple factors to the risk of mortality in the 90 days following the start of hemodialysis. These factors

include initial vitamin D levels ($\geq$ 10 ng/ml – High; < 10 ng/ml – Low), treatment with intravenous vitamin D therapy (IV D+), age, mean arterial pressure (MAP), serum albumin, and means of dialysis access (Graft, Fistula, or Catheter). Again, it was observed that patients with low initial 25-OH D levels who did not receive intravenous vitamin D therapy were almost six times as likely (odds ratio 5.66, P = 0.002) to not survive for 90 days on hemodialysis compared to patients with high initial 25-OH D levels who did receive vitamin D therapy. Survival was found to decrease with age (odds ratio 1.03 per year, P = 0.005), and increase with higher mean arterial pressure (odds ratio 0.97 per unit mmHg, P < 0.001) and higher serum albumin concentration (odds ratio 0.50 per g/dl, P = 0.001). Patients with graft or catheter dialysis access had poorer survival rates than those with fistula access (odds ratio 8.8 and 22.5, respectively).

Table 7. MV analysis using D, no D, 25-D cut point ($\geq$ or < 10 ng/ml) and significant covariates (parsimonious models)

	Odds Ratio	95% CI	P
High D – IV D+	1	-	-
High D – IV D-	1.62	0.99, 2.67	0.06
Low D – IV D+	2.13	0.80, 5.09	0.16
Low D – IV D-	5.66	1.93, 16.60	0.002
Age (per year)	1.03	1.01, 1.05	0.005
MAP (per unit mmHg)	0.97	0.96, 0.99	< 0.001
Albumin (per g/dl)	0.50	0.32, 0.76	0.001
Graft v Fistula	8.8	1.03, 74.6	0.05
Catheter v Fistula	22.5	2.9, 175.8	0.003

Taken together, these results indicate that administration of vitamin D therapy to dialysis patients with low 25-hydroxyvitamin D levels (< 10 ng/ml) significantly improves their survival.

Example 2. Vitamin D Therapy Alleviates an Increased Risk of Mortality in Patients with Low Levels of 25-OH D and/or 1,25-$(OH)_2$ D

Methods

Study Population

For this study, we selected 250 consecutive hemodialysis patients who died within 90 days of initiating dialysis. A random selection of 750 consecutive patients who initiated hemodialysis contemporaneously and survived for at least 90 days served as the control group. Sixteen subjects had insufficient serum samples for vitamin D assays. Given that the priority was to measure 25-hydroxyvitamin D (25D) first and then 1,25-dihydroxyvitamin D (1,25D), 984 subjects were included in the analyses of 25D, whereas 703 had adequate remaining samples to assay 1,25D levels. Data were not retrospectively abstracted from patient records.

Exposures and Outcomes

The primary exposures were circulating serum levels of 25D and 1,25D measured at base-line (within 14 days of initiating hemodialysis), prior to the start of any oral or injectable vitamin D. 25D and 1,25D levels were measured using radioimmunoassay (DiaSorin Inc., Stillwater, MN). The coefficients of variation (CVs) for 25D measurements were < 3% at levels < 30 ng/ml and for 1,25D the CVs were < 6.5% at levels < 32.5 pg/ml. Based on clinically accepted definitions (Holick, *Mayo Clin. Proc.*, 81:353-73 (2006); Feldman et al., *Vitamin D*, MA: Elsevier (2005)) and for purposes of interpretability, 25D levels ≥ 30 ng/ml were considered replete, whereas vitamin D deficiency was defined as a level of < 30 ng/ml. Severe deficiency was defined as a level < 10 ng/ml. Since cut-points for 1,25D levels in a kidney disease population are unclear, we analyzed tertiles of 1,25D levels according to its distribution in the overall control population. The primary outcome was death within the first 90 days after initiating dialysis and the secondary outcome was cardiovascular mortality. Death was verified by confirmation of discharge diagnosis reports from dialysis centers and cardiovascular deaths were determined based on ICD-9 mortality codes as has been done in prior studies (Teng et al., *N. Engl. J. Med.*, 349:446-56 (2003); Teng et al., *J. Am. Soc. Nephrol.*, 16:1115-25 (2005)).

Analysis

Standard descriptive statistics were used to present demographic and laboratory characteristics of the overall study population at the initiation of chronic hemodialysis. We examined the prevalence of vitamin D deficiency in the overall study population and according to gender, race, and diabetes history. To test whether routine laboratory tests of mineral metabolism could predict decreased vitamin D levels, we examined correlation between vitamin D levels and biomarkers of mineral metabolism, and compared levels of these parameters according to the 25D and 1,25D cut points.

Cox proportional hazards analysis was used to examine survival on hemodialysis according to base-line vitamin D levels. Subjects were censored if they underwent kidney transplantation or recovered renal function within the study period. We performed separate analyses for 25D and 1,25D. We examined 25D and 1,25D as continuous exposures and also considered them as categorical exposures using the cut points described above to test for potential nonlinear associations with mortality. Although injectable vitamin D is frequently used in dialysis populations, the first dose may not be administered for weeks or months after initiation of dialysis. Therefore, we considered active injectable vitamin D as a time-dependent covariate, attributing survival time before initiating injectable vitamin D to the "no" vitamin D arm, and only attributing survival time to the "yes" vitamin D arm after injectable vitamin D was begun. We tested for effect modification between vitamin D levels and active injectable vitamin D in multivariable Cox models. When significant interaction was detected, subsequent survival analyses were stratified according to "any" versus "no" active injectable vitamin D therapy, and models with interaction terms (n – 1) were examined.

Multivariable analyses were used to adjust for potential confounders, including age, gender, race, etiology of renal failure, blood pressure, body mass index, dialysis access at initiation (arteriovenous fistula, graft or venovenous catheter), dialysis dose assessed by the urea reduction ratio, facility specific standardized mortality rates (SMR) (Lacson et al., *Am. J. Kidney Dis.*, 37:267-75 (2001)), and comorbidities at the initiation of dialysis (diabetes, hypertension, coronary artery disease, myocardial infarction, peripheral vascular disease, stroke, congestive heart failure, chronic obstructive

pulmonary disease, noncutaneous malignancy and liver disease). To further capture overall health status at the initiation of dialysis we adjusted for the first results of laboratory tests obtained after initiating dialysis (serum sodium, potassium, bicarbonate, blood urea nitrogen, creatinine, calcium, phosphate, PTH, albumin, hemoglobin, white blood cell count, ferritin and total cholesterol levels) and whether patients initiated hemodialysis as an outpatient in the dialysis center versus in the hospital. Since the primary exposures were vitamin D levels, which are influenced by climate and season, we adjusted for the season (summer: April 1 – September 30 versus winter: October 1 – March 30) and the states (divided categorically according to latitude) in which patients initiated dialysis as has been done in previous vitamin D studies (Holick, *Mayo Clin. Proc.*, 81:353-73 (2006); Holick et al., *J. Clin. Endocrinol. Metab.*, 90:3215-24 (2005); Zittermann et al., *Br. J. Nutr.*, 94:483-92 (2005)). Finally, we measured vitamin D binding protein (DBP, the primary binding protein for circulating vitamin D) in a randomly selected subset of 100 cases and 200 controls using an automated immunonephelometric method (Dade Behring BNII) with CV's < 7%, which permitted analyses of the association between serum levels of vitamin D and mortality independent of its carrier protein. Intercooled Stata 7.0 (Stata Corporation, College Station, TX) and SAS (Cary, North Carolina) were used to perform the analyses. P values < 0.05 were considered statistically significant.

Results

Demographics and Circulating 25D and 1,25D Levels

Of the 10,018 prospectively enrolled patients representing 1,056 dialysis units throughout the U.S., 19 (0.2%) had serum levels of either 25D or 1,25D measured for routine clinical purposes within the first 90 days of initiating hemodialysis. The nested case-control subset represented 569 unique chronic hemodialysis units in 37 different states. Base-line characteristics of the nested subset at the initiation of dialysis are presented in Table 8. Distributions of serum 25D and 1,25D levels are shown in FIGs. 1 and 2. The mean serum levels of 25D were 21 ± 13 ng/ml. Only 21% of subjects had 25D levels > 30 ng/ml, while 60% had levels between 10 and 30 ng/ml; the remaining 19% were severely vitamin D deficient (< 10 ng/ml). Compared with men, women were

more likely to be severely 25D deficient (23% vs. 15%; $P < 0.01$) and less likely to have normal levels (14% vs. 26%; $P < 0.01$). Compared to patients without diabetes, those with diabetes were more likely to be severely 25D deficient (22% vs. 17%; $P = 0.05$) and less likely to have normal levels (16% vs. 23%; $P < 0.01$). The mean serum levels of 1,25D were 11 ± 10 pg/ml; 14% of subjects had undetectable 1,25D levels; 24% had levels > 15 pg/ml. There was no difference in 1,25D levels between men and women or between patients with and without diabetes.

Table 8. Base-line characteristics of the overall study population (n = 1,000) at the initiation of chronic hemodialysis

Demographic characteristics	
Age (yrs)	64.7 ± 15.3
Gender (% women)	46.8
Race (%)	
White	62.6
Black	30.5
Other	6.8
Ethnicity (% Hispanic)	13.2
Mean arterial pressure (mm Hg)	96 ± 18
Body mass index (kg/m2)	27.2 ± 6.9
Etiology of renal failure (%)	
Diabetes mellitus	43.2
Hypertension	34.8
Glomerulonephritis	9.0
Polycystic kidney disease	2.2
Other	10.8
Access at the initiation of hemodialysis (%)	
Arteriovenous fistula	22.9
Arteriovenous graft	11.2
Catheter	61.8
Dialysis initiation site (% inpatient)	78.9
Comorbid conditions (%)	
Coronary artery disease/myocardial infarction (%)	16.7
Congestive heart failure (%)	22.2
Peripheral vascular disease (%)	10.2
Stroke (%)	4.8
Malignancy (%)	5.4
Chronic obstructive pulmonary disease (%)	4.0
Liver disease (%)	6.1
Hepatitis B surface antigen + (%)	0.4
Laboratory tests	
Calcium (mg/dl)	8.4 ± 0.8
Phosphate (mg/dl)	4.6 ± 1.6
Parathyroid hormone (intact; pg/ml)	252 ± 217
Albumin (g/dl)	3.4 ± 0.6
Creatinine (mg/dl)	6.1 ± 2.7
Total cholesterol (mg/dl)	150 ± 49
Urea reduction ratio (%)	68 ± 11
White blood cell count (cells/mm^3)	9.0 ± 4.8
Platelet count ($10^3/mm^3$)	235 ± 100
Hemoglobin (g/l)	10.4 ± 1.4
Ferritin (ng/ml)	416 ± 1119

25D levels correlated weakly with serum levels of calcium (r = 0.19), PTH (r = – 0.13), and albumin (r = 0.30); there was no correlation with serum phosphate or creatinine. Table 9 illustrates mineral and nutrition metabolites according to 25D levels. Subjects with vitamin D deficiency had slightly lower serum calcium and albumin levels, and increased PTH and alkaline phosphatase levels compared to subjects with normal 25D stores.

Table 9. Markers of mineral metabolism, nutrition and renal function according to serum 25D levels*

	Severely Deficient < 10 ng/ml n = 187	Deficient 10 – 30 ng/ml n = 594	Replete > 30 ng/ml n = 203	P
Calcium (mg/dl)	8.3 ± 0.8	8.4 ± 0.8	8.7 ± 0.8	< 0.01
Phosphate (mg/dl)	4.6 ± 1.6	4.5 ± 1.5	4.7 ± 1.6	NS
Mean PTH (pg/ml)*	285 ± 220	257 ± 232	202 ± 165	0.02
Alkaline phosphatase (U/L)	116 ± 220	107 ± 232	97 ± 165	< 0.01
Albumin (g/dl)	3.2 ± 0.6	3.4 ± 0.5	3.7 ± 0.5	< 0.01
Creatinine (mg/dl)	6.1 ± 2.5	6.0 ± 2.7	6.1 ± 2.9	NS

* To convert values for 25D to nanomoles per liter, multiply by 2.50. To convert values for parathyroid hormone to picomoles per liter, multiply by 0.11.

The strongest correlate of 1,25D levels was the serum 25D (r = 0.31). Other factors that were correlated, albeit weakly, with 1,25D included serum calcium (r = 0.13) and albumin (r = 0.23); there was no correlation with serum PTH, phosphate, or creatinine. Importantly, 78% of patients with PTH levels < 300 pg/ml (above which vitamin D therapy is usually started and below which it is not) had blood vitamin D levels < 30 ng/ml. Table 10 illustrates mineral and nutrition metabolites according to tertiles of serum 1,25D. Compared to subjects in the upper 1,25D tertile, those in the lower tertiles had decreased serum calcium and albumin levels. There was no association between 1,25D tertiles and serum phosphate, PTH, alkaline phosphatase or creatinine. FIGs. 3 and 4 illustrate the overlap in the distributions of 25D and 1,25D levels, respectively,

according to base-line PTH levels, and suggest that PTH levels are poor surrogate measures of vitamin D levels.

Table 10. Markers of mineral metabolism, nutrition and renal function according to serum 1,25D levels*

	Tertile 1 < 5 pg/ml n = 220	Tertile 2 5 – 15 pg/ml n = 248	Tertile 3 > 15 pg/ml n = 235	P
Calcium (mg/dl)	8.3 ± 0.9	8.4 ± 0.8	8.5 ± 0.8	< 0.01
Phosphate (mg/dl)	4.7 ± 1.7	4.5 ± 1.6	4.5 ± 1.5	NS
Mean PTH (pg/ml)*	234 ± 203	272 ± 238	244 ± 230	NS
Alkaline phosphatase (U/L)	105 ± 65	111 ± 105	100 ± 61	NS
Albumin (g/dl)	3.2 ± 0.6	3.5 ± 0.6	3.6 ± 0.5	< 0.01
Creatinine (mg/dl)	6.4 ± 2.8	5.9 ± 2.5	5.8 ± 2.8	NS

* To convert values for 1,25D to picomoles per liter, multiply by 2.40. To convert values for parathyroid hormone to picomoles per liter, multiply by 0.11.

Circulating 25D, 1,25D and Hemodialysis Survival

There were no significant differences in mean levels of 25D (20.7 ± 11.5 vs. 21.3 ± 13.1 ng/ml; P = NS) or 1,25D (10.3 ± 7.7 vs. 11.9 ± 10.5 pg/ml; P = NS) comparing subjects who died with those who survived. The results did not change when comparing log-transformed values or when using non-parametric tests. We then examined potential nonlinear associations between levels of 25D, 1,25D and survival using categories defined by cut points described above. As shown in Table 11, subjects with severe 25D deficiency (< 10 ng/ml) were at significantly increased risk of all-cause and cardiovascular related mortality compared to subjects with normal 25D levels in the age, gender and race-adjusted analyses. The increased risk of all-cause mortality remained significant in the multivariable analyses. There was also a trend towards increased risk for early mortality with decreased 1,25D levels (Table 12). Adjusting the models for vitamin D binding protein levels did not alter the results.

Table 11. Risk of death within 90 days of initiating chronic hemodialysis according to base-line 25D levels

25D	< 10 ng/ml	10 – 30 ng/ml	> 30 ng/ml	P for trend
All Cause Mortality				
Age, gender, race-adjusted	1.92	1.42	1.0	< 0.01
95% CI	(1.30 – 2.86)	(1.03 – 1.95)	(REF)	
Multivariable-adjusted*	1.56	1.27	1.0	0.31
95% CI	(1.01 – 2.42)	(0.90 – 1.79)	(REF)	
Cardiovascular Mortality				
Age, gender, race-adjusted	1.87	1.81	1.0	0.01
95% CI	(1.03 – 3.39)	(1.15 – 2.85)	(REF)	
Multivariable-adjusted*	1.55	1.59	1.0	0.21
95% CI	(0.81 – 2.96)	(0.97 – 2.59)	(REF)	

* Multivariable analyses were adjusted for age, gender, race, ethnicity, BMI, vascular access, BP, cause of ESRD, comorbidities, serum albumin, calcium, phosphate, PTH, white blood cells, hemoglobin, vitamin D binding protein levels, season, latitude, and facility-specific standardized mortality rates.

Table 12. Risk of death within 90 days of initiating chronic hemodialysis according to base-line 1, 25D levels

1,25D	< 5 pg/ml	5 – 15 pg/ml	> 15 pg/ml	P for trend
All Cause Mortality				
Age, gender, race-adjusted	1.41	1.23	1.0	0.01
95% CI	(1.01 – 1.97)	(0.83 – 1.81)	(REF)	
Multivariable-adjusted*	1.28	1.27	1.0	0.06
95% CI	(0.90 – 1.84)	(0.85 – 1.88)	(REF)	
Cardiovascular Mortality				
Age, gender, race-adjusted	1.55	1.18	1.0	0.22
95% CI	(0.98 – 2.43)	(0.69 – 1.99)	(REF)	
Multivariable-adjusted*	1.42	1.13	1.0	0.33
95% CI	(0.86 – 2.32)	(0.66 – 1.94)	(REF)	

* Multivariable analyses were adjusted for age, gender, race, ethnicity, BMI, vascular access, BP, cause of ESRD, comorbidities, serum albumin, calcium, phosphate, PTH, white blood cells, hemoglobin, vitamin D binding protein levels, season, latitude, and facility-specific standardized mortality rates.

Relationship between Vitamin D Levels, Injectable Vitamin D Therapy, and Mortality

Compared to no treatment, therapy with injectable vitamin D was associated with a 90-day survival advantage following initiation of hemodialysis (HR 0.41; 95% CI 0.31, 0.54; $P < 0.01$). Although the survival advantage associated with injectable vitamin D therapy was greatest among subjects with PTH > 300 pg/ml (HR 0.28; 95% CI 0.14, 0.53), there was also a significant survival advantage associated with therapy at lower levels of base-line serum PTH (PTH 150-300: HR 0.36; 95% CI 0.21, 0.61; PTH < 150: HR 0.57; 95% CI 0.33, 0.97; P for trend < 0.01), suggesting that PTH does not identify all patients who may benefit from injectable vitamin D.

Given the association between injectable vitamin D therapy and outcomes, and that therapy with injectable vitamin D can correct vitamin D deficiency, we tested for potential effect modification between serum vitamin D levels and survival by injectable vitamin D therapy. There was significant interaction between active injectable vitamin D therapy, vitamin D levels ($P < 0.01$ for *25D x injectable vitamin D*; $P = 0.01$ for *1,25D x injectable vitamin D*), and survival. Therefore, we performed additional analyses using 3 (n – 1) interaction categories, defining binary cut-points based on analyses in Tables 11 and 12. FIGs. 5 and 6 illustrate the risk of all-cause and cardiovascular mortality among groups defined by their vitamin D levels and whether or not they received injectable vitamin D therapy. In these multivariable-adjusted analyses, the reference groups included subjects who were treated with injectable vitamin D and who had 25D levels ≥ 10 ng/ml (FIG. 5) or were in the upper tertile (≥ 15 pg/ml) for serum 1,25D (FIG. 6), respectively. Compared to patients with serum 25D ≥ 10 ng/ml or 1,25D ≥ 15 pg/ml who received injectable vitamin D, those who were untreated and had base-line 25D levels < 10 ng/ml (HR 5.6; 95% CI 2.5, 12.2) or 1,25D levels < 15 pg/ml (HR 3.1; 95% CI 1.7, 5.6) were at significantly increased risk of early all-cause mortality. The results were comparable for cardiovascular-related mortality.

Vitamin D Levels and Hemodialysis Survival among African-Americans

Compared with Caucasians, African Americans had significantly lower mean 25D levels (17 ± 10 vs. 24 ± 13 ng/ml; $P < 0.01$) and were significantly more likely to be severely 25D deficient (30% vs. 14%; $P < 0.01$). Although there was no significant

difference in base-line 1,25D levels, African-Americans also had significantly increased base-line PTH levels compared with Caucasians (349 ± 272 vs. 218 ± 181; $P < 0.01$), were significantly more likely to have a PTH level > 300 (40% vs. 19%; $P < 0.01$), and were significantly more likely to be treated with injectable vitamin D (75% vs. 57%; $P < 0.01$) within 90 days of initiating hemodialysis. African-Americans who did not receive injectable vitamin D and had 25D levels < 10 ng/ml were at markedly increased risk for all-cause (HR 9.4, 95% CI 2.8, 32.1) and cardiovascular-related (HR 11.9, 95% CI 2.5, 57.1) mortality compared to those who were treated and had adequate 25D stores. Those with similarly low levels of 25D but who were treated had risks for all-cause (HR 2.7, 95% CI 1.0, 2.7) and cardiovascular related (HR, 2.9, 0.8, 10.1) mortality that were lower than untreated patients. Similarly, African-Americans with 1,25D levels < 15 pg/ml who did not receive injectable vitamin D therapy were at markedly increased risk of all-cause (HR 5.4, 95% CI 1.4, 21.5) and cardiovascular-related (HR 15.0, 95% CI 2.5, 95.7) mortality compared with those who had 1,25D levels ≥ 15 pg/ml and who received injectable vitamin D therapy. Those with similarly low levels of 1,25D but who were treated were at slightly lower risk for all-cause (HR 4.6, 95% CI 2.6, 12.7) and cardiovascular related mortality (HR 7.4, 95% CI 1.9, 29.5) compared to patients who were treated. Importantly, there was poor correlation between PTH and 25D ($r = -0.11$) or 1,25D ($r = 0.05$), even among African-Americans who were most vitamin D deficient. Indeed, in 88% of African-Americans with 25D levels < 10ng/ml and 85% with 1,25D levels < 15 pg/ml, serum PTH levels were < 300 pg/ml, indicating that PTH was a poor predictor of low vitamin D levels in African-Americans.

OTHER EMBODIMENTS

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A kit for determining whether to administer vitamin D therapy to a dialysis patient or a patient in need of dialysis, comprising:

a reference standard corresponding to a threshold level of vitamin D; and

instructions to compare an amount of endogenous vitamin D in a sample from the patient to the threshold level, and administer vitamin D therapy to the patient if the amount in the patient sample is below the threshold level.

2. The kit of claim 1, further comprising a protein that binds to vitamin D.

3. A kit for determining whether to administer vitamin D therapy to a dialysis patient or a patient in need of dialysis, comprising:

a protein that binds specifically to vitamin D; and

instructions to compare an amount of endogenous vitamin D in a sample from the patient to the threshold level, and administer vitamin D therapy to the patient if the amount in the patient sample is below the threshold level.

4. The kit of any of claims 1-3, wherein the endogenous vitamin D is 25-OH vitamin D.

5. The kit of claim 4, wherein the threshold level is a level between 8 and 12 ng/ml 25-OH vitamin D.

6. The kit of claim 5, wherein the threshold level is about 10 ng/ml 25-OH vitamin D.

7. The kit of any of claims 1-3, wherein the endogenous vitamin D is 1,25-dihydroxyvitamin D.

8. The kit of claim 7, wherein the threshold level is a level between 10 and 20 pg/ml 1,25-dihydroxyvitamin D.

9. The kit of claim 8, wherein the threshold level is about 15 pg/ml 1,25-dihydroxyvitamin D.

10. A method of reducing the risk of mortality associated with dialysis, the method comprising:

(a) assaying an amount of endogenous vitamin D in a sample from a dialysis patient or a patient in need of dialysis;

(b) comparing the amount of endogenous vitamin D to a threshold level; and

(c) if the amount of endogenous vitamin D is below the threshold level, administering to the patient an amount of vitamin D effective to reduce the patient's risk of mortality.

11. The method of claim 10, wherein the endogenous vitamin D is 25-OH vitamin D.

12. The method of claim 11, wherein the threshold level is a level between 8 and 12 ng/ml 25-OH vitamin D.

13. The method of claim 12, wherein the threshold level is about 10 ng/ml 25-OH vitamin D.

14. The method of claim 10, wherein the endogenous vitamin D is 1,25-dihydroxyvitamin D.

15. The method of claim 14, wherein the threshold level is a level between 10 and 20 pg/ml 1,25-dihydroxyvitamin D.

16. The method of claim 15, wherein the threshold level is about 15 pg/ml 1,25-dihydroxyvitamin D.

17. The method of any of claims 10-16, wherein the mortality is cardiovascular- or infectious-related mortality.

18. The method of any of claims 10-17, wherein the active vitamin D is selected from calcitriol, doxercalciferol, paricalcitol, a compound that activates the vitamin D receptor, and 1,25-dihydroxyvitamin D.

19. A method of monitoring and treating a dialysis patient, the method comprising:

(a) identifying a patient who is undergoing dialysis, will undergo dialysis, or is diagnosed to require dialysis; and

(b) assaying an amount of endogenous vitamin D in the a sample from the patient;

(c) comparing the amount of vitamin D to a threshold level; and

(d) if the amount of vitamin D is below the threshold level, administering an effective amount of vitamin D to the patient.

20. The method of claim 19, wherein the patient is undergoing dialysis.

21. The method of claim 19, wherein the patient will undergo dialysis.

22. The method of claim 19, wherein the patient is diagnosed to require dialysis.

23. The method of any of claims 19-22, wherein the endogenous vitamin D is 25-OH vitamin D.

24. The method of claim 23, wherein the threshold level is a level between 8 and 12 ng/ml 25-OH vitamin D.

25. The method of claim 24, wherein the threshold level is about 10 ng/ml 25-OH vitamin D.

26. The method of any of claims 19-22, wherein the endogenous vitamin D is 1,25-dihydroxyvitamin D.

27. The method of claim 26, wherein the threshold level is a level between 10 and 20 pg/ml 1,25-dihydroxyvitamin D.

28. The method of claim 27, wherein the threshold level is about 15 pg/ml 1,25-dihydroxyvitamin D.

29. The method of any of claims 19-28, wherein the exogenous active vitamin D is selected from calcitriol, doxercalciferol, paricalcitol, a compound that activates the vitamin D receptor, and 1,25-dihydroxyvitamin D.

30. A method for treating a patient to reduce the risk of mortality associated with dialysis, the method comprising:

selecting the patient on the basis that the patient is undergoing dialysis, will undergo dialysis, or is diagnosed to require dialysis, and on the basis that the patient is known to have a level of endogenous vitamin D below a threshold level; and

administering to the patient a vitamin D composition in an amount effective to lower the risk of mortality associated with dialysis.

31. A method for characterizing a patient's risk of mortality associated with dialysis, the method comprising:

obtaining a level of endogenous vitamin D in the patient;

comparing the level of vitamin D to a threshold value; and

characterizing the patient's risk of mortality associated with dialysis.

32. A method for evaluating the likelihood that an patient will benefit from treatment with an agent for reducing the risk of mortality associated with dialysis, the agent selected from the group consisting of calcitriol, doxercalciferol, paricalcitol, a compound that activates the vitamin D receptor, and 1,25-dihydroxyvitamin D, the method comprising:

obtaining a level of endogenous vitamin D in the patient; and

comparing the level of endogenous vitamin D to a threshold value, wherein a level of endogenous vitamin D below the threshold value indicates that the patient will benefit from treatment with said agents.

33. A kit comprising a reference standard that corresponds to a threshold level of vitamin D and instructions to perform the method of any of claims 10-32.

34. A kit comprising a protein that binds to vitamin D and instructions to perform the method of any of claims 10-32.

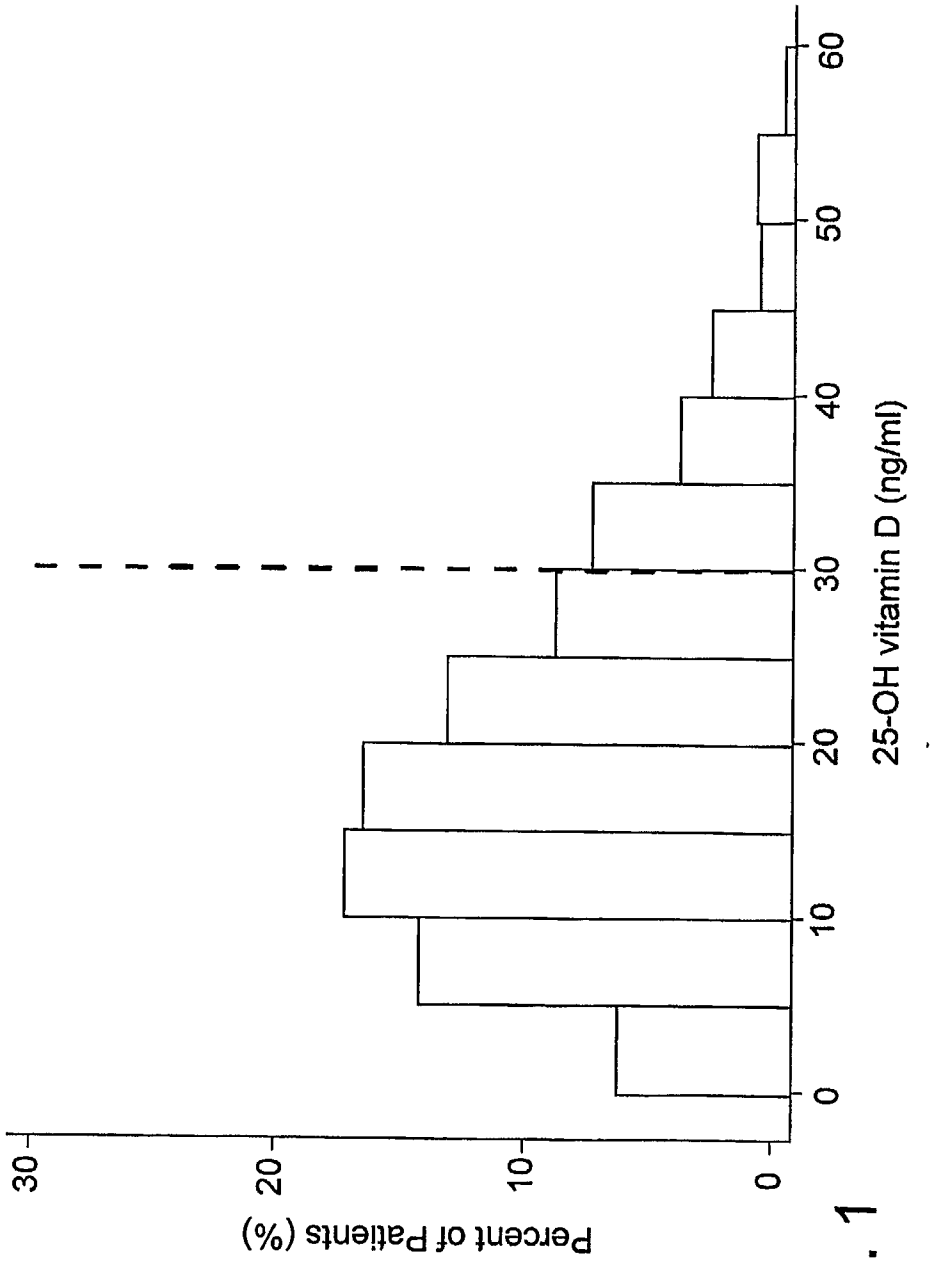
Percent of Patients (%)
0
10
20
30
0
10
20
30
40
50
60
25-OH vitamin D (ng/ml)

FIG. 1

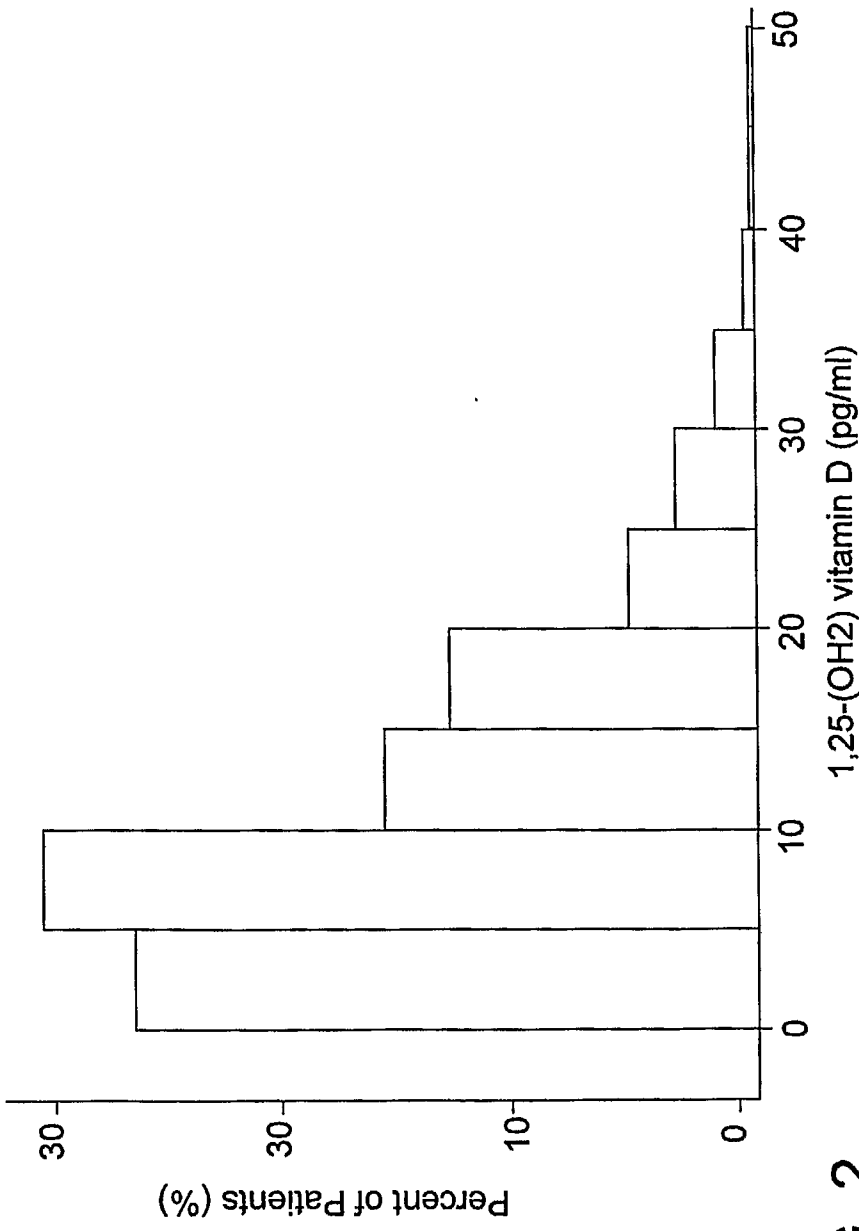
Percent of Patients (%)
0
10
30
30
0
10
20
30
40
50
1,25-(OH2) vitamin D (pg/ml)

FIG. 2

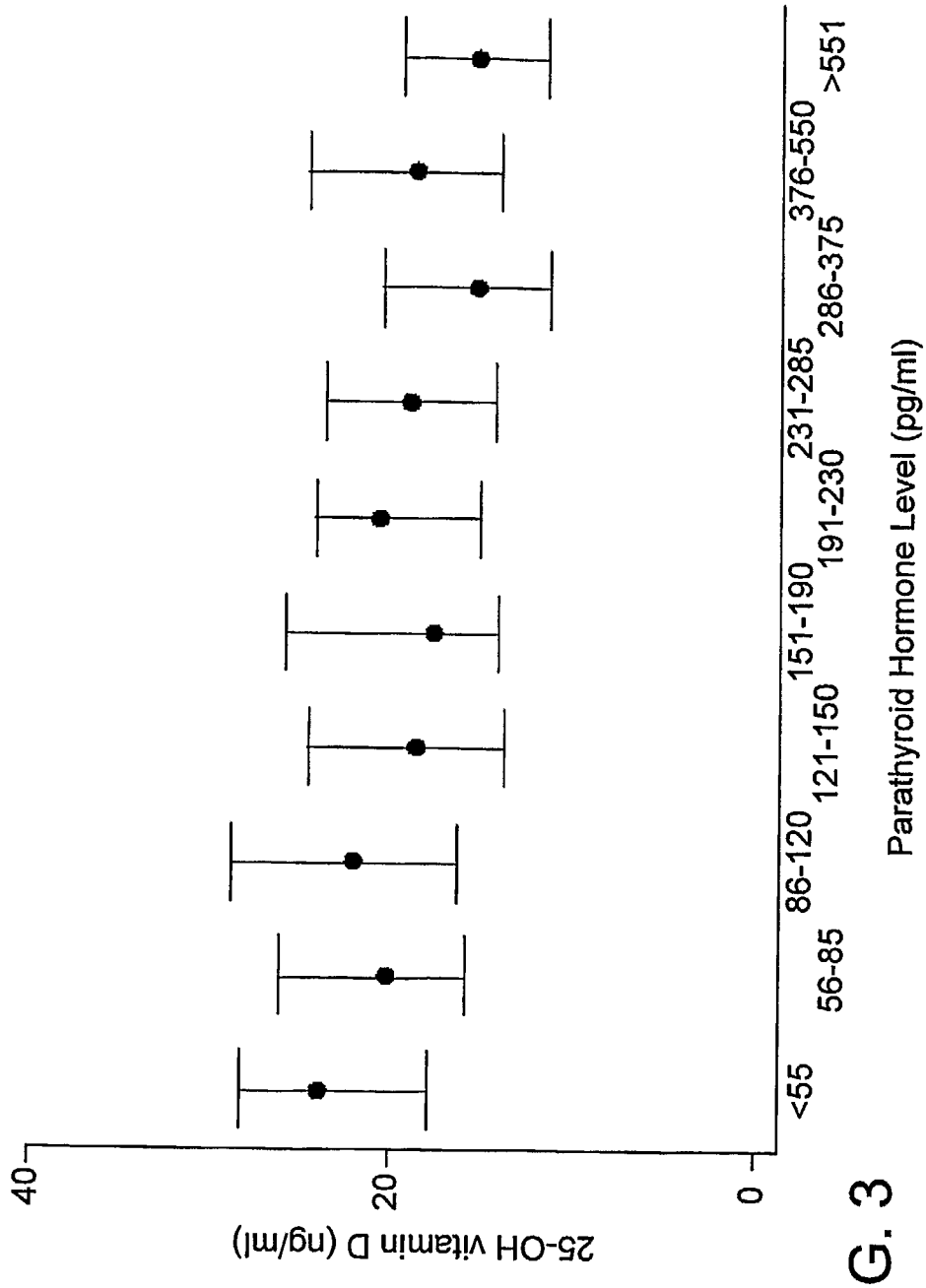
25-OH vitamin D (ng/ml)
0
20
40
<55
56-85
86-120
121-150
151-190
191-230
231-285
286-375
376-550
>551
Parathyroid Hormone Level (pg/ml)

FIG. 3

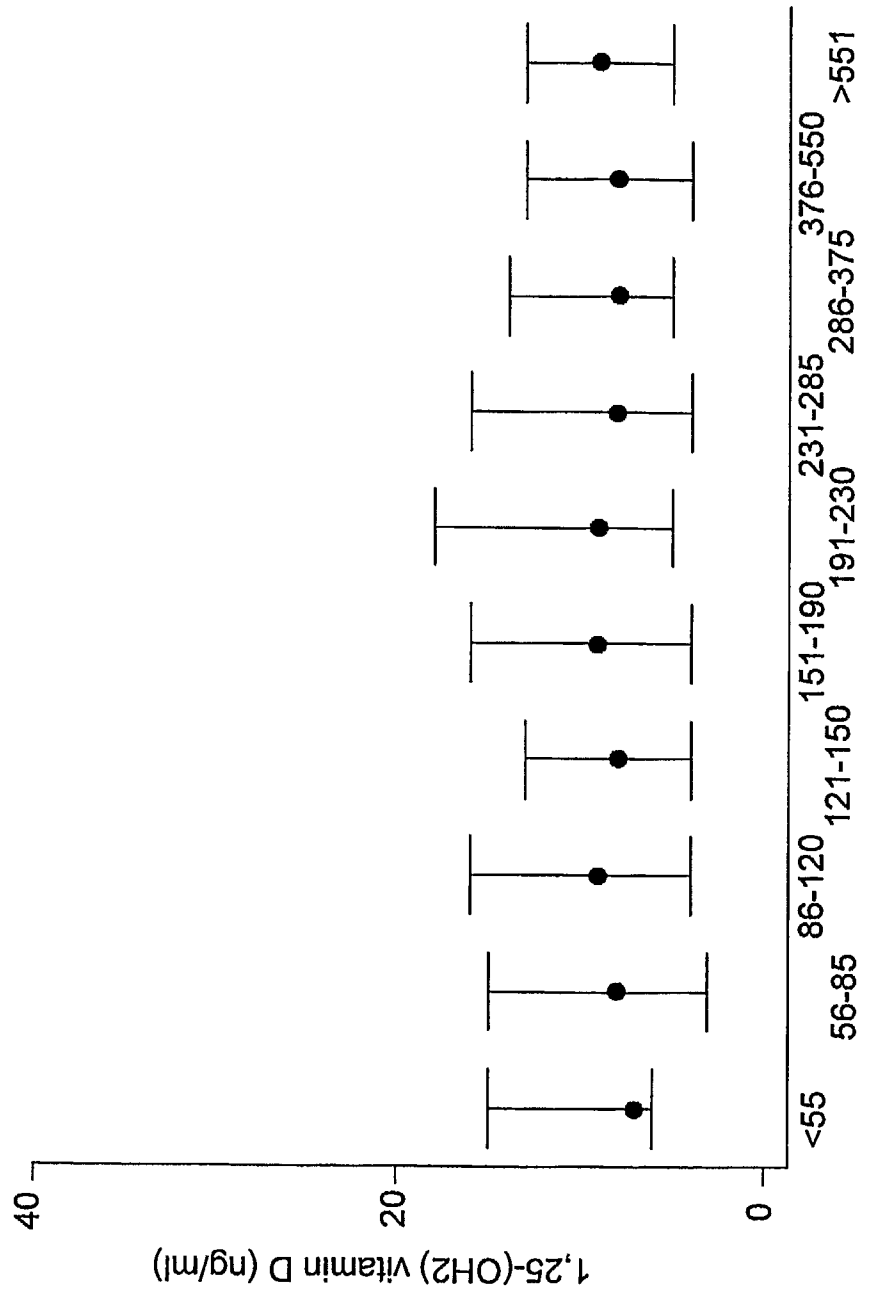
1,25-(OH2) vitamin D (ng/ml)
40
20
0
<55
56-85
86-120
121-150
151-190
191-230
231-285
286-375
376-550
>551
Parathyroid Hormone Level (pg/ml)

FIG. 4

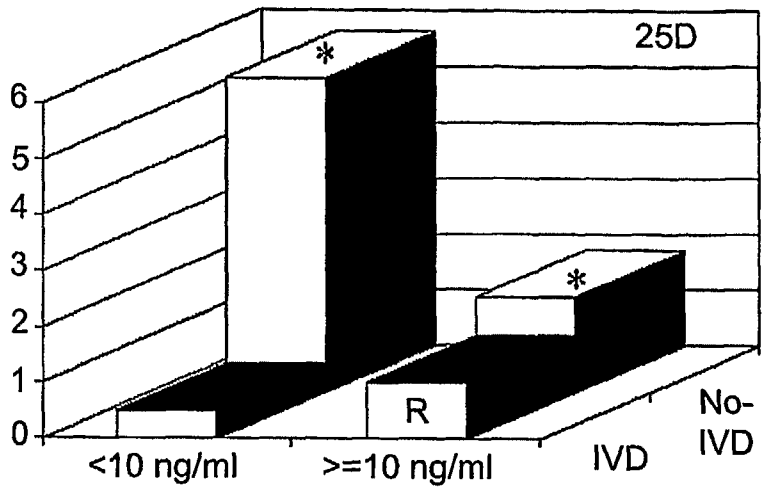
25D
*
*
6
5
4
3
2
1
0
R
<10 ng/ml
>=10 ng/ml
IVD
No-IVD

FIG. 5A

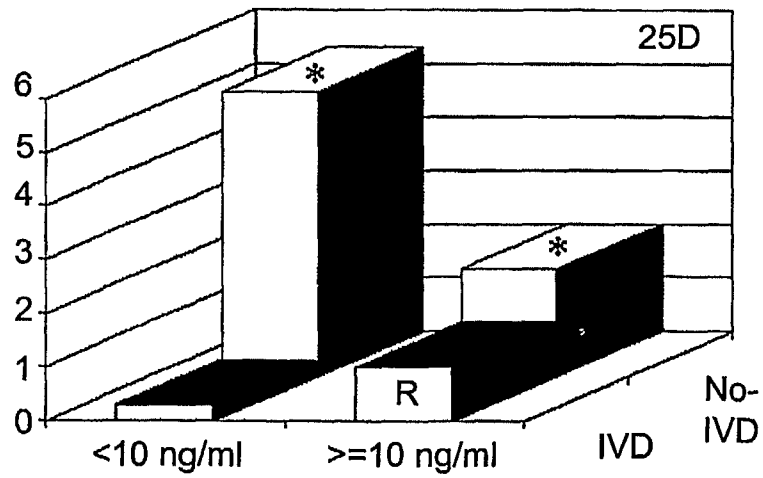
25D
*
*
6
5
4
3
2
1
0
R
<10 ng/ml
>=10 ng/ml
IVD
No-IVD

FIG. 5B

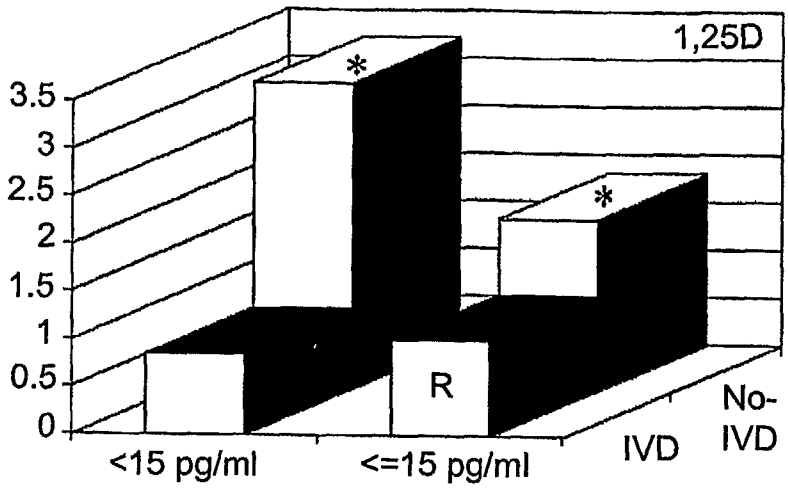
1,25D
3.5
3
2.5
2
1.5
1
0.5
0
*
*
R
<15 pg/ml
<=15 pg/ml
IVD
No-IVD

FIG. 6A

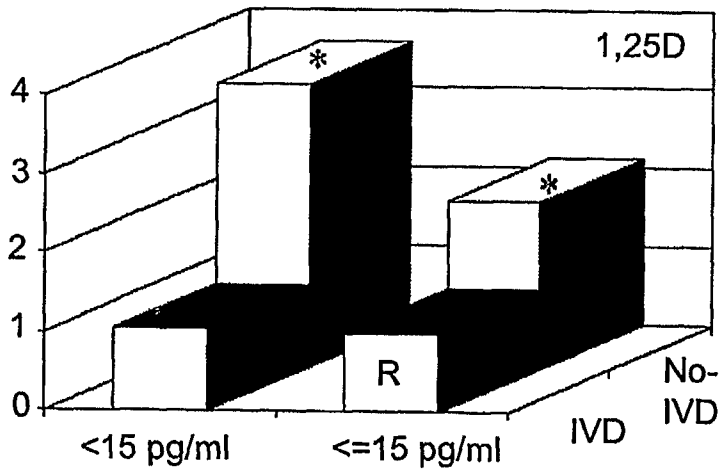
1,25D
4
3
2
1
0
*
*
R
<15 pg/ml
<=15 pg/ml
IVD
No-IVD

FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/23774

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A01N 45/00**(2006.01)

USPC: 514/167
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/167, 552/653

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PUBMED, SCIENCEDIRECT, EAST/WEST, PALM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0124591 A1 (TIAN et al.) 09 June 2005 (09.06.2005), see entire document	1-34
A	TENG, M. et al. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. J. Am. Soc. Nephrol. June 2005, Vol. 16, pages 1115-1125, see entire document	1-34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
02 October 2006 (02.10.2006)

Date of mailing of the international search report
13 NOV 2006

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (571) 273-3201

Authorized officer
David J. Venci
Valerie Bell-Harris for

Telephone No. (571) 272-1600

Form PCT/ISA/210 (second sheet) (April 2005)