

**eAppendix. ACVID Study Protocol**

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**ACVID (Asthma Control with Vitamin D Supplementation)****Study protocol****2015**

**A study to determine if the addition of vitamin D is superior to placebo in vitamin D insufficient asthma patients with persistent symptoms in improving the degree of asthma control**

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## 1. Justification of the study.

Inhaled corticosteroids (ICS) are the main treatment for persistent asthma and a large part of patients are treated successfully with such therapeutic agents (1). However, there is a great variability in response to ICS, with a large group of subjects who do not get an adequate control of their respiratory disease (1-3).

For this purpose, different therapeutic alternatives have been searched, such as the possible role of vitamin D.

This theory initially emerged after finding that the specific receptors of vitamin D (VDR) were distributed in a variety of tissues and immune cells, including the airway. Later, numerous observational studies have emerged and showed an association between plasmatic 25-OH-D3 levels and asthma severity and the number of asthma exacerbations, both in children and adults.

Few randomized clinical trials (RCTs) have been carried out in this sense, and they found different results; which probably may due to the great heterogeneity in the design of the studies, using different sample populations, objectives, protocols and vitamin D preparations.

The availability of properly designed clinical trials would help to clarify whether there is a causal link between asthma and vitamin D. So, this is why we have designed this study, with which carry out a triple-blind RCT, whose results could provide good quality of evidence to determine if vitamin D supplementation may help in improving asthma control.



## 2. Vitamin D: classical and nonclassical actions.

Vitamin D has been traditionally linked to phospho-calcium metabolism and maintenance of bone health, but for some years have aroused great interest in its possible extra-skeletal actions, such as potential role in promoting the immune system and participating in certain respiratory diseases, including asthma.

### 2.1. Classical actions of vitamin D.

In human, vitamin D comes from two main sources: orally, by eating vitamin D- enriched food, and by a cutaneous route, in which the hormone is synthesized from 7-dehydrocholesterol after exposure to UV rays.

This latter route has been considered the main way to get vitamin D, but in recent years and due to a variety of changes in lifestyle, such as the increased use of sunscreens and less sun exposure, have led to increasingly more important dietary sources of vitamin D. These sources are fortified cereals, dairy products, fish oils and egg yolks.

Regardless of origin, either digestive or skin, vitamin D enters the circulation bound to its binding protein (VDBP), a globulin that is synthesized in liver. About 88% of 25-OH-D3 circulates bound to VDBP, 0.03% is in free state and the rest circulates bound to albumin.

This complex (vitamin D-VDBP) travels to liver, where it undergoes a 25-hydroxylation by CYP27A1 enzyme, resulting in 25-hydroxyvitamin D (25-OH-D3), which is the main circulating and storage form of vitamin D.

The half-life of 25-OH-D3 is 2-3 weeks, but may be shortened as concentrations of VDBP are reduced, as may occur when increased urinary losses in nephrotic syndrome.

The second hydroxylation necessary for the active form of vitamin D, the 1,25-dihydroxyvitamin D (1,25-OH-D3) or calcitriol, occurs in kidney and is

conducted by the enzyme CYP27B1 or 25-OH-D3-1 $\alpha$  hydroxylase. This enzyme is an oxidase which is tightly regulated in cells of the proximal renal tubule, being stimulated by parathyroid hormone (PTH), and inhibited by calcium and 1,25-OH-D3.

The main route of inactivating vitamin D metabolites is by hydroxylation, carried out by the vitamin D-24-hydroxylase or CYP24A1, an enzyme that is expressed in almost all tissues. This enzyme is induced by 1,25-OH-D3, thus promoting its own inactivation and thus limiting its biological effects.

#### Actions of 1,25-OH vitamin D.

This active form of vitamin D performs its biological effects by binding to a member of the superfamily of nuclear receptors, the vitamin D receptor (VDR).

As 1,25-OH-D3 enters into the cell, it binds to the nuclear VDR and forms a heterodimer with the retinoid X receptor (RXR). The complex vitamin D-VDR-RXR then binds to a response element vitamin D (VDRE) within the genome of the cell, and starts a large transcriptional complex that can activate or repress transcription of certain genes, depending on the composition of the transcriptional complex.

### **2.2. non-classical actions of vitamin D.**

The discovery of VDR in numerous tissues (4), has suggested that this vitamin could play functions out of phospho-calcium metabolism and that receptors could be activated by different ligands (5).

It has also been described the CYP21B1 activity in numerous extra-renal cells, which facilitate the activation of 25-OH-D3, transforming into its active form 1,25-OH-D3 (6).

The binding of vitamin D to its specific receptor regulates transcription of 200 genes involved in numerous functions, such as regulation of cell growth and maturation, inhibiting the renin-angiotensin axis and angiogenesis, the secretion of insulin and the sensitivity to it.

Therefore, it has been studied the association of vitamin D with numerous pathologies, finding associations between that vitamin and certain neoplasms, and cardiovascular, pulmonary, metabolic and autoimmune diseases.

Within these extra-bone actions of vitamin D, and due to the objective of this work, we will focus on the major studies conducted between vitamin D and asthma (section 4).

### **3. Vitamin D deficiency and supplementation.**

#### **3.1. Vitamin D deficiency**

##### 3.1.1. Definition of vitamin D deficiency

Nowadays there is no unanimous consensus on the optimal plasma levels of vitamin D and the definition of vitamin D deficiency (7, 8).

A large majority of experts believe that the level should be equal to or greater than 30ng/ml, to promote bone and extra-bone health (7, 9-12); although there is a great debate about this, considering even then for extra-bone health should probably be recommended higher values.

In 2011, the IOM (Institute Of Medicine) published the definition of vitamin D deficiency in serum levels of 25-OH-D<sub>3</sub> less than 20 ng/ml (13). And later in the same year, the US Endocrine Society (14) published their clinical guidelines about the management of vitamin D deficiency, using the cutoff value lower than 30 ng/ml.

The vast majority of scientific societies, including the IOF (International Osteoporosis Foundation) (15) also defines this deficit with plasma levels of 25-OH-D<sub>3</sub> below 30ng/ml.

##### 3.1.2. Epidemiology of vitamin D deficiency

It is believed that vitamin D deficiency is an "epidemic" worldwide, affecting more than half of the population (16, 17) and including children, youth, adults, and elderly people; especially if they have osteoporotic fractures, where the prevalence of low 25-OH-D<sub>3</sub> reaches almost 100%.

In a systematic review published in 2014 (18) that included 195 studies about the prevalence of vitamin D deficiency in 44 countries around the world (belonging to Europe, Asia, North and South America, and Oceania), found that 88% of the samples tested had serum levels 25-OH-D3 below 30ng/ml, 37% had average values below 20 ng/ml and 7% had lower average values at 10 ng/ml (18). The great heterogeneity of the studies made it difficult to generalize the results, without having allowed significant differences between countries or continents, nor in terms of age or sex.

### 3.1.3. Etiology of vitamin D deficiency

The vitamin D deficiency may be due to low skin production, because of lack of sun exposure or increased use of sunscreens preventing penetration of UV light on skin. Similarly, it could be due to low dietary intake.

Elderly have particularly high risk for this deficiency because of a lower efficiency in the cutaneous synthesis and intestinal absorption of vitamin D.

Also, there are several conditions that may increase the risk of such vitamin deficiency, as accelerated loss of vitamin in nephrotic syndrome; resistance to its biological effects, as in the case of mutation of VDR; intestinal disturbances that reduce fat absorption, such as celiac disease, inflammatory bowel disease (IBD) or bariatric surgery; alterations in activating vitamin D, such as kidney failure, hypoparathyroidism, certain drugs like ketoconazole, mutations in the 1 $\alpha$ hydroxylase, oncogenic osteomalacia and the hypophosphatemic rickets X-linked (19).

There are some drugs, such as barbiturates, phenytoin and rifampicin, which induce liver oxidases, and thereby accelerate inactivation of metabolites of vitamin D.

### 3.2. Supplementation with vitamin D preparations

In numerous studies conducted in the US use a vitamin D2 preparation (ergocalciferol), however this formulation is not available in our country (Spain), where we have only available vitamin D3 supplements.

It has been described that vitamin D2 is less effective than vitamin D3 (20), so the triple dose of the first one would be required to achieve similar effectiveness. Heaney et al (21) conducted a study with various formulations of vitamin D, concluding that vitamin D3 was 87% more potent than vitamin D2 in raising and maintaining desired levels of 25-OH-D3 in plasma.

#### 3.2.1. Vitamin D3 preparations marketed in Spain:

- Vitamin D3, calciferol or cholecalciferol.

They are liposoluble prepared with long half-life (approximately 1 to 3 months), which are deposited in adipose tissue, from where they are released slowly.

To develop its action, liver and kidney must work properly, to carry out the two hydroxylation to transform it into the active form of vitamin D.

There are two preparations available in our country: Deltius® (oral solution containing 10.000UI per ml, in vials with 2.5 ml) and vitamin D Kern Pharma® (oily solution containing 2,000 IU/ml, in vials of 10ml).

- 25-OH-D3 or calcifediol.

It is a derivative of vitamin D hydroxylation at position 25, such as the produced by hepatic metabolism, but still precise renal hydroxylation for its activation.

It has a half-life intermediate between natural vitamin D (cholecalciferol described above) and those prepared by hydroxylation at position 1 to be discussed later; which lasts approximately 15 to 30 days.

In our country and to the date, there is only one company that markets this formulation: Hidroferol®, with three preparations forms (drops: 1 drop being equivalent to 240UI; in ampoules of 0,266mg, equivalent to 16.000UI; and shock-blisters with 3mgr, equivalent to 180.000UI).

- There are two derivatives of vitamin D hydroxylation at position 1, and which are 1,25-OH-D3 (or calcitriol) and D3 1alpha-OH (or alfacalcidol); which ones have a shorter mean half-life.

Calcitriol is available in Spain in generic formulations (Teva®, Kern®) in ampoules of 1 and 2 mcg/ml and in capsules (Rocaltrol®) at doses of 0.25 and 0.5 mcg.

This is the active form of vitamin D, and does not require subsequent liver or kidney transformations.

It is effective in microgram dose, and speed of action is the main advantage, but also its main drawback, being only 3 hours, which can cause major variations in serum calcium.

Alfacalcidol (1-OH-D3) is available in our country under the name of Etalpha®, in ampoules 1mcg/0.5ml and 2mcg/1ml, and capsules of 0.25, 0.5 and 1 mcg.

It needs to hepatic transformation for activation but can be used in cases of renal insufficiency.

Its power is less than calcitriol, requiring double dose for similar efficacy, but has a longer half-life (1 to 5 days).

Equivalences between different presentations of vitamin D are as follows:

1ng / ml = 2.5 nmol / l

1 drop of calcifediol (Hidroferol®) = 240 IU = 4 mcg

1mcg calcifediol (Hidroferol ®) = 60 IU

1 IU vit D3 = 3 IU vitamin D2

The presentation of vitamin D that will be used in this study is calciferol (Hidroferol®) at a dose of 0,266mg/weekly (16.000UI/week), of which there are studies supporting its effectiveness compared to cholecalciferol, inducing an more rapid increase and sustained serum levels of 25-OH-D3 (22).

There are very few published trials using this preparation and therefore there is little evidence on the optimal recommended dose of calcifediol to be used.

### 3.2.2. Guidelines supplementation with vitamin D.

Considering vitamin D deficiency as serum levels of 25-OH-D3 <30ng/ml, several protocols can be found in the literature, highlighting the guidelines of the US Endocrine Society (14), whose recommendations in adults are as follows:

- Dose of 50,000 IU/week of vitamin D2 or D3 for 8 weeks to reach the target level of 25-OH-D3, followed by 1,500-2,000 IU/day for maintenance.
- In patients with obesity, malabsorption syndromes or treated with drugs that may affect the metabolism of vitamin D: 6.000-10.000UI dose/day to achieve the target values, followed by 3.000-6.000UI/day for maintenance.
- The maximum tolerable maintenance dose should not be taken without medical supervision is 4.000UI/day.

For its part, the International Osteoporosis Foundation (15) recommends consuming 800-1000 IU/day of vitamin D2 or D3, but believes that may be required up to 2,000 IU/day in patients at risk, such as obesity, osteoporotic, low sun exposure (as institutionalized), malabsorption problems ...

### 3.2.3. Safety of vitamin D.

They are not known accurately doses of vitamin D from which toxic effects may occur.



Both IOM and the American Society of Endocrinology consider 4.000UI dose/day, which should not be exceeded without medical supervision.

As for serum 25-OH-D3, the Endocrine Society believes that it should exceed the levels of 150ng/ml so that there could be risk of hypercalcemia.

There have been numerous studies that have shown the safety of various preparations of vitamin D at much higher doses than those commonly used, such as some studies conducted by Hathcock et al (23), where they used 10.000UI/day, without finding hypercalcemia or other manifestations of toxicity. Nor hypervitaminosis occurred in another study using single dose of 500.000UI (24).

The symptoms described in acute intoxication vitamin D are generally due to hypercalcemia and include confusion, polyuria, polydipsia, anorexia, vomiting and muscle weakness. Chronic intoxication can cause nephrocalcinosis, demineralization and bone pain (23).

Although vitamin D toxicity is rare, it is still much unknown about it, since most of the available information comes from sporadic cases (25-27).

## 4. Vitamin D and asthma.

### 4.1. Experimental studies.

There are several mechanisms by which vitamin D might influence asthma, according to results of several experimental studies, and we could summarize in the following sections:

#### - Influence of vitamin D on innate immunity.

Certain infectious agents and allergens can stimulate the receptors of antigen presenting cells, which in turn increase the expression of CYP27B1 enzyme in macrophages, monocytes and epithelial cells (28, 29). That improve the active form of vitamin D, 1,25-OH-D3, that binds VDR and genomic VDRE, modulating various proinflammatory cytokines and increasing the production of antimicrobial peptides, as cathelicidin and beta-defensin2 (30-32).

#### - Influence of vitamin D on adaptive immunity.

The type of T cell response is directly influenced by the antigen presenting cells, which are themselves modulated by vitamin D. In addition, vitamin D has direct effects on T cells: inhibits Th1 cytokines associated to the phenotype of T cells in vitro and animal studies (33); while the effects on Th2 cells are more complex, but particularly important because Th2 cytokines lead to IgE synthesis, essential in allergic asthma pathogenesis. Vitamin D may either inhibit or promote Th2 response in different animal models and in vitro human T cells (34). Hypponen et al (35) demonstrated that plasma levels of 25-OH-D3 did not have a linear relationship with serum IgE levels, so that IgE levels were elevated only with very low or very high levels of 25-OH-D3.

In vitro, it has been observed that calcitriol reduces IgE production in peripheral human B cells and increases the synthesis of IL-10 by these cells (36, 37). Besides producing B cells also synthesize IL-10 IgG4, isotype associated with beneficial results after desensitization with certain allergen immunotherapy (38).

Some cytokines such as IL-17 play a central role in epithelial defense against bacterial and fungal infections. However, excessive levels of this cytokine has been implicated in severe asthma, associated with neutrophilic present in many patients with severe asthma (39-42).

That IL-17 has been shown to promote bronchial hyperresponsiveness and remodeling, resistance to steroids and synthesis of proinflammatory cytokines (41, 43, 44). Meanwhile, vitamin D has been shown to reduce IL-17 responses in both mice and humans with severe asthma (39, 45).

- Effects on regulatory T cells.

Regulatory T cells (Tregs) are essential for the prevention and control of excessive and inappropriate immune responses, including allergy-related processes and asthma (46). In asthmatic patients has observed decreased levels of these cells Tregs (47, 48) and also observed lower levels of IL-10 compared to healthy subjects (49, 50). In vitro vitamin D showed improving several actions of Tregs (51), including promoting those that synthesize IL-10 and depending of existing levels of vitamin D (52).

- Effects on response to corticosteroids.

It has been described that the CD4<sup>+</sup> T cells in peripheral blood of corticosteroid-resistant asthmatic patients were not able to increase the secretion of IL-10 in vitro when they received dexamethasone, as opposite that in corticosteroid-sensible asthmatic patients or in healthy subjects (50). In addition, supplementation with vitamin D, either by addition of 1,25-OH-D3 to the culture or by oral supplementation for 7 days of asthmatic patient's refractory to steroids, allowed to restore the induction of IL-10 by dexamethasone (50).

Interestingly it has been found that dexamethasone may increase production of IL-17 from mononuclear peripheral blood cells and CD4<sup>+</sup> T cells in vitro, showing a positive association between the dose of inhaled corticosteroid and synthesis of IL-17. These data suggest that corticosteroids could contribute to disease progression in severe asthma for increased response mediated IL-17 (53); while vitamin D has been shown to strongly inhibit the production of IL-17 induced by corticosteroids in vitro (39).

- Effects on airway remodeling.

Remodeling of the airway in asthma causes reduction in lung function irreversibly and is poorly controlled with current therapies (54). Some studies have found that vitamin D is associated with reduced smooth muscle mass airway (ASM) (55, 56).

It has been found an inverse relationship between ASM and serum levels of 25-OH-D3 in pediatric patients with asthma (57); and so the addition of vitamin D to cultures of human ASM has been found to reduce the proliferation of such muscle cells (58).

#### **4.2. Vitamin D and asthma: Descriptive studies.**

In addition to experimental studies described above, there are observational studies, that found a relationship between plasma levels of 25-OH-D3 with different aspects of asthma, in both children and adults; and only some of them will be exposed here.

Bener et al (59) in 2012, conducted a large study of 966 asthmatic children and their healthy controls, finding that vitamin D deficiency was higher in the group of asthmatic children (AOR = 4.82, 2.41-8.63).

Six studies investigated the association between plasma levels of 25-OH-D<sub>3</sub> and asthma exacerbations, defined by need of hospitalization or use of systemic corticosteroids (oral or parenteral) (57, 60-64).

In all of them, except in the study by Gergen et al (63), found that low 25-OH-D3 plasma levels were associated with an increased risk of asthma exacerbations.

In adults, Devereux et al (65), conducted a case-control study in the UK in 2010, selecting 160 adults between 18 and 50 years old and found no significant association between serum vitamin D levels and the prevalence of asthma (AOR = 0.98 [0.91 to 1.06]).

In the same year, Sutherland et al (66) carried out a cross sectional study in 54 asthmatic patients over 18 years, non-smoking; measuring the relationship

between plasma levels of vitamin D and the values obtained in pulmonary function tests, and found a significant association between higher serum 25-OH-D3 and better results in pulmonary function tests, measured as FEV1 and the methacholine test.

In another cross-sectional study conducted in 2013 by Korn et al (67), serum vitamin D in 280 asthmatics adult patients from Germany were determined, and stratified depending on the degree of control (in three subgroups: well controlled, partially controlled and uncontrolled) and asthma severity (four subgroups: intermittent, mild persistent, moderate persistent and severe), using the GINA criteria (68). The results obtained were as follows: plasma levels of vitamin D were lower in the greater severity of asthma and in the poorer disease control. The odds ratio of patients with severe or uncontrolled asthma to present inadequate levels ( $<30$  ng / ml) of plasma vitamin D was 1.9 (95% CI: 1.2 to 3.2) and 2.1 (1.3-3.5) respectively.

In 2014 Confino-Cohen et al (69), selected 21237 asthmatic adults 22-50 years, from Israel, and measured their plasma levels of 25-OH-D3 and so the number of asthma exacerbations who had submitted during the previous year (defining exacerbation as prescription oral corticosteroids, prescription  $> 5$  short-term beta agonists, or more than 4 visits to a doctor for asthma-related symptoms). Levels of 25-OH-D3 were also measured in non-asthmatic patients, finding a similar prevalence in both groups. However, found an inverse linear association between the ratio of asthmatic exacerbations and plasma levels of 25-OH-D3 ( $p < 0.0001$ ).

#### **4.3. Vitamin D and asthma: Clinical Trials.**

Among the main clinical trials Vitamin D in asthmatic children till the date, three of them investigated the influence of vitamin D on asthma exacerbations (70-72) and another clinical trial (73) studied their relationship to the degree of asthma control determined by the ACT questionnaire (74).

In the latter RCT, conducted in 2012, by Lewis et al (73), they selected 30 children with persistent asthma, aged between 6 and 17 years. They were then randomized in the intervention group (IG), which was administered 1000 IU/day of vitamin D orally, and in the control group (CG), receiving placebo. They measured at baseline, at 6 and 12 months later, the serum levels of 25-OH-D3 and the ACT questionnaire (74). As a result, it was noted that all patients except one, had plasma initial values of 25-OH-D3 below 15 ng/ml and they increased throughout the study in both groups, but without any of them getting higher values to 30ng/ml, and also presenting significant variations in these levels depending on the season (summer or winter).

For the score on the ACT questionnaire in both groups, no significant differences were found. As conclusion the authors believed that they probably should have used higher doses of vitamin D, in order to achieve serum levels of 25-OH-D3 > 30ng/ml, and so they may have observed most significant results.

We can find 3 RCTs that investigated the association between vitamin D supplementation and asthma exacerbations in children (70-72).

In the first one in chronological order, in 2010, by Urashima et al (70), they selected 167 asthmatic children between 6-15 years of age, being randomized into the IG, which received oral supplements 1200UI/day of vitamin D, and the CG receiving placebo. Follow-up lasted 16 weeks, during the winter months, and for which the incidence of influenza A (determined in nasopharyngeal aspirate) and the number of asthma exacerbations (defined as wheezing improved with short-acting beta2 agonists) were measured. The results obtained were as follows: The incidence of influenza A in the group supplemented with vitamin D was 10.8%, compared with 18.6% of affected children in the placebo group (RR = 0.58; CI 95% 0.17 to 0.79; p = 0.006). As conclusion, the authors suggested that supplementation with vitamin D preparations for the winter season, could reduce the incidence of influenza A and the number of asthma attacks in children, but consider that studies should be conducted with a larger sample size.

The second RCT, in 2011, by Majak et al (71), selected 48 children with newly diagnosed asthma in children aged 5-18 years. They were randomized into two groups: IG (which was prescribed 500UI/day of vitamin D orally) and CG (with placebo). Both groups received 800mcg/day budesonide inhalation. The follow

up period was 6 months, measuring at the beginning and at the end of the study, the plasma levels of 25-OH-D3 and the incidence of asthma attacks (defined by the need for use of short-acting beta2-agonists).

As a result, it was found that the average levels of 25-OH-D3 at baseline were greater than 30 ng/ml in both groups (35,1ng/ml in the placebo group and 36,1ng/ml in IG), with a subsequent decrease in the levels on the first group at the end of the study (31,9ng/ml) and a slight increase in the group supplemented with vitamin D (37,6ng/ml) after 6 months of treatment. They presented 4 asthma attacks in IG (17%) and 11 in CG (46%) ( $p = 0.029$ ).

The conclusion of their authors was that supplementation of vitamin D may reduce the risk of asthma exacerbations, but recommended further studies.

The last of these RCT in children, was published in 2014 (72) in which was selected 100 asthmatic children (according to the criteria of the GINA 2011), aged between 3 and 14 years. They were randomized between IG (which received 60.000UI/month oral vitamin D) and CG (placebo also monthly). The follow-up period was 6 months, making monthly reviews and found that the number of asthma attacks during the study was significantly lower in the vitamin D group compared with the placebo group.

In adults and till the date, we find four main RCTs that studied the influence of supplementation with vitamin D in adult patients with asthma.

The first one was the VIDA study (Vitamin D Add-on Corticosteroid Therapy Enhances Responsiveness in Asthma) (75), published in 2014. It was a multicenter double-blind study, in which 408 patients with symptomatic asthma (median score on the ACT of 20points and a slight spirometric alteration) and vitamin D deficiency were included (25-OH-D3 plasma <30ng / ml).

For this study, the researchers created a period of "bleach", in which the previous asthma treatment of patients was suspended, and all of them were changed to inhaled ciclesonide (320mcg/day) and levalbuterol for 4 weeks, and added oral prednisone 40mg/day of in the last week of that month of "bleach".

The patients were assigned randomly into two groups: the IG that received vitamin D (once 100,000 IU initial, followed by 4000 IU/day orally) and the CG,

who took placebo in a similar way. Following this randomization, the only treatment that was maintained for asthma control was inhaled ciclesonide 320mcg/day for 12 weeks; after which patients were retested and those with good control were reduced the dose to half (ciclesonide 160mcg/day), which was kept for another 8 weeks; and again were reevaluated and those with good control were reduced their dose of ICS to half (ciclesonide 80mcg/day) for another 8 weeks. The total length of patient follow-up was 28 weeks.

The main objective in this VIDA study, was the rate of treatment failure and number of asthma exacerbations among patients who took vitamin D supplementation or placebo, without finding differences between them. The researchers only found significant differences in the total dose of ICS at the end of the study, which was a 25% smaller in the IG with respect to the CG; although in absolute terms, the total difference in the accumulated dose between the groups was small (15mcg/day).

In the same year, S.Arshi et al (76), carried out another RCT, in which selected 108 patients, 10-50 years old, with mild-moderate persistent asthma as criteria GINA 2012. All patients were treated with ICS (budesonide) with / without formoterol depending on the degree of control, and then were divided 2 groups: IG, who received vitamin D (a single dose of 100,000 IU IM, followed by 50,000 IU/week orally) and CG, who were not added any treatment apart from ICS (+/- beta2). It was therefore an open unblinded study.

All of them were measured FEV1 and plasma levels of 25-OH-D3, prior to randomization and at weeks 8 and 24 of the study.

As a result, they found an improvement in FEV1 similar in both groups at week 8, while in week 24 the improvement in FEV1 was significantly higher in the vitamin D group compared to the placebo group.

In the conclusion, the authors described that vitamin D supplementation could help to improve response to the ICS in the control of asthma at long term, specifying that at least 24 weeks of supplementation with these preparations should be used before to observe their immune effects.

In 2015, JC de Groot et al (77), selected 44 non atopic asthmatics patients, whose induced sputum were eosinophilic (> 3%) and / or neutrophilic (> 53%),



and all of them had serum 25-OH-D3 were less than 40ng/ml. These patients were randomized into two groups: the IG, receiving vitamin D (a single dose of 400.000UI) and CG, which received one dose of placebo. They all continued their regular medication for asthma. At baseline and after 9 weeks, the analysis of induced sputum, pulmonary function tests and questionnaires AQLQ (Asthma Quality of Life Questionnaire was conducted(78)) And ACQ (Asthma Control Questionnaire (79)) were determined. As a result, it was observed that treatment with vitamin D did not significantly affect the total percentage of neutrophils or eosinophils between IG and CG. Although, in the group of patients with higher levels of sputum eosinophils (> 26%), it was observed that this percentage was reduced following treatment with vitamin D (from 41% to 11.8%) compared with the placebo group, whose percentage increased (from 51% to 63%,  $p = 0.034$ ). Also the group of vitamin D showed slightly better score in the ACQ questionnaire (0.8 in IG and 1.1 in CG;  $p = 0.08$ ).

In conclusion, the authors suggested that vitamin D could be an adjunctive therapy to the ICS effective in the subgroup of patients with severe asthmatic eosinophilic inflammation of the airway.

In the last RCT, carried out by Martineau et al (80), in 2015 and in the UK, 250 asthmatic patients were selected, aged between 16 and 80 years. They were randomized into two groups: the IG, which received vitamin D (120.000UI 2 times/month orally for 3 months, followed by a dose of 120,000 IU vo every 2 months until one year), and CG who took placebo in a similar pattern. The main objective of this study was to investigate whether there were differences between the two groups in the time to first severe exacerbation (defined as requiring treatment with oral corticosteroids, hospitalization, consultation in the emergency department, or decreased PEF>25%) (81).

The results were as follows:

- The prevalence of vitamin D deficiency (<30 ng / ml) in asthmatic patients was 83%.
- No significant differences between IG and CG was observed in time to first exacerbation; and there were no differences in the overall rate of exacerbations or respiratory infections throughout follow-up.

-The only significant difference between the two groups, was the improvement in quality of life as measured by SGRQ (questionnaire Sant George (82)) In the IG respect to CG.

The researchers concluded that supplementation with bolus of vitamin D did not influence the time to first severe asthma attack or first respiratory infection in the upper airway in a population of adult asthmatic patients with a high prevalence of vitamin deficit D.

## 5. Objectives of the ACVID study.

- Primary: Evaluate the efficacy of vitamin D supplementation in asthmatic patients with vitamin D deficiency in the degree of asthma control.

- Secondary: Evaluate the efficacy of vitamin D supplementation in those patients in improving the quality of life, reducing the number of exacerbations, decreasing the dose of inhaled corticosteroids, the number of oral corticosteroid cycles, the number of emergency visits, and the number of unscheduled consultations with the primary care physician and hospitalizations for asthma.

## **6. Design and study protocol.**

### **6.1. Design.**

It will be a prospective, randomized and triple-blind study, to be carry out in Morales Meseguer Hospital of Murcia, Spain; which gives health coverage to an estimated population of 240,000 inhabitants.

Sample size calculation, randomization and analysis of the results will be performed by a computer program and by a professional statistician, independent and outside of the studio.

Researchers and patients throughout the study will be unaware which group of patients belong to IG, who will receive vitamin D supplementation, and who to CG, who will receive placebo.

### **6.2. Equipment and funding.**

The research team consists of two specialists in Endocrinology, one specialist in Pneumology and one expert in statistical analysis and data processing.

Blisters vitamin D and placebo will be provided by the pharmaceutical company FAES FARMA, essential for blinding them, but without receiving from it or any other industry any funding or donations. Therefore, the research team for this study declares no conflict of interest.

### 6.3. Randomization and recruitment.

Patients will be selected from lists of income hospital and in the emergency department during the years 2013 and 2014, with the main or secondary diagnosis of asthma.

After this search, all patients will be contacted by telephone and invited to participate in the study, sending them a flyer for a blood extraction, to identify those with vitamin D deficiency, and being cited those who accept in a first visit in consultations of Endocrinology in Morales Meseguer Hospital of Murcia, Spain.

In that first recruiting visit the inclusion and exclusion criteria will be assessed, and patients will be asked for signing the informed consent (Annex I).

For randomization, random numbers will be generated by computer and by an external researcher to the study. Each patient will be assigned to each group by a system of opaque numbered envelopes, that will be held by the principal researcher.

In the recruiting visit, if considered the inclusion of patients in the study, the main researcher will open consecutively an envelope with the group of each patient, identified as "A" or "B" to maintain blinding, without knowing which of them would correspond to CG or IG.

Patients assigned to one group or another, will be given the ampoules named as "A" or "B", ignoring if they contain vitamin D or placebo, while continuing with their usual medical assistance and habitual treatments for asthma.

The study was approved by the Ethics Committee for Clinical Research Morales Meseguer Hospital dated 29 April 2015 (Annex II).

#### 6.4. Inclusion and exclusion criteria.

Patients must be over 18 years, with a medical diagnosis of bronchial asthma, and have vitamin D deficiency (25-OH-D3 < 30 ng/ml).

Exclusion criteria:

- Tobacco use > 10 pack-years.
- Be already taking vitamin D supplementation.
- Renal-disease (serum creatinine> 2 mg / dl)
- Hypercalcemia (protein corrected serum calcium> 10.5mg / dl).
- Repeated kidney stones (> 2 episodes).
- Diseases affecting ability of intestine to absorb vitamin D (as Crohn's disease or great intestinal resections).
- Pregnancy or breastfeeding.
- Severe psychosocial problems (dementia, alcoholism or other drug abuse psychiatric disorders such as major active depression or schizophrenia).

#### 6.5. Study variables.

Data to be collected can be grouped into several subtypes: socio-demographic, clinical, laboratory and respiratory function; as summarize below:

##### 6.5.1. Socio-demographic data.

- Age.
- Sex.
- Educational level: completed basic, medium or university studies.

### 6.5.2. clinical variables.

- Body mass index (BMI): calculated using the following formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Size (m)}^2}$$

- Immunization history: Influenza and pneumococcal vaccination of patients.

- Smoking: smoking history accumulated, expressed in pack-years, in order to exclude possible diagnostic confusion with COPD; and so there will be excluded all patients whose consumption is equal to or greater than 10 pack-years. This index is calculated by the following formula:

$$\text{Packyears index} = \frac{\text{N}^\circ \text{ cigarettes smoked per day} \times \text{N}^\circ \text{ smoking years}}{\text{twenty}}$$

- Degree of dyspnea: measured by the scale Medical Research Council (MRC) (Fletcher, BMJ, 1959) (83, 84) reviewed by Schilling in 1955 and recommended for use in obstructive diseases (85). This instrument has the advantage of simplicity and reflect breathlessness caused by activities of daily life, and helps in predicting quality of life and survival (86-88). We will use this scale to measure baseline dyspnea of patients, and check if there are differences between the two groups.

- Clinical factors related to asthma:
  - Years of development asthma.
  - Extrinsic or intrinsic asthma.

- Classification of asthma severity, according to the criteria defined by GINA, in four groups: Intermittent, mild persistent, moderate persistent or severe persistent.

Severity of asthma will be determined only at the initial visit to check the possible difference between the two groups, but without being repeated at the end of the study, since there will be not changes in the specific treatment for asthma over the 6-month study.

## GINA Classification of Asthma Severity

	Symptoms/Day	Symptoms/Night	PEF or FEV1	PEF variability
<b>STEP 1</b> <b>Intermittent</b>	< 1 time a week  Asymptomatic and normal PEF between attacks	<= 2 times a month	>= 80%	< 20%
<b>STEP 2</b> <b>Mild Persistent</b>	> 1 time a week but < 1 time a day  Attacks may affect activity	> 2 times a month	>= 80%	20-30%
<b>STEP 3</b> <b>Moderate Persistent</b>	Daily  Attacks affect activity	> 1 time a week	60%-80%	> 30%
<b>STEP 4</b> <b>Severe Persistent</b>	Continuous  Limited physical activity	Frequent	<= 60%	> 30%

- The degree of asthma control, assessed by questionnaire ACT score (Asthma Control Test) (Annex III).

It is a simple self-administered questionnaire by the patient, to quantify the degree of asthma control.

Developed in 2004 by Nathan et al (74), it has been validated in different populations (89) and different measurement criteria. It has also been validated the Spanish version (90).

It consists of 5 questions that assess: the limitations on physical activity, the degree of dyspnea, nocturnal symptoms, use of rescue medication and the



perception of patients with respect to the control of their disease; all these aspects for the four weeks preceding the survey. These questions are graded with a score ranging from 1 (worst) to 5 (best), and the resulting score is the sum of responses, that may range from 5 (poor control) and 25 (excellent control). Cutoff scores have been studied, in order to establish a relationship with the degree of control with the GINA and other criteria (such as Exhaled Nitric Oxide Fraction -FENO- or spirometry), concluding that a score equal or greater than 20 is consistent with very well controlled asthma, a score between 19-16 with partially controlled asthma, and ratings at or below 15 with poorly controlled asthma (91).

Similarly has been established minimally significant difference, defined as the smallest difference in that test score representing a clinically significant change in patients, and whose value is equal to or greater than 3 points (92).

In our study, we will collect the score on the ACT in both groups at the beginning and at the end of the study. In the first case, to determine the possible differences between the two study groups at the beginning of the trial. Then we will compare the final score between the groups, to assess whether the vitamin D supplementation could have caused significant differences between them; and we will analyze the variation of the final score with respect to the initial in each of the groups.

As a last aspect in this point, we will value the necessary number of patients to treat (NNT) required to achieve a clinically meaningful improvement (3 points) (92) in ACT score (74) using the formula proposed by Suissa (93) in 2012. This parameter, the NNT, has been described as a simple and valid tool to assess the impact of an intervention (94).

- Quality of life, which will be measured with the questionnaire Mini-AQLQ (Annex IV).

This is the short version of the validated questionnaire AQLQ (Asthma Quality of Life Questionnaire) (95), whose shorter version has also been validated (96) and so its adaptation in Spanish (97).

It is a self-administered questionnaire that includes 15 questions with a score from 1 (always) to 7 (ever), through which four asthma-related aspects are evaluated:

- Respiratory symptoms (in items 1,4,6,8,10);
- possibility to perform activities (items 12, 13, 14, 15);
- environmental stimuli (items 2,7,11);
- emotions against these symptoms (items 3,5,9).

It has been studied in relation to the AQLQ and all versions (mini-AQLQ, AQLQ (S) and Acute AQLQ) the difference in score considered minimally important, defined as the smallest difference in the test score that patients perceive as beneficial (98, 99), and which has been set in 0.5 for minimal; a difference equal to or greater than 1 point would be considered moderate, and a difference equal to or greater than 1.5 points would be considered important.

- Specific treatment for asthma, both inhaled and systemic; specifying the number of cycles of oral corticosteroids in the 6 months prior to baseline and during the trial. In the case of ICS we will use a conversion of equivalent doses of different preparations, according to the criteria defined by the GINA, to be able to classified them in three groups: low, medium or high doses.

	LOW DOSES ( $\mu\text{g}/\text{día}$ )	MEDIUM DOSES ( $\mu\text{g}/\text{día}$ )	HIGH DOSES ( $\mu\text{g}/\text{día}$ )
Beclometasona dipropionato	200-500	501-1.000	1.001-2.000
Beclometasona extrafina	100-200	201-400	> 400
Budesónida	200-400	401-800	801-1.600
Ciclesonida	80-160	161-320	321-1.280
Fluticasona furoato	-	92	184
Fluticasona propionato	100-250	251-500	501-1.000
Mometasona furoato	100-200	201-400	401-800

- Number of asthma attacks

Crisis or asthma exacerbation is defined retrospectively as the need of increase the specific treatment for asthma for at least 3 days. For this, we will ask directly to the patients and consult the medical record.

-Number of unscheduled consultations with primary care physician for asthma-related causes.

Similarly, to obtain such data we will directly ask the patients and consult the medical record.

-Number of consultations in the emergency department for asthma-related causes.

We will ask patients and then check the information with the hospital computer program.

- Number of hospital admissions for asthma-related causes.

We will ask the patients and check this information with the medical record.

#### 6.5.3. Laboratory data.

- Plasma levels of 25-OH-D3 (ng / ml).
- total serum protein (mg / dl), to calculate corrected calcium by proteins.
- Total serum calcium (mg / dl).
- Calcium corrected by proteins (CCP), calculated using the following

formula, to detect possible cases of hypercalcemia:

$$\text{CCP} = \text{Total measured calcium} / [0,6 + (\text{proteins}/18.5)].$$

- Hemogram, with particular interest in the white series, in particular eosinophils (both absolute number and percentage).

- Immunoglobulin E (IgE).

#### 6.5.4. Spirometry.

Spirometry is a diagnostic test of choice in both the diagnosis and monitoring of asthma patients.

In our study, we will collect the following data: Forced vital capacity (FVC), FEV1

and index FEV1/FVC, indexed by height and age, and according to the reference values of the European Respiratory Society (ERS).

These data will be obtained to verify the similarity of the two study groups, giving value to randomization, and to test whether vitamin D supplementation would have any impact on the respiratory function tests.

Measurements will be made with the spirometer MIR® Spirobank type, version 1.6 with A23-04P.01282 number series.

#### 6.5.5. Other data.

- Any data on possible side untoward effects throughout the study will be collected patients and so the dropout rate thereof.

- We will collect data on all-cause mortality that could occur from the time of recruitment and until the end of the study.

- Days to first hospitalization for asthma-related causes. Defined as the number of days until patients present the first hospital admission for asthma.

- Days until the first emergency care for asthma-related causes. Defined as the number of days until patients need to be attended for the first time in the emergency department for asthma-related causes.

#### 6.6. Initial visit.

After contacting patients by telephone, they will be cited in consultation for recruitment, checking the criteria for inclusion and exclusion of each patient. Previously they should had received a steering wheel for analytical extraction, to include only those patients whose serum 25-OH-D3 levels < 30ng/ml.

All candidates to participate in the trial will be adequately informed about the purpose and development of the study, and asked for signing the informed consent (CI) (Annex I). Then we will open the opaque envelopes, assigning randomly and blindly patients to group "A" or "B", without knowing which would be the GC and which the intervention group, nor by patients or researchers.

After inclusion, we will collect the different baseline socio-demographic, clinical, analytical and spirometric data, and after that we will give to patients blisters "A" or "B", making them know how to take it: once a week orally. The composition of ampoules containing vitamin D will be in a presentation of 266 mcg calcifediol (equivalent to vitamin D3 16.000U) while placebo blisters will contain the same amount (1.5ml in each ampoule) but only with an oily substance.

On this visit, the pulmonologist researcher will collect the clinical variables, the spirometry tests and questionnaires, to establish the degree of asthma control patients at that time; and if necessary will adjust treatment in both groups to ensure that patients take the proper specific treatment for asthma in both groups, equally, without knowing who belong to the CG or the IG.

Finally, we will provide to patients a new steering wheel for their next analytical extraction at the end of the study, and give a scheduled 6-month review date.

### **6.7. Monitoring.**

During follow-up, a mobile phone will be activated and available, so patients could directly call the researchers if they had any questions.

Patients will be telephoned once a month, to remind proper medication adherence and ask them about the appearance of side effects.

### **6.8. Final visit.**

The last visit will be 6 months after the initial visit, and patients should have already taken the "A" or "B" blisters. Then, we will collect the final data: clinical, analytical and spirometric, and so the possible side effects associated with the

blisters, the number of dropouts, mortality, the number of days until the first emergency care for asthma or until the first hospital admission for reasons related with asthma.

### **6.9. Data Collect**

We will use a database SPSS® for Mac® environment, that will be created by the researchers.

The database will allow to collect the data during the initial and final visits, and later will be analyzed by an independent statistician, who also will not know the treatment received in groups “A” or “B”.

### **6.10. Statistical analysis.**

The data analysis will be performed by intent-to-treat. To calculate the sample size, and accepting an alpha risk of 0.05 and a beta risk of 0.2 (80% power), 100 participants would be required in total, 50 in each group, to detect an absolute difference of 3 points in the ACT questionnaire, considering the mean of 19 and a standard deviation of +/- 5. A loss rate of 7% of patients will be estimated.

The baseline characteristics and results will be expressed as numbers (%) for qualitative variables and mean (SD) or median (interquartile range [IQR]) for quantitative variables, depending of their distribution. The 95% confidence intervals will be calculated for the outcome endpoints. The comparison between qualitative variables will be performed using Pearson's chi-squared test or Fisher's exact test (two-tailed). The comparison between quantitative and qualitative variables will be performed using the Student's t-test or the Mann-Whitney U test, depending on whether the qualitative variable is distributed normally or not. The comparison between quantitative variables will be performed with a test to compare the means for paired data. The final ACT score will be compared between groups by analysing the covariance (ANCOVA), with the

initial ACT score as a covariate. For the correlations between qualitative variables, Spearman's rho will be used, expressing it with the correlation coefficient and statistical significance. The statistical package SPSS® version 15.0 ® was used for the analysis.

### **6.11. Ethical and legal aspects.**

The development of research is based on the *Declaration of the Helsinki World Medical Association* and the Code of Ethics of the Association of Medical Colleges of Spain. Patients and relatives will be informed about the nature of the study, voluntary participation in it, the proposed objectives and potential adverse effects that might occur in their realization. Each patient or family member will be provided with a sheet with the information, and will be asked for signing the Informed Consent (Annex I).

Treatment, communication and transfer of personal data of all participants subject will be adjusted to the provisions of Law 15/1999 of 13 December Protection of Personal Data (LOPD). According to the provisions of the above legislation, patients could exercise the rights of access, modification, opposition and cancellation of data, for which should be addressed to the study doctor in case of medical emergency or legal requirement.

Access to personal information will be restricted to the researcher / collaborators, health authorities (Spanish Agency for Medicines and Health Products), the Ethics Committee for Clinical Research and authorized by the sponsor personnel when necessary to check the data and procedures study, but always maintaining the confidentiality of the same in accordance with current legislation.

Similarly, patients who will be taken by the study test all security measures established by Royal Decree 1720/2007, of 21 December approving the Regulations implementing the Organic Law approving 15 reported / 1999 of

December 13, Protection of Personal data and is subject to all the provisions applicable to them under Law 41/2002, of 14 November, regulating basic patient autonomy and rights and obligations regarding information and clinical documentation.

This RCT was approved by the Ethics Committee for Clinical Research (CEIC) of Morales Meseguer Hospital in 29 April 2015 (Annex II).



## 7. References.

1. Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. *American journal of respiratory and critical care medicine*. 2004;170(8):836-44.
2. Adcock IM, Barnes PJ. Molecular mechanisms of corticosteroid resistance. *Chest*. 2008;134(2):394-401.
3. Szeffler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *The Journal of allergy and clinical immunology*. 2002;109(3):410-8.
4. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? *Archives of biochemistry and biophysics*. 2012;523(1):123-33.
5. Ryan JW, Anderson PH, Morris HA. Pleiotropic Activities of Vitamin D Receptors - Adequate Activation for Multiple Health Outcomes. *The Clinical biochemist Reviews / Australian Association of Clinical Biochemists*. 2015;36(2):53-61.
6. Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horst RL. Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. *The New England journal of medicine*. 1981;305(8):440-3.
7. Holick MF. Vitamin D deficiency. *The New England journal of medicine*. 2007;357(3):266-81.
8. IOM, Institute of Medicine. Dietary Intakes for Calcium and Vitamin D. Committee to Review Dietary Reference Intakes for Calcium and Vitamin D. 2011.
9. Holick MF. Optimal vitamin D status for the prevention and treatment of osteoporosis. *Drugs & aging*. 2007;24(12):1017-29.
10. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *The American journal of clinical nutrition*. 2006;84(1):18-28.
11. National Osteoporosis Foundation. Clinician's guide to prevention and treatment of osteoporosis. 2010.
12. Pérez Edo L ARA, Roig Vilaseca D, García Vellido A, Guañabens Gay N, Peris P, et al. Actualización 2011 del consenso Sociedad Española de Reumatología de osteoporosis. *Reumatol Clin*. 2011;7:357-79.
13. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *The Journal of clinical endocrinology and metabolism*. 2011;96(1):53-8.
14. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism*. 2011;96(7):1911-30.
15. Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt P, Fuleihan GE, et al. IOF position statement: vitamin D recommendations for older adults. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2010;21(7):1151-4.

16. Rosen CJ, Adams JS, Bikle DD, Black DM, Demay MB, Manson JE, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocrine reviews*. 2012;33(3):456-92.
17. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clinic proceedings*. 2006;81(3):353-73.
18. Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, et al. A systematic review of vitamin D status in populations worldwide. *The British journal of nutrition*. 2014;111(1):23-45.
19. Dan L, Longo M, Harrison, 18<sup>a</sup> ed. 2012;2:3092-5.
20. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *The Journal of clinical endocrinology and metabolism*. 2004;89(11):5387-91.
21. Heaney RP, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D(3) is more potent than vitamin D(2) in humans. *The Journal of clinical endocrinology and metabolism*. 2011;96(3):E447-52.
22. Bischoff-Ferrari HA, Dawson-Hughes B, Stocklin E, Sidelnikov E, Willett WC, Edel JO, et al. Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2012;27(1):160-9.
23. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *The American journal of clinical nutrition*. 2007;85(1):6-18.
24. Bacon CJ, Gamble GD, Horne AM, Scott MA, Reid IR. High-dose oral vitamin D3 supplementation in the elderly. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2009;20(8):1407-15.
25. Pirotte B, Stifkens F, Kaye O, Radermacher L, Putzeys V, Deflandre J, et al. [Hypercalcemia and acute renal failure: a case report of vitamin D intoxication]. *Revue medicale de Liege*. 2015;70(1):12-6.
26. van den Ouweland J, Fleuren H, Drabbe M, Vollaard H. Pharmacokinetics and safety issues of an accidental overdose of 2,000,000 IU of vitamin D3 in two nursing home patients: a case report. *BMC pharmacology & toxicology*. 2014;15:57.
27. Marins TA, Galvao Tde F, Korkes F, Malerbi DA, Ganc AJ, Korn D, et al. Vitamin D intoxication: case report. *Einstein*. 2014;12(2):242-4.
28. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *Journal of immunology*. 2008;181(10):7090-9.
29. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006;311(5768):1770-3.
30. Lopez-Garcia B, Lee PH, Yamasaki K, Gallo RL. Anti-fungal activity of cathelicidins and their potential role in *Candida albicans* skin infection. *The Journal of investigative dermatology*. 2005;125(1):108-15.
31. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*. 2011;50(3):194-200.
32. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked

chemokines and cytokines in airway epithelium while maintaining the antiviral state. *Journal of immunology*. 2010;184(2):965-74.

33. Cantorna MT. Mechanisms underlying the effect of vitamin D on the immune system. *The Proceedings of the Nutrition Society*. 2010;69(3):286-9.

34. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. *Expert review of clinical immunology*. 2009;5(6):693-702.

35. Hypponen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE - a significant but nonlinear relationship. *Allergy*. 2009;64(4):613-20.

36. Heine G, Niesner U, Chang HD, Steinmeyer A, Zugel U, Zuberbier T, et al. 1,25-dihydroxyvitamin D(3) promotes IL-10 production in human B cells. *European journal of immunology*. 2008;38(8):2210-8.

37. Hartmann B, Heine G, Babina M, Steinmeyer A, Zugel U, Radbruch A, et al. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy*. 2011;66(4):540-8.

38. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *The Journal of allergy and clinical immunology*. 2013;131(4):1204-12.

39. Nanzer AM, Chambers ES, Ryanna K, Richards DF, Black C, Timms PM, et al. Enhanced production of IL-17A in patients with severe asthma is inhibited by 1alpha,25-dihydroxyvitamin D3 in a glucocorticoid-independent fashion. *The Journal of allergy and clinical immunology*. 2013;132(2):297-304 e3.

40. Zhao J, Lloyd CM, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling. *Mucosal immunology*. 2013;6(2):335-46.

41. McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *Journal of immunology*. 2008;181(6):4089-97.

42. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. *Respiratory medicine*. 2010;104(8):1131-7.

43. Zijlstra GJ, Ten Hacken NH, Hoffmann RF, van Oosterhout AJ, Heijink IH. Interleukin-17A induces glucocorticoid insensitivity in human bronchial epithelial cells. *Eur Respir J*. 2012;39(2):439-45.

44. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *American journal of respiratory and critical care medicine*. 2009;180(8):720-30.

45. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, et al. Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. *The Journal of biological chemistry*. 2011;286(2):997-1004.

46. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity*. 2009;31(3):438-49.

47. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nature reviews Immunology*. 2005;5(4):271-83.

48. Hartl D, Koller B, Mehlhorn AT, Reinhardt D, Nicolai T, Schendel DJ, et al. Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T

- cells in pediatric asthma. *The Journal of allergy and clinical immunology*. 2007;119(5):1258-66.
49. Gupta A, Dimeloe S, Richards DF, Chambers ES, Black C, Urry Z, et al. Defective IL-10 expression and in vitro steroid-induced IL-17A in paediatric severe therapy-resistant asthma. *Thorax*. 2014;69(6):508-15.
50. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *The Journal of clinical investigation*. 2006;116(1):146-55.
51. Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. *Current allergy and asthma reports*. 2011;11(1):29-36.
52. Urry Z, Chambers ES, Xystrakis E, Dimeloe S, Richards DF, Gabrysova L, et al. The role of 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and cytokines in the promotion of distinct Foxp3<sup>+</sup> and IL-10<sup>+</sup> CD4<sup>+</sup> T cells. *European journal of immunology*. 2012;42(10):2697-708.
53. Pfeffer PE, Mann EH, Hornsby E, Chambers ES, Chen YH, Rice L, et al. Vitamin D influences asthmatic pathology through its action on diverse immunological pathways. *Annals of the American Thoracic Society*. 2014;11 Suppl 5:S314-21.
54. Durrani SR, Viswanathan RK, Busse WW. What effect does asthma treatment have on airway remodeling? Current perspectives. *The Journal of allergy and clinical immunology*. 2011;128(3):439-48; quiz 49-50.
55. Lai G, Wu C, Hong J, Song Y. 1,25-Dihydroxyvitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>) attenuates airway remodeling in a murine model of chronic asthma. *The Journal of asthma : official journal of the Association for the Care of Asthma*. 2013;50(2):133-40.
56. Banerjee A, Panettieri R, Jr. Vitamin D modulates airway smooth muscle function in COPD. *Current opinion in pharmacology*. 2012;12(3):266-74.
57. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. *American journal of respiratory and critical care medicine*. 2011;184(12):1342-9.
58. Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, et al. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. *British journal of pharmacology*. 2009;158(6):1429-41.
59. Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D deficiency as a strong predictor of asthma in children. *International archives of allergy and immunology*. 2012;157(2):168-75.
60. Brehm JM, Acosta-Perez E, Klei L, Roeder K, Barmada M, Boutaoui N, et al. Vitamin D insufficiency and severe asthma exacerbations in Puerto Rican children. *American journal of respiratory and critical care medicine*. 2012;186(2):140-6.
61. Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *American journal of respiratory and critical care medicine*. 2009;179(9):765-71.
62. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *The Journal of allergy and clinical immunology*. 2010;126(1):52-8 e5.

63. Gergen PJ, Teach SJ, Mitchell HE, Freishtat RF, Calatroni A, Matsui E, et al. Lack of a relation between serum 25-hydroxyvitamin D concentrations and asthma in adolescents. *The American journal of clinical nutrition*. 2013;97(6):1228-34.
64. Cheng HM, Kim S, Park GH, Chang SE, Bang S, Won CH, et al. Low vitamin D levels are associated with atopic dermatitis, but not allergic rhinitis, asthma, or IgE sensitization, in the adult Korean population. *The Journal of allergy and clinical immunology*. 2014;133(4):1048-55.
65. Devereux G, Wilson A, Avenell A, McNeill G, Fraser WD. A case-control study of vitamin D status and asthma in adults. *Allergy*. 2010;65(5):666-7.
66. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. *American journal of respiratory and critical care medicine*. 2010;181(7):699-704.
67. Korn S, Hubner M, Jung M, Blettner M, Buhl R. Severe and uncontrolled adult asthma is associated with vitamin D insufficiency and deficiency. *Respiratory research*. 2013;14:25.
68. Global Initiative for Asthma: GINA Report, Global Strategy for Asthma Management and Prevention. 2010.
69. Confino-Cohen R, Brufman I, Goldberg A, Feldman BS. Vitamin D, asthma prevalence and asthma exacerbations: a large adult population-based study. *Allergy*. 2014;69(12):1673-80.
70. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *The American journal of clinical nutrition*. 2010;91(5):1255-60.
71. Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. *The Journal of allergy and clinical immunology*. 2011;127(5):1294-6.
72. Yadav M, Mittal K. Effect of vitamin D supplementation on moderate to severe bronchial asthma. *Indian journal of pediatrics*. 2014;81(7):650-4.
73. Lewis E, Fernandez C, Nella A, Hopp R, Gallagher JC, Casale TB. Relationship of 25-hydroxyvitamin D and asthma control in children. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. 2012;108(4):281-2.
74. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *The Journal of allergy and clinical immunology*. 2004;113(1):59-65.
75. Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, et al. Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *Jama*. 2014;311(20):2083-91.
76. Arshi S, Fallahpour M, Nabavi M, Bermanian MH, Javad-Mousavi SA, Nojomi M, et al. The effects of vitamin D supplementation on airway functions in mild to moderate persistent asthma. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. 2014;113(4):404-9.
77. de Groot JC, van Roon EN, Storm H, Veeger NJ, Zwinderman AH, Hiemstra PS, et al. Vitamin D reduces eosinophilic airway inflammation in nonatopic asthma. *The Journal of allergy and clinical immunology*. 2015;135(3):670-5 e3.



78. Juniper EF BA, Cox FM, Ferrie PJ, el rey DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest*. 1999;115 (5):1265-70.
79. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *The European respiratory journal*. 1999;14(4):902-7.
80. Martineau AR, MacLaughlin BD, Hooper RL, Barnes NC, Jolliffe DA, Greiller CL, et al. Double-blind randomised placebo-controlled trial of bolus-dose vitamin D3 supplementation in adults with asthma (ViDiAs). *Thorax*. 2015;70(5):451-7.
81. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. *AMA archives of internal medicine*. 1958;101(2):267-78.
82. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *The American review of respiratory disease*. 1992;145(6):1321-7.
83. Fletcher CM, Elmes PC, Fairbairn AS, Wood CH. The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. *British medical journal*. 1959;2(5147):257-66.
84. Mahler DA, Rosiello RA, Harver A, Lentine T, McGovern JF, Daubenspeck JA. Comparison of clinical dyspnea ratings and psychophysical measurements of respiratory sensation in obstructive airway disease. *The American review of respiratory disease*. 1987;135(6):1229-33.
85. Barbera JA, Peces-Barba G, Agusti AG, Izquierdo JL, Monso E, Montemayor T, et al. [Clinical guidelines for the diagnosis and treatment of chronic obstructive pulmonary disease]. *Archivos de bronconeumologia*. 2001;37(6):297-316.
86. Celli BR, MacNee W, Force AET. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *The European respiratory journal*. 2004;23(6):932-46.
87. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS, Committee GS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *American journal of respiratory and critical care medicine*. 2001;163(5):1256-76.
88. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest*. 1988;93(3):580-6.
89. Gaga M, Papageorgiou N, Zervas E, Gioulekas D, Konstantopoulos S. Control of asthma under specialist care: is it achieved? *Chest*. 2005;128(1):78-84.
90. Rodrigo GJ, Arcos JP, Nannini LJ, Neffen H, Broin MG, Contrera M, et al. Reliability and factor analysis of the Spanish version of the asthma control test. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology*. 2008;100(1):17-22.
91. See KC, Christiani DC. Normal values and thresholds for the clinical interpretation of exhaled nitric oxide levels in the US general population: results from the National Health and Nutrition Examination Survey 2007-2010. *Chest*. 2013;143(1):107-16.
92. Schatz M, Kosinski M, Yarlas AS, Hanlon J, Watson ME, Jhingran P. The minimally important difference of the Asthma Control Test. *The Journal of allergy and clinical immunology*. 2009;124(4):719-23 e1.
93. Suissa S. Number needed to treat in COPD: exacerbations versus pneumonias. *Thorax*. 2013;68(6):540-3.

94. Laupacis A, Sackett DL, Roberts RS. An assessment of clinically useful measures of the consequences of treatment. *The New England journal of medicine*. 1988;318(26):1728-33.
95. Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest*. 1999;115(5):1265-70.
96. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. *The European respiratory journal*. 1999;14(1):32-8.
97. Sanjuas C, Alonso J, Ferrer M, Curull V, Broquetas JM, Anto JM. Adaptation of the Asthma Quality of Life Questionnaire to a second language preserves its critical properties: the Spanish version. *Journal of clinical epidemiology*. 2001;54(2):182-9.
98. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. *Journal of clinical epidemiology*. 1994;47(1):81-7.
99. Wyrwich KW, Tierney WM, Wolinsky FD. Using the standard error of measurement to identify important changes on the Asthma Quality of Life Questionnaire. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation*. 2002;11(1):1-7.

## **8. Annexes.**

### **8.1. ANNEX I: INFORMED CONSENT.**

#### **INFORMED CONSENT, English version:**

**"Effectiveness calcifediol supplementation in asthma control in patients with vitamin D deficiency".**

**Researcher: Lourdes Salinero González.**

Please read this information, and ask anything you do not understand.

We ask for your permission to include you in a study aimed to evaluate the efficacy of vitamin D supplementation in asthmatic patients with vitamin D deficiency, in relation to the degree of asthma control.

Vitamin D supplementation used (Hidroferol®) is used in common for treating vitamin D deficiency states on habitual clinical practice.

The study methodology consists in taking a preparation in the form of drinkable ampoule, which may or not contain vitamin D supplement with a probability of 50%, and should be taken once a week until the end of the study. The follow-up period ends at 6 months after, and then you will be prescribed dose vitamin D supplement that needs to normalize the deficiency.

If you decide not to participate, we will recommend you taking this vitamin D supplement anyway, but your data and results of treatment will not be used for our study.

If you become pregnant during the 6 months of follow-up, you should inform the principal researcher to be removed from this study.

#### **WILLFULNESS:**

Participation in this study is completely voluntary. If at any time you decide not to continue, simply notify the specialist with whom you spoke.



**DISADVANTAGES AND BENEFITS:**

The benefits you can get are the possible improvement in the control of your respiratory disease due to interventions in the study, and whose knowledge is the ultimate goal of this study.

Drawbacks you could get would be the extractions of analytical variables, performing pulmonary function tests (spirometry), answer two specific questionnaires on asthma and will be called by phone one monthly to check adherence to treatment.

**SIDE EFFECTS:**

No side effects of this study are expected, since no different vitamin D supplements are used to those used today.

Medical care will be performed by a specialist in Endocrinology and another in Pneumology, and does not vary regarding recommended in these types of queries, so it can not be obtained negative health effects.

**WITHDRAWAL OF STUDY:**

The patient and his family may void the consent at any time, without incurring any negative consequences for later attention on this or any other hospital.

**ALTERNATIVE TREATMENTS:**

On the market other business presentations vitamin D, also effective in normalizing vitamin D deficiency.

In case of not wanting to participate, your data will not be taken into account for the results and conclusions of the study.

**CONFIDENTIALITY** (Clause is provided).

Treatment, communication and transfer of personal data of all participants

subject is governed by the provisions of Law 15/1999 of 13 December protection of personal data, Royal Decree 1720/2007 of 21 December, approving the Regulation implementing the Law 15/1999 and Law 41/2002, of 14 November, a basic regulatory Patient Autonomy and Rights and Obligations approved information and clinical documentation.

Only they are transmitted to third countries and other data collected for the study, which in any case contain information that can identify you directly, such as name, initials, address, etc. In the event that such transfer occurs, will be for the same purposes of the study described and ensuring the confidentiality and at least the level of protection equivalent to that provided by current legislation in our country.

Data collected for the study will be identified by a code and only your study doctor or collaborators of health workers can relate these data with you and your medical history. Therefore, your identity will not be disclosed to any person except exceptions, medical emergency or legal requirement. The data of the data is the principal investigator of the project.

You may contact at any time with the principal investigator of the study, to exercise their rights of access, rectification, cancellation and opposition (ARCO rights) regarding your personal data, according to the aforementioned law 15/1999.

Access to their personal information will be restricted to the study doctor, employees, health authorities (Spanish Agency for Medicines and Health Products), the Ethics Committee for Clinical and Research staff authorized by the promoter, when they need to check the data and procedures study, but always keeping them confidential according to the legislation referred to above. Access

to their medical records will be for study purposes only.

**INFORMED CONSENT:**

Mr./Ms.....

or Mr./Ms.....as your representative

**EXPOSE:**

that Dra. Lourdes Salinero Gonzalez, a specialist in Endocrinology informed me the opportunity to participate in this study:

She has ensured that all medical care I receive will dictate the current recommendations of good medicine, so the treatment or testing are equal to those of any other patient with my illness that was not in this study.

She has explained that the objective is to evaluate the effectiveness of supplementation with calcifediol (vitamin D) in patients with vitamin D deficiency, in relation to the degree of control of asthma; and a totally confidential Data will be collected to analyze the results and to compare them with other studies.

**MANIFEST:**

I asked what I considered necessary to be fully informed about the advantage that participate in this study and has been answered to my satisfaction.

And so I give consent for them to use data from my clinical and analytical evolution, understanding that this consent may be revoked by me at any time.

Murcia, of 201

Firm:

**INFORMED CONSENT, Spanish version:****“Eficacia de la suplementación con calcifediol en el control del asma en pacientes con déficit de vitamina D.”****Investigador principal: Lourdes Salinero González.**

Por favor, lea esta información, y pregunte todo aquello que no entienda.

Le pedimos su autorización para incluirle en un estudio que pretende evaluar la eficacia de la suplementación con vitamina D en pacientes asmáticos que presentan dicho déficit, respecto al grado de control del asma.

En ningún momento, se probará ningún tipo de preparado ni medicamentos nuevos; el suplemento de vitamina D utilizado (hidroferol®) se usa en la práctica clínica habitual para tratar los estados deficitarios de dicha vitamina.

La metodología del estudio consiste en la toma de un preparado en forma de ampolla bebible, que puede contener o no dicho suplemento vitamínico con una probabilidad del 50%, y que deberá tomar inicialmente una vez a la semana, y posteriormente con la periodicidad que se le indique.

Cuando finalice dicho periodo de seguimiento de 6 meses, e independientemente del tipo de preparado recibido, se le prescribirá la dosis de suplemento de vitamina D que precise, para normalizar dicho déficit.

Si usted decide no participar, de igual modo se le recomendará la toma de dichos suplementos vitamínicos, pero sus datos y los resultados de su tratamiento no serán utilizados para nuestro estudio.

Si se quedara embarazada durante los 6 meses de seguimiento, debería comunicarlo al investigador principal, para ser retirada de este estudio.

**VOLUNTARIEDAD:**

La participación en este estudio es totalmente voluntaria. Si en cualquier momento decide no continuar, sólo tiene que comunicarlo al especialista con el que habló.

**BENEFICIOS E INCONVENIENTES:**

→ Los beneficios que puede obtener son la posible mejoría en el control de su enfermedad, debido a las intervenciones realizadas en el estudio, y cuyo conocimiento es el objetivo final de dicho estudio.

→ Los inconvenientes que podría obtener sería la extracción de variables analíticas, la realización de pruebas de función pulmonar (espirometría y FeNO), responder 2 cuestionarios específicos sobre el asma, así como se le llamará por teléfono en una ocasión para comprobar la adherencia al tratamiento, revisión de resultados analíticos o cualquier otra incidencia que pudiera aparecer.

**EFFECTOS SECUNDARIOS:**

No se espera ningún efecto secundario de este estudio, puesto que no se utilizan suplementos vitamínicos diferentes a los utilizados en la actualidad.

La atención médica será realizada por un especialista en Endocrinología y Nutrición, y otro en Neumología, y no varía respecto a la recomendada en este tipo de consultas, por lo que no puede obtenerse efectos negativos para su salud.

**RETIRADA DEL ESTUDIO:**

El enfermo y su familia pueden anular el consentimiento en cualquier momento, sin que suponga ninguna consecuencia negativa para su atención posterior en este u otro hospital.

**TRATAMIENTOS ALTERNATIVOS:**

Existen en el mercado otras presentaciones comerciales de vitamina D, igualmente eficaces a la hora de normalizar dicho déficit vitamínico.

En caso de no querer participar, sus datos no serán tenidos en cuenta para obtener los resultados y conclusiones del estudio.

**CONFIDENCIALIDAD** (se aporta cláusula).

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se rige por lo dispuesto en la Ley Orgánica 15/1999, de 13 de diciembre de protección de datos de carácter personal, el Real Decreto 1720/2007, de 21 de diciembre, por el que se aprueba el Reglamento de desarrollo de la citada Ley Orgánica 15/1999, y la Ley 41/2002, de 14 de noviembre, básica reguladora de la Autonomía del Paciente y de Derechos y Obligaciones en materia de información y documentación clínica.

Sólo se transmitirán a terceros y a otros países los datos recogidos para el estudio, que en ningún caso contendrán información que le pueda identificar directamente, como nombre y apellidos, iniciales, dirección, etc. En el caso de que se produzca esta cesión, será para los mismos fines del estudio descrito y garantizando la confidencialidad y, como mínimo, con el nivel de protección equivalente al que contempla la legislación vigente en nuestro país.

Los datos recogidos para el estudio estarán identificados mediante un código y sólo su médico del estudio o colaboradores del personal sanitario podrán relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones, en caso de urgencia médica o requerimiento legal. El responsable del fichero de los datos es el investigador principal del proyecto.

Usted podrá ponerse en contacto en cualquier momento con el investigador principal del estudio, para ejercer sus derechos de acceso, rectificación, cancelación y oposición (derechos ARCO) respecto a sus datos personales, según la ley ya citada 15/1999.

El acceso a su información personal quedará restringido al médico del estudio, colaboradores, autoridades sanitarias (Agencia Española del Medicamento y Productos Sanitarios), al Comité Ético de Investigación Clínica y personal autorizado por el promotor, cuando lo precisen para comprobar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo a la legislación referida anteriormente. El acceso a su historia clínica será sólo para los fines del estudio.

**CONSENTIMIENTO INFORMADO:**

D./D<sup>a</sup>.....  
o D./D<sup>a</sup>.....como su representante

**EXPONGO:**

Que la Dra. Lourdes Salinero González, médico especialista en Endocrinología y Nutrición, me ha informado de la posibilidad de participar en el estudio:

Me ha garantizado que toda la atención médica que reciba será la que dictan las recomendaciones actuales de la Buena Medicina, por lo que el tratamiento o las pruebas serán iguales a las de cualquier otro paciente con mi enfermedad que no estuviera en este estudio.

Me explican que el objetivo es evaluar la eficacia de la suplementación con calcifediol (vitamina D) en pacientes con dicho déficit vitamínico, respecto al grado de control del asma; y que de forma totalmente confidencial se recogerán

los datos para analizar los resultados obtenidos y poder compararlos con otros estudios.

**MANIFIESTO:**

Que he preguntado lo que he considerado necesario para estar perfectamente informado acerca de la ventaja que tiene participar en el presente estudio y se me ha contestado satisfactoriamente.

Y por ello **OTORGO MI CONSENTIMIENTO** para que puedan utilizar los datos de mi evolución clínica y analítica, entendiendo que este consentimiento puede ser revocado por mí en cualquier momento.

Murcia, a                      de                      de 201

Firma:



## 8.2. ANNEX II:

## DOCUMENT OF ACCEPTANCE OF THE STUDY BY THE CEIC and address of H. MORALES MESEGUER.



Informe Dictamen Protocolo Favorable  
Otros Estudios  
C.P. - C.I. EST:18/15  
29 de Abril de 2015

CEIC Hospital General Universitario José María Morales Meseguer

Dra. María Dolores Nájera Pérez  
Presidenta del CEIC Hospital General Universitario José María Morales Meseguer

## CERTIFICA

1º. Que el CEIC Hospital General Universitario José María Morales Meseguer en su reunión del día 29/04/2015, acta ORDINARIA ha evaluado la propuesta del promotor referida al estudio:

Título: Proyecto de Investigación: "Eficacia de la suplementación con calcifediol en el control del asma en pacientes con déficit de vitamina D".

Código Interno: EST:18/15

Promotor: Investigador Principal.

Versión Protocolo Evaluada: Marzo-2015

Versión Hoja Información al Paciente Evaluada: GENERAL / Marzo-2015

Fecha Entrada de Aclaraciones: 27/03/2015

Investigador Principal: Dra. Lourdes Salinero González, Endocrinología y Nutrición, H.G.U. "Morales Meseguer".

1º. Considera que:

- Se respetan los principios éticos básicos y es adecuado el procedimiento para obtener el consentimiento informado.

2º. Por lo que este CEIC emite un **DICTAMEN FAVORABLE**.

3º. Para la realización del Estudio, como prueba extra y fuera de la práctica clínica habitual, es necesaria la realización de 270 analíticas de Vitamina D a los participantes.

Lo que firmo en Murcia, a 29 de Abril de 2015



Dra. María Dolores Nájera Pérez  
Presidenta del CEIC Hospital General Universitario José María Morales Meseguer

Hospital General Universitario J.M. Morales Meseguer  
Plaza de los Váleros s/n Murcia 30008 Murcia España  
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APROBACIÓN DE REALIZACIÓN DE ESTUDIO  
POST AUTORIZACION DE TIPO OBSERVACIONAL

**CEIC Hospital General Universitario Morales Meseguer**

CONFORMIDAD DE LA DIRECCIÓN DEL CENTRO

D. Tomás Salvador Fernández Pérez, Director Gerente del Área VI, vista la autorización del Comité Ético de Investigación Clínica del Hospital Morales Meseguer,

**CERTIFICA**

Que conoce la propuesta realizada sobre el Estudio: **Proyecto de Investigación: "Eficacia de la suplementación con calcifediol en el control del asma en pacientes con déficit de vitamina D"**.

**Código Interno: ESTUDIO: 18/15**

Y que será realizado por la Dra. Lourdes SALINERO GONZÁLEZ, Endocrinología y Nutrición del Hospital Morales Meseguer.

Que está de acuerdo con los aspectos económicos de este estudio.

Que acepta la realización de dicho estudio.

Fecha de aprobación por el CEIC del Área VI Hospital General Universitario "Morales Meseguer" el día 29 de Abril de 2015 para la realización del Estudio, como prueba extra y fuera de la práctica clínica habitual, es necesaria la realización de 270 analíticas de Vitamina D a los participantes.


Lo que firma en Murcia, a 25 de mayo de 2015.

Director Gerente del Área VI

Fdo.: D. Tomas Salvador Fernández Pérez

8.3. APPENDIX III: ACT (Asthma Control Test)

English Version:



Name: \_\_\_\_\_

Today's Date: \_\_\_\_\_

# ASTHMA CONTROL TEST™

**Know your score.**

The Asthma Control Test™ provides a numerical score to help you and your healthcare provider determine if your asthma symptoms are well controlled.

Take this test if you are 12 years or older. Share the score with your healthcare provider.

**Step 1:** Write the number of each answer in the score box provided.

**Step 2:** Add up each score box for the total.

**Step 3:** Take the completed test to your healthcare provider to talk about your score.


**IF YOUR SCORE IS 19 OR LESS, Your asthma symptoms may not be as well controlled as they could be. No matter what the score, bring this test to your healthcare provider to talk about the results.**

**NOTE:** If your score is 15 or less, your asthma may be very poorly controlled. Please contact your healthcare provider right away. There may be more you and your healthcare provider could do to help control your asthma symptoms.

1. In the <u>past 4 weeks</u> , how much of the time did your <u>asthma</u> keep you from getting as much done at work, school or at home?					SCORE
All of the time [1]	Most of the time [2]	Some of the time [3]	A little of the time [4]	None of the time [5]	_____
2. During the <u>past 4 weeks</u> , how often have you had shortness of breath?					
More than Once a day [1]	Once a day [2]	3 to 5 times a week [3]	Once or twice a week [4]	Not at all [5]	_____
3. During the <u>past 4 weeks</u> , how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?					
4 or more nights a week [1]	2 to 3 nights a week [2]	Once a week [3]	Once or twice [4]	Not at all [5]	_____
4. During the <u>past 4 weeks</u> , how often have you used your rescue inhaler or nebulizer medication (such as albuterol)?					
3 or more times per day [1]	1 to 2 times per day [2]	2 or 3 times per week [3]	Once a week or less [4]	Not at all [5]	_____
5. How would you rate your asthma control during the past 4 weeks?					
Not Controlled at All [1]	Poorly Controlled [2]	Somewhat Controlled [3]	Well Controlled [4]	Completely Controlled [5]	_____
TOTAL:					_____

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**ACT questionnaire: Spanish version:****Asthma Control Test™ (La Prueba de Control del Asma) es:**

- Una prueba rápida que produce un resultado numérico para evaluar el control del asma.
- Reconocida por los Institutos Nacionales de la Salud (National Institutes of Health - NIH) en sus directrices sobre el asma de 2007.<sup>1</sup>
- Convalidada clínicamente por espirometría y evaluaciones de especialistas.<sup>2</sup>

**Para pacientes de 12 años de edad en adelante:**

1. Conteste cada pregunta y escriba el número de la respuesta en el cuadro que aparece a la derecha de la pregunta.
2. Sume sus respuestas y escriba el puntaje total en el cuadro del TOTAL que se muestra abajo.
3. Hable con su médico sobre sus resultados.

1. En las últimas **4 semanas**, ¿cuánto tiempo le ha impedido su **asma** hacer todo lo que quería en el trabajo, en la escuela o en la casa?

Siempre	1	La mayoría del tiempo	2	Algo del tiempo	3	Un poco del tiempo	4	Nunca	5
---------	---	-----------------------	---	-----------------	---	--------------------	---	-------	---

**PUNTAJE**

2. Durante las últimas **4 semanas**, ¿con qué frecuencia le ha faltado el aire?

Más de una vez al día	1	Una vez al día	2	De 3 a 6 veces por semana	3	Una o dos veces por semana	4	Nunca	5
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3. Durante las últimas **4 semanas**, ¿con qué frecuencia sus síntomas del **asma** (respiración sibilante o un silbido en el pecho, tos, falta de aire, opresión en el pecho o dolor) lo/la despertaron durante la noche o más temprano de lo usual en la mañana?

4 o más noches por semana	1	De 2 a 3 noches por semana	2	Una vez por semana	3	Una o dos veces	4	Nunca	5
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4. Durante las últimas **4 semanas**, ¿con qué frecuencia ha usado su inhalador de rescate o medicamento en nebulizador (como albuterol)?

3 o más veces al día	1	1 ó 2 veces al día	2	2 ó 3 veces por semana	3	Una vez por semana o menos	4	Nunca	5
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5. ¿Cómo evaluaría el control de su **asma** durante las últimas **4 semanas**?

No controlada en absoluto	1	Mal controlada	2	Algo controlada	3	Bien controlada	4	Completamente controlada	5
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Si obtuvo 19 puntos o menos, es probable que su asma no esté bajo control. Hable con su médico sobre sus resultados. Las siguientes respuestas no deben sumarse al puntaje total. Converse con su médico acerca de estas respuestas.

En los últimos 12 meses, ¿cuántas veces ha acudido al servicio de emergencias debido al asma (que no hayan resultado en una hospitalización)? \_\_\_\_\_

En los últimos 12 meses, ¿cuántas veces ha estado hospitalizado debido al asma? \_\_\_\_\_

**TOTAL**

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La Prueba de Control del Asma es para personas asmáticas de 12 años de edad en adelante.

**Referencias:** 1. Departamento de Salud y Servicios Humanos de EE. UU., Institutos Nacionales de la Salud, Instituto Nacional del Corazón, los Pulmones y la Sangre. *Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma (EPR-3 2007)*. Ítem de NIH No. 08-4051. <http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm>. Consultado el 10 de septiembre de 2007. 2. Nathan RA y otros. *J Allergy Clin Immunol*. 2004;113:59-65.



HM2701R0 May 2010



**8.4. ANNEX IV: mini-AQLQ (Asthma Quality of Life Questionnaire).****Spanish version:**

CUESTIONARIO DE CALIDAD DE VIDA EN  
PACIENTES CON ASMA - VERSIÓN REDUCIDA  
(SPANISH VERSION)  
AUTO-ADMINISTRADO

ID PACIENTE: \_\_\_\_\_

FECHA: \_\_\_\_\_

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Le rogamos responda **a todas** las preguntas señalando con un círculo la respuesta que mejor describa cómo se ha encontrado **durante las dos últimas semanas, debido al asma.**

EN GENERAL, ¿CON QUÉ FRECUENCIA DURANTE LAS 2 ÚLTIMAS SEMANAS:

	Siempre	Casi siempre	Gran parte del tiempo	Parte del tiempo	Poco tiempo	Casi nunca	Nunca
1. NOTÓ QUE LE FALTABA EL AIRE debido al asma?	1	2	3	4	5	6	7
2. Sintió que le molestaba el POLVO, o tuvo que evitar un lugar debido al POLVO?	1	2	3	4	5	6	7
3. Se sintió FRUSTRADO O IRRITADO debido al asma?	1	2	3	4	5	6	7
4. Sintió molestias debido a la TOS?	1	2	3	4	5	6	7
5. TUVO MIEDO DE NO TENER A MANO SU MEDICACIÓN PARA EL ASMA?	1	2	3	4	5	6	7
6. Notó una sensación de AHOGO U OPRESIÓN EN EL PECHO?	1	2	3	4	5	6	7
7. Sintió que le molestaba el HUMO DEL TABACO, o tuvo que evitar un lugar debido al HUMO DEL TABACO?	1	2	3	4	5	6	7
8. Tuvo DIFICULTADES PARA DORMIR BIEN POR LA NOCHE debido al asma?	1	2	3	4	5	6	7



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## EN GENERAL, ¿CON QUÉ FRECUENCIA DURANTE LAS 2 ÚLTIMAS SEMANAS:

	Siempre	Casi siempre	Gran parte del tiempo	Parte del tiempo	Poco tiempo	Casi nunca	Nunca
9. Se sintió PREOCUPADO POR TENER ASMA?	1	2	3	4	5	6	7
10. Sintió SILBIDOS O PITOS en el pecho?	1	2	3	4	5	6	7
11. Sintió que le molestaba o tuvo que evitar salir de casa debido AL TIEMPO O A LA CONTAMINACIÓN ATMOSFÉRICA ?	1	2	3	4	5	6	7

## ¿HASTA QUÉ PUNTO EL ASMA LE HA LIMITADO PARA HACER ESTAS ACTIVIDADES DURANTE LAS 2 ÚLTIMAS SEMANAS?

	Totalmente limitado	Extremadamente limitado	Muy limitado	Moderadamente limitado	Algo limitado	Poco limitado	Nada limitado
12. ESFUERZOS INTENSOS (como darse prisa, hacer ejercicio, subir escaleras corriendo, hacer deporte)	1	2	3	4	5	6	7
13. ESFUERZOS MODERADOS (como caminar, hacer las tareas del hogar, trabajar en el jardín o en el huerto, hacer la compra, subir escaleras sin correr)	1	2	3	4	5	6	7

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## ¿HASTA QUÉ PUNTO EL ASMA LE HA LIMITADO PARA HACER ESTAS ACTIVIDADES DURANTE LAS 2 ÚLTIMAS SEMANAS?

	Totalmente limitado	Extremadamente limitado	Muy limitado	Moderadamente limitado	Algo limitado	Poco limitado	Nada limitado
14. ACTIVIDADES SOCIALES (como hablar, jugar con niños/animales domésticos, visitar a amigos/familiares)	1	2	3	4	5	6	7
15. ACTIVIDADES RELACIONADAS CON SU TRABAJO (tareas que tiene que hacer en su trabajo*)	1	2	3	4	5	6	7

\* Si no está trabajando, responda a esta pregunta pensando en las tareas que tiene que hacer la mayoría de los días.

## CLAVE DE LAS DIMENSIONES:

Síntomas: 1, 4, 6, 8, 10  
 Limitación de actividades: 12, 13, 14, 15  
 Función emocional: 3, 5, 9  
 Estímulos ambientales: 2, 7, 11