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VITAMIN D IN PALLIATIVE CARE – ASPECTS ON INFECTIONS, PAIN AND QUALITY OF LIFE

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Vitamin D in Palliative Care – Aspects on Pain, Infections and Quality of Life

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To my family

ABSTRACT

The World Health Organization's (WHO's) definition of palliative care stresses the importance of early identification and assessment of physical symptoms in the palliative patient, to prevent and relieve suffering. The overall goal is to improve quality of life, and it is thus important that side effects of medical treatment do not outweigh beneficial effects. Vitamin D has few and mild side effects, and vitamin D treatment has well defined uses in medicine. However, the role of vitamin D supplementation to patients with advanced or metastatic cancer has rarely been studied. The papers included in this thesis aim at understanding the possible effects and optimal use of vitamin D in symptom management in palliative cancer patients, and to study effects of antibiotics in end-of-life cancer patients.

In study I, we investigated the effect of antibiotic use in patients in the last week of life. Almost 50 % in the studied cohort were treated with antibiotics during the last week in life; 37% of all patients and 50% of patients with septic symptoms experienced symptom relief.

Based on a study showing association between lower vitamin D levels and higher doses of opioids in patients with advanced cancer, we performed a pilot study to investigate effects of vitamin D in pain management, Quality of Life (QoL) and infections in patients with advanced or metastatic cancer, **study II**. Participants (n=39, with 25-hydroxyvitamin D < 75 nmol/L) received vitamin D 4000 IU/day for twelve weeks and were matched to untreated controls. After one month, the mean change in opioid dose was significantly lower in the vitamin D supplemented group -46 μg/h (95% CI -24-78), and difference increased over time. Differences in antibiotic use and QoL were also significant, in favor of the supplementation group.

The encouraging results from study II were used to plan **study III**, a multicentre, randomized, double blind placebo-controlled trial investigating the effect of vitamin D supplementation (4000 IU/day) on pain, infections, fatigue and QoL for 12 weeks. Last patient out was in June 2020, and results (submitted but not yet accepted manuscript) indicate a significant effect on opioid use and fatigue, but not on infections and QoL.

In a post-hoc analysis of a previously studied cohort of immunodeficient patients, **study IV,** we compared the effectiveness of vitamin D supplementation administered as oil drops versus powdered tablets in raising the individual's vitamin D level. There was no significant difference between groups ($p = 0.77$). In a subgroup of patients without immunoglobulin replacement, vitamin D supplementation with oil drops ($n = 34$) but not with tablets ($n = 60$) resulted in significantly less antibiotic use ($p < 0.001$ and $p = 0.58$).

LIST OF SCIENTIFIC PAPERS

I. **Antibiotic treatment in End-of-Life Cancer Patients – A Retrospective Observational Study at a Palliative Care Center in Stockholm**

Helde-Frankling M, Bergqvist J, Bergman P, Björkhem-Bergman L. *Cancers, 2016, Vol.8(9)*

II. **Vitamin D supplementation to palliative cancer patients shows positive effects on pain and infections-Results from a matched case-control study.**

Helde-Frankling, M., Höijer, J., Bergqvist, J., & Björkhem-Bergman, L. *PloS One.2017, Vol.12(8).*

III. **Vitamin D supplementation reduces opioid-use and fatigue in cancer patients admitted to palliative care - results from the Palliative-D study, a double blind, randomized placebo-controlled multi-center trial.**

Helde Frankling M, Klasson C, Sandberg C, Nordström M, Warnqvist A, Bergqvist J, Bergman, Björkhem-Bergman L. *Manuscript.*

IV. **Are Vitamin D3 Tablets and Oil Drops Equally Effective in Raising S-25-Hydroxyvitamin D Concentrations? A Post-Hoc Analysis of an Observational Study on Immunodeficient Patients**

Helde Frankling M, Norlin AC, Hansen S, Wahren Borgström E, Bergman P, Björkhem-Bergman L. *Nutrients. 2020 Apr 26;12(5):1230.*

ADDITIONAL PAPERS NOT INCLUDED IN THE THESIS

1. Vitamin D supplementation to palliative cancer patients: protocol of a double-blind, randomised controlled trial 'Palliative-D'.
Helde-Frankling M, Bergqvist J, Klasson C, Nordstrom M., Höijer J, Bergman P, Bjorkhem-Bergman L. *BMJ Support Palliat Care. 2017;7(4):458-463*

2.Vitamin D and Fatigue in Palliative Cancer: A Cross-Sectional Study of Sex Difference in Baseline Data from the Palliative D Cohort

Klasson C, Helde-Frankling M, Sandberg C, Nordström M, Lundh-Hagelin C, Björkhem-Bergman L. *J Palliat Med. 2020 Sep 1.*

3. Death at home: predictive factors in a medical home care unit.
Rasch-Westin M, Helde-Frankling M, Björkhem-Bergman L.
BMJ Support Palliat Care. 2019 Sep 19:bmjspcare-2019-001932

4. Differences in discontinuation of statin treatment in women and men with advanced cancer disease

Bergström H, Brånvall E, Helde-Frankling M, Björkhem-Bergman L. *Biol Sex Differ. 2018 Oct 20;9(1):47.*

CONTENTS

LIST OF ABBREVIATIONS

$1,25\text{-}(OH)_2D$	1,25-dihydroxyvitamin D
25-OHD	25-hydroxyvitamin-D
AMP	Antimicrobial peptide
ASIH	Avancerad sjukvård i hemmet (Swedish)
CI	Confidence interval
CLIA	Clinical laboratory improvement amendments
CONSORT	Consolidated standards of reporting trials
CPS	Clinician predicted survival
CRP	C-reactive protein
DBP	Vitamin D binding protein
DDD	Defined daily dose
ECOG-PS	Eastern Cooperative Oncology Group – Performance Status
EORTC QLQ-C15-PAL	European Organization for Research and Treatment of Cancer – Quality of Life Questionnaire-Core15-Palliative
ESAS	Edmonton Symptom Assessment Scale
ESAS-r	Revised Edmonton Symptom Assessment Scale
ESBL	Extended spectrum B-lactamase
FACT	Functional Assessment of Cancer Therapy
GMP	Good manufacturing practice
HPC	Specialized home-based palliative care services
HRQoL	Health related Quality of life
IFN-Y	Interferon Y
IQR	Inter-quartile range
ITT	Intention to treat
IU	International unit
LC-MS/MS	Liquid-chromatography – mass spectrometry
mGPS	Modified Glasgow Palliative Scale
MCID	Minimal clinically important difference
MED	Minimal erythemal dose
MEDD	Morphine equivalent daily dose

MRSA	Methicillin resistant Staphylococcus Aureus
NA	Not applicable
NK-cells	Natural killer cells
NRS	Numeric rating scale
PGE2	Prostaglandin E2
PROM	Patient reported outcome measure
PTH	Parathyroid hormone
RCC	Regionalt Cancercentrum (Swedish)
RCT	Randomized controlled trial
SI	Système Internationale
UVB	Ultraviolet B light
VDR	Vitamin D receptor
WHO	World Health Organization

1 BACKGROUND

1.1 PALLIATIVE CARE

1.1.1 Definition

According to the WHO's revised definition of palliative care from 2002, the main goal of palliative care is to improve quality of life in patients and their families facing life-threatening illness (1). The definition emphasizes the importance of early detection, impeccable assessment and treatment of symptoms; physical, psychosocial and spiritual.

In 2017, the Lancet Commission recommended that the WHO's definition of palliative care should be revised (2). After a thorough global consensus process, an updated definition with a broader scope and more emphasis on organization health care and training was proposed by the International Organization for Hospice and Palliative Care. The wording of the core sentence is as follows: "Palliative care is the active holistic care of individuals across all ages with serious health-related suffering due to severe illness and especially of those near the end of life. It aims to improve the quality of life of patients, their families and their caregivers." (3) .

1.1.2 Palliative care in Sweden

Nearly 90 000 people die in Sweden every year. It is estimated that 70 000 of these require palliative care (4) and that 20 000 patients have more complex needs warranting specialized palliative care (5). Swedish healthcare is decentralized, and responsibility for providing citizens with healthcare lies mostly with the 21 regional councils. For elderly and disabled receiving care in their homes or in special accommodation, responsibility lies with local councils or municipalities (6). Organization of both general and specialized palliative care differs between different regions and between rural and urban areas. Specialized home-based palliative care services (HPCs) with multi-professional teams are more common in urban areas.

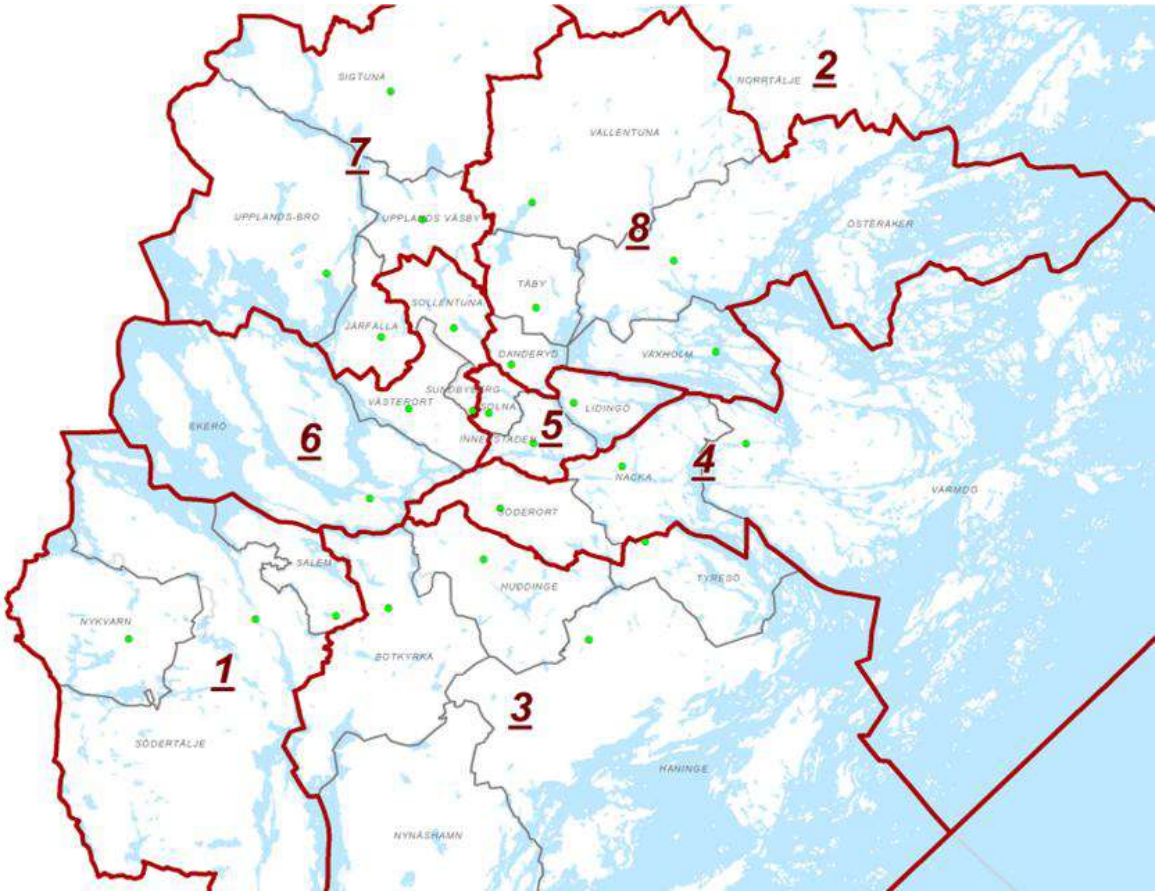
The National Board of Health and Welfare (Socialstyrelsen) provides a national knowledge support base for good palliative care, and national guidelines regarding palliative care are regularly updated under the supervision of Regional Cancer Centers (RCC).

More than 5 300 facilities report data on end-of-life care to the National Quality Registry for Palliative care in Sweden. In 2019, data on 60% of all deaths in Sweden were recorded. Amongst these deceased patients, nearly 20% had received specialized palliative care during their last week of life (7).

1.1.3 Organization of specialized palliative care in Stockholm

The Stockholm Region has almost 2.4 million citizens, nearly a quarter of the population of Sweden (8). Referral to an HPC in Stockholm is initiated by a hospital or primary care physician (and in rare cases by a nurse). Patients can choose any of the HPCs authorized to provide care in their residential area (eight different areas, Fig. 1), and the choice is made at time of referral. The facilities do not only tend to palliative needs, they also offer supportive care to patients undergoing curative treatment. The specialized home care facilities are all government-funded but can be owned and operated by the Stockholm Region Council as well as by private providers.

Figure 1. Residential areas covered by different HPC facilities in the Stockholm Region



https://www.medscinet.com/Belport/

In 2015, a total of 2780 different patients were at one time or another cared for by a HPC, and 2087 of these had cancer (9). Most, but not all, specialized palliative home care providers also offer inpatient care in a palliative ward. In October 2020, approximately 250 beds were available for inpatient care and almost 2 700 patients were under outpatient care in HPCs in the Stockholm Region (10).

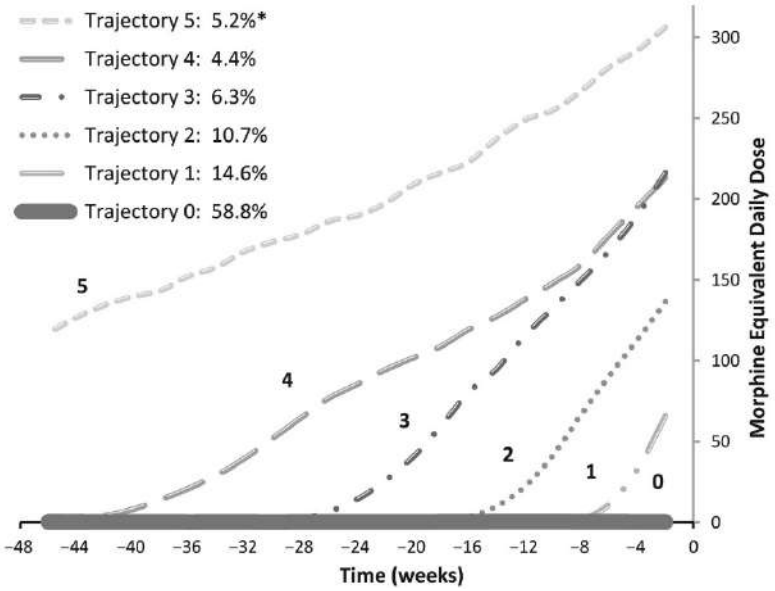
The palliative care team comprises nurses, physicians, dieticians, occupational therapists, physiotherapists, and social workers. Patients admitted to specialized palliative home care are visited regularly by a nurse, most often weekly visits are planned. If needed, patients receive more frequent visit, sometimes several times per day. Physicians meet patients at more irregular intervals, and other team members are consulted as needed.

1.2 OUR ENDPOINTS – PAIN/OPIOID USE, INFECTIONS/ANTIBIOTIC USE, FATIGUE AND QOL

1.2.1 Pain and opioid use in patients with cancer

In patients with advanced cancer, 70-80% suffer from moderate or severe pain, and it is recommended to use opioids in this setting to relieve suffering (11). A recent register-based study on Swedish prostate cancer patients showed that 67% used strong opioids during their last months of life (12). In the last week of life, 97% of Swedish patients are prescribed opioids for breakthrough pain (13). In a retrospective population-based Canadian study, prescription of opioids to cancer patients increased in all quintiles over time in patients' last eleven months of life (14). Still, a large group did not use opioids at all until the final weeks of life (14), Fig 2.

Figure 2. Patterns of community based opioid prescription filling in people dying of cancer during their last months of life, principal trajectories of opioid use.



Still, opioids have side effects such as nausea and vomiting, constipation, tiredness, and dizziness. It is therefore important to limit opioid doses by using all possible resources to relieve pain, pharmacological as well as non-pharmacological.

Opioids are available as peroral capsules or tablets, both fast and slow-release, as transdermal patches and as subcutaneous or intravenous injections. Patients with chronic moderate or

severe pain, who are prescribed continuous slow-release opioid medication, should be offered 1/6 of the daily dose as short-acting extra dose in case of breakthrough pain. Morphine equivalent daily dose (MEDD) is a metric that can be used to follow opioid use in an individual or in a cohort over time. It is calculated as strength per unit x number of units x MEDD conversion factor (15). In pharmacoepidemiology, defined daily doses or DDD:s are often used to assess the expected total daily doses of both long- and short-acting opioids (16). DDD:s may however underestimate the daily total dose of opioids used (17).

In order to switch between different preparations and types of opioids, as well as to calculate extra doses when combining different types of long-acting and short-acting opioids, equianalgesic conversion schemes based on MEDDs have been developed to facilitate clinical work (18). A commonly used conversion scheme in Sweden is presented in the Methods-section (Table 3, p 33).

1.2.2 Infections in the dying cancer patient – epidemiology and aetiology

1.2.2.1 Epidemiology of infections and antibiotic use

Infections occur frequently in the last days and weeks of patients dying from cancer. A small autopsy study (n=38) on both non-cancer and cancer patients revealed pneumonia in four out of five patients and assessed infections as cause of death in 44% of cases (19). Most studies on use of antibiotics in this late palliative phase are retrospective, observational, single centre studies (20-22). These studies reveal differences in intensity of care in different parts of the world and over time rather than best practice. A systematic review from 2013 illustrates this well; prevalence of antimicrobial use ranges between 4 and 84% in reviewed cohorts (23).

In a more recent multicentre study comprising 2091 patients with a median survival time of 15 days admitted to inpatient hospice care in France, 36,4 % received antibiotic treatment (24).

1.2.2.2 Benefits and risks of antibiotic treatment in end-of-life cancer patients

Possible benefits of antibiotic treatment in palliative care are symptom relief and prolonged survival. Possible adverse effects are suffering from side effects including reaction to he administered antibiotic, drug-drug interactions, the risk of development of multidrug resistant bacteria, superinfection with C difficile, increased use of intravenous devices and increased costs (25).

1.2.2.3 Aetiology

Patients with advanced cancer are at great risk of infections. In Table 1, a list of factors explaining this increased risk are presented (26).

Table 1. Factors that increase the risk of infections in patients with solid tumours.

Risk factor	**Additional explanatory comments**
Neutropenia	Chemotherapy, radiation therapy, bone marrow infiltration with tumor, drugs (e.g., ganciclovir)
Disruption of anatomic barriers (e.g., skin, mucosal surfaces)	Chemotherapy (mucositis), radiation therapy, vascular access catheters, urinary catheters, percutaneous endoscopic gastrostomy tubes and other medical devices, surgical/diagnostic procedures
Obstruction due to primary or metastatic tumor	Airways: post-obstructive pneumonia, lung abscess, empyema, fistula formation (e.g., broncho-pleural or trachea-esophogeal) Biliary tract: ascending cholangitis, hepatic and pancreatic abscess Bowel: bowel obstruction, necrosis, perforation, peritonitis, hemorrhage Urinary tract: urinary tract infection, renal abscess, prostatitis or prostatic abscess
Procedure and devices	Diagnostic/therapeutic surgery: surgical site infections, wound dehiscence, abscess formation Shunts: disseminated infection (bacteremia) shunt-related infections such as meningitis/ventriculitis, hepato-biliary infections, complicated urinary tract infections Prosthetic devices: infected prosthesis, osteomyelitis and/or septic arthritis, local abscess formation, disseminated infection

Adapted from Rolston, K.V.I. Infections in Cancer Patients with Solid Tumors: A Review. *Infect Dis Ther* **6,** 69–83 (2017). Reprinted with permission under the terms of the CC BY

1.2.3 Patient reported assessment of symptoms and QoL

Self-assessment of symptoms and health-related quality of life (HRQoL) in patients with advanced cancer are patient recorded outcome measures (PROMs) that can be assessed using standardized and validated questionnaires. The instruments can be generic, disease-specific, or aspect-specific.

One of the oldest general instruments developed for symptom assessment in palliative care that is still in use is the Edmonton Symptom Assessment Scale (ESAS) (27). A disease-specific assessment tool for palliative cancer patients is The European Organization of Research and Treatment of Cancer, Quality of Life Questionnaire Core 15 Palliative or EORTC QLQ-C15-PAL (28), a shortened version of EORTC QLQ-C30-PAL (29).

1.2.3.1 ESAS

ESAS is an instrument that was developed for standardized assessment of symptoms in patients admitted to a palliative care unit in the early 1990s (27). It has been translated to and psychometrically validated in more than 20 languages and is used in clinical routine to quantify symptom burden in both cancer and non-cancer patients (30-35). Nine prespecified symptoms and one optional symptom, often obstipation, are assessed on an eleven point numeric rating scale (NRS), rangning from 0 to 10. An assessment of zero is equivalent to absence of the specific symptom, and an assessment of ten represents the worst imaginable intensity of the specific symptom (36).

Since evaluation of the tool revealed that patients found some questions difficult to interpret, a revised version (ESAS-r) was proposed in 2011 (37). The revised version has proven to be easier for patients to comprehend (38). In ESAS-r, instructions emphasize that assessment of symptoms refers to "now", and thus offers a snapshot of the patient's situation (38). There are large inter-individual differences in how patients interpret scores, as well as differences in cut-off levels between different symptoms. However, it is generally accepted to consider symptom intensity scored as 1-3 as mild, 4-6 as moderate and 7-10 as severe (39-42). Minimal clinically important difference (MCID) is considered to be one point on the 11-point scale (43, 44).

A review of 39 publications published up until 2007 discusses advantages and disadvantages of ESAS (45). The authors address the issue of different versions, making aggregation of data difficult. The lack of theoretical framework presents difficulties in defining constructs and interpreting statistical data. There is also lack of data on responsiveness and validity. Still, ESAS is up to this day used in clinical routine in many centers (46). In Ontario, Canada, with a population of 13.5 million and more than 50 000 new cancer cases/year, ESAS-r is assessed regularly by all cancer patients with the aim of aggregating data from routine symptom screening to improve symptom management (47). Data from this large-scale screening is presently being used for registry based studies, for example by identifying groups at risk of higher symptom burden after cancer diagnosis (48), and symptom burden in end of life in rare tumors (49).

ESAS-r has been translated into and psychometrically validated into Swedish (50). Still, ESAS is not protected by copyright, and many permutations of ESAS exist. At ASIH Stockholm Södra, an older version of ESAS is used. The permuation of ESAS used in study II and III in this thesis are presented in the Methods-section (Fig 6, p 32).

1.2.3.2 EORTC QLQ-C15-PAL

Unlike the symptom assessment instrument ESAS, EORTC QLQ-C15-PAL was specifically developed to assess the construct of HRQoL in cancer patients in a palliative setting. The shortened questionnaire, based on the twice as long instrument EORTC QLQ-C30, was proposed for use as a 'core questionnaire' addressing the most important domains of HrRQoL in this patient group as identified thorough item response theory methods on patients and health care professionals (28). The questionnaire comprises 15 items. Fourteen of these are symptom-items that are assessed on a four point verbal scale ranging from "not at all" (1), to "a little" (2), "quite a bit" (3) and finally "very much" (4). The last item is global quality of life, assessed on a seven-digit numeric scale with the verbal anchors very poor (1) and excellent (7) (28). In contrast to ESAS, the patient is asked to assess symptoms and functioning as well as QoL during the past week (28). The two instruments thus cover different time frames.

The scoring system is based on the scoring system for the longer version, and the self-assessment tool offers information on two functional scales, physical and emotional functioning, as well as five single-symptom items and global QoL (28). The longer version, EORTC QLQ-C30, has been translated to and psychometrically validated in Swedish. There are also EORTC QLQ C30 reference values from the general Swedish population (51, 52), as well as values in correlation to self-reported chronic health problems in a Swedish cohort (53). The questionnaire is presented in the Methods section (Fig 7, p 34).

1.3 VITAMIN D

1.3.1 Synthesis of vitamin D in the skin

Vitamin D_3, or cholecalciferol, is a steroid hormone synthesized from 7-dehydrocholesterol in the plasma membrane of epidermal cells of the skin using energy from ultraviolet-B light (UVB, wavelength 280-320 nm) (54, 55). The photodynamic (in contrast to enzymatic) process includes an intermediated step (previtamin D_3) and has negative feedback loops to prevent toxic levels of vitamin D in the individual due to extensive sun exposure (55).

1.3.2 Dietary sources of vitamin D

Endogenous synthesis of vitamin D_3 is the most important source of vitamin D. However, nutritional intake of vitamin D_3 from fatty fish, meat and fortified dairy products add to the individual's vitamin D levels. Vitamin D_2 (ergocalciferol) is synthesized in plants and fungi from ergosterol irradiated with UVB-light. The molecule has a methyl group at C24 and an extra double bond (C22-23) compared with vitamin D_3 (56, 57).

Vitamin D_3 and D_2 is considered to have the same effect in the human body and is activated by the same hydroxylation steps (54). However, according to findings in animal studies vitamin D_3 might be more potent than D_2 (58).

1.3.3 Transportation and activation of vitamin D

After excretion of Vitamin D_3 from the plasma membrane of epithelial skin cells, it is absorbed into the capillary circulation where it binds to the vitamin D binding protein (DBP) (54). Vitamin D_3 is not biologically active, and is either transported to fat cells for storage or to the liver for first stage metabolism. Vitamin D_2 and D_3 from foodstuffs are absorbed in the intestine, transported to the liver in chylomicrons, and then undergo the same conversions steps as vitamin D_3 synthesized in the skin (54).

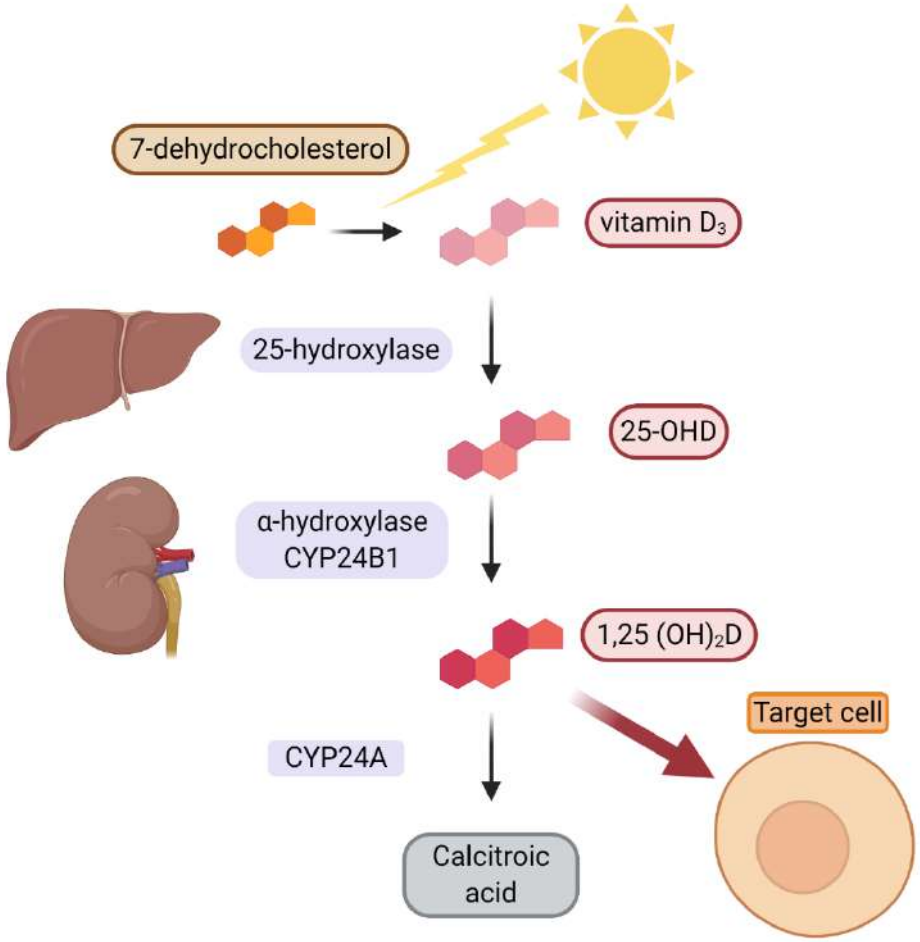
Enzymatic conversions of vitamin D in the liver is mediated through a number of different enzymes from the Cytochrome P 450 system. 25-hydroxylases (mainly CYP27A1, CYP2R1, but also CYP3A4, CYP2C11, CYP2J1, and CYP2D25) add an -OH group to the vitamin D molecule (59). This conversion is not regulated by any negative feedback loops.

In contrast, the second hydroxylation step to 1,25-$(OH)_2$D in the kidney is mediated by a single, tightly controlled enzyme, 1-α hydroxylase (CYP27B1). The conversion is regulated by calcium, phosphate, 1,25-$(OH)_2$D itself and parathyroid hormone (PTH) (56, 60). CYP27B1 is however present in many other cells and tissues as well (colon, placenta, skin, pancreas, monocytes and macrophages) (60). In immune cells the local conversion to active vitamin D can modulate immune response (61).

1.3.4 Degradation of vitamin D

CYP24A is the only enzyme that can peform the fist step in catabolizing both 25-OHD and 1,25$(OH)_2$D. This enzyme is present in many tissues, such as kidneys, intestines, bone etc. After further degradation steps, calcitriol or lactone are end-products. Calcitrol is water-soluble and can be excreted through bile (59).

Figure 3: A simplified overview of Vitamin D synthesis and degradation



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1.3.5 Vitamin D physiology

Vitamin D is the only known ligand to the Vitamin D receptor (VDR), a nuclear transcription factor. The complex regulates more than 1000 genes in the nucleus of target cells and has both direct genetic and epigenetic effects on the translational output. (62, 63). The classic effect of vitamin D on regulation of the steroid hormone endocrine system maintaining mineral homeostasis and skeletal health is well established. Presence of active vitamin D in the intestines increase absorption of calcium and phosphorus from 10-15 and 60 to 30-40 and 80% respectively. Vitamin D also induces osteocalcin-production in bone and downregulates bone resorption (54, 64).

The scope of this introductory chapter is to give a background to the studies included in my thesis. I will therefore focus on the effects of vitamin D on the immune system and on its role in pain physiology, rather than the "classic" role that vitamin D plays in skeletal health.

1.3.6 Vitamin D - effects on the immune system

Immunity is achieved through close cooperation between the innate and the adaptive immune systems. The innate immune system constitutes the individual's first-line defense against microbes and responds quickly. It consists of physical and anatomical barriers, where epithelial and endothelial cells play an important role, immune cells (monocytes, macrophages, dendritic cells, natural killer (NK)-cells and granulocytes), cytokines and proteins in the complement system. The adaptive immune system responds more slowly as it recognizes specific antigens on the surface of cells, and immune response is thus acquired throughout an individual's lifespan. The adaptive immune system consists of B-cells, which produce antibodies, and T-cells (61). The vitamin D receptor is abundantly expressed in monocytes and activated B- and T-lymphocytes (63, 65), indicating that vitamin D plays a role in both parts of the immune system.

Activation of the innate immunity through toll-like receptor pathways and exposure to cytokines such as IFN-Y results in increased activity of the hydroxylating enzyme CYP27B1 (alfa1-hydroxylase) in macrophages and dendritic cells, thus increasing levels of the active form 1,25 $(OH)_2$ D. (66-69).

In the innate immune system, increased levels of active vitamin D binding to the VDR result in induction of antimicrobial peptides (AMPs) produced by macrophages (68, 70). Also, increased production of cytokines alerts other components of the immune system and direct them to the site of the infection. Thirdly, active vitamin D may increase autophagy of cells with intracellular pathogens such as mycobacteria and viruses (66).

Low levels of circulating vitamin D induce a more inflammatory immune response with higher levels of Th1 and Th17 and lower levels of Th2 and Treg (68). On the other hand, lower serum levels of circulation inflammatory cytokines and prostaglandins are seen when adequate amounts of vitamin D are present (71). A simplified and schematic presentation of the effects of vitamin D on innate and adaptive immune response is presented in Fig 4 (72).

Figure 4 Immune response modulation

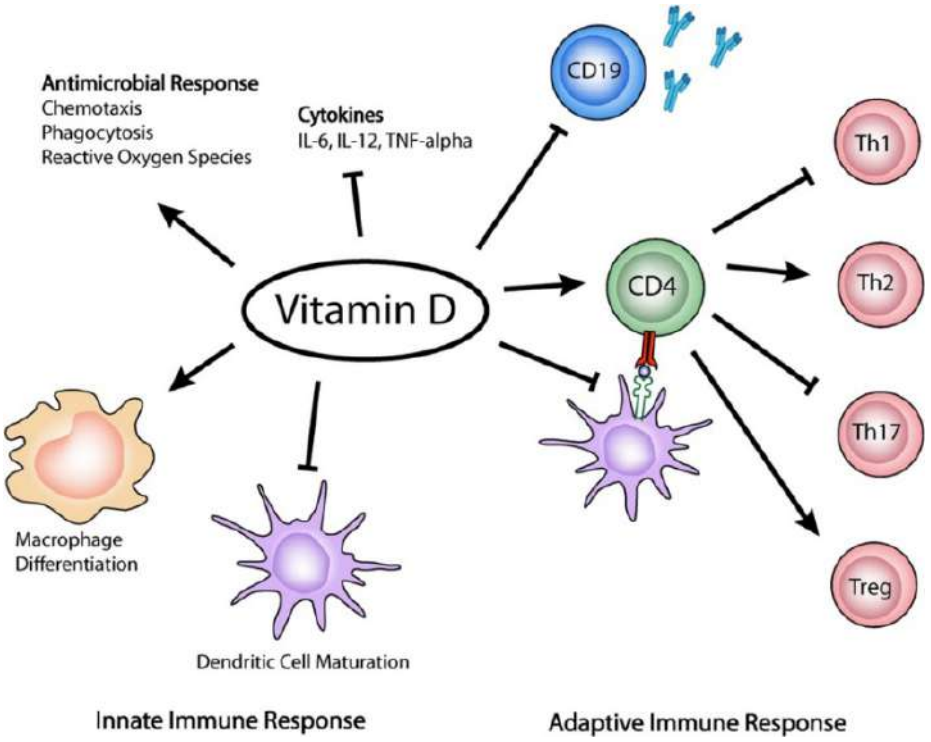

Figure 4. Effects of vitamin D on the innate and adaptive immune systems

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1.3.5 Extraskeletal effects - hypotheses in pain physiology

There are two main hypotheses linking vitamin D to pain. First, the fact that vitamin D, as shown above, modulates inflammatory T-cell response may have an impact on perceived inflammatory pain (68). In addition to the concepts discussed above, synthesis of prostaglandin E2 (PGE2) was inhibited in the presence of vitamin D in an experimental setting (73). This finding is in line with the results from a clinical study on healthy individuals, where vitamin D supplementation reduced levels of circulating prostaglandins (74).

Secondly, clinical studies on humans as well as animal studies suggest that low levels of vitamin D might affect both peripheral and parasympathetic nerve function and thus play a role in neuropathic pain (75-77). A third and altogether different hypothesis linking vitamin D to pain, suggests that vitamin D is nothing but a surrogate marker for UVB-exposure. In a rodent model, UVB-exposure increased production of analgesic Beta-endorphins, which act as endogenous analgesics (78).

1.3.6 Measurement of Vitamin D

Measurement of vitamin D focuses on the measurement of fraction of vitamin D present in the circulation. 25-OHD is the summation of $25(OH)D_2$ and $25(OH)D_3$, has a half-life of about 3 weeks and is more stable than $1,25(OH)_2D$, with a half -life of a few hours (79-81). Since 25-OHD is also abundant, it is technically easier to measure with acceptable accuracy (79). Generally, 25-OHD in the circulation is consider to reflect the vitamin D status in an individual (81).

Liquid chromatography - mass spectrometry (LC-MS/MS) is a method of analysing 25-OHD that can discriminate between different contributing fractions of total 25-OHD (82). Routine clinical measurement of 25-OHD in Stockholm today is however performed through a fully automated immune-assay-based method, without the capacity to discriminate between vitamin D_2 and D_3. This is also the most commonly used method with a relationship in 3:1 in number of laboratories performing immune-assays compared to LC-MS/MS (83). Since vitamin D_2 generally constitutes a very small fraction of total vitamin D, at least in a Swedish population, it is usually sufficient to measure the total Vitamin D with the immune-assay method (82).

1.4 LEVELS OF 25-OHD IN INDIVIDUALS

1.4.1 Adequate levels of 25-OHD in individuals

There is continuous debate on adequate levels of vitamin D in the individual. Different US organizations arrived at different cut-off levels for vitamin D insufficiency some years ago; the Institute of Medicine at 50 nmol/L (84) , the Endocrine Society and the Infectious Diseases Society of America at 75 nmol/L (85, 86). The higher cut-off level is proposed to ensure a well-functioning immune system, the lower cut-off level to be sufficient for skeletal health. Very low levels of 25-OHD, below 25 nmol/L, increase the risk of rickets a in children and of osteomalacia in adults (87). On the contrary, levels above 250 nmol/L are considered toxic, resulting in hypercalcemia, hyperphosphatemia and impaired renal function.

The SI unit for 25-OHD is nmol/L. Some clinical laboratories present levels of 25-OHD as ng/mL, the conversion factor being 2,5: 1 ng/mL = 2,5 nmol/mL. In the following presentation of background and in the discussion, 25-OHD are presented in nmol/L. Cut-off levels using both units are presented in Table 2.

Table 2 Cut-off levels for vitamin D deficiency

	nmol/L	ng/mL
Severe vitamin D deficiency	< 30	< 12
Vitamin D deficiency	30-50	12-20
Sufficient levels	50-75	20-29
Optimal levels	75-125	30-50
Toxic levels	> 250	100

1.4.2 Sun exposure and 25-OHD levels

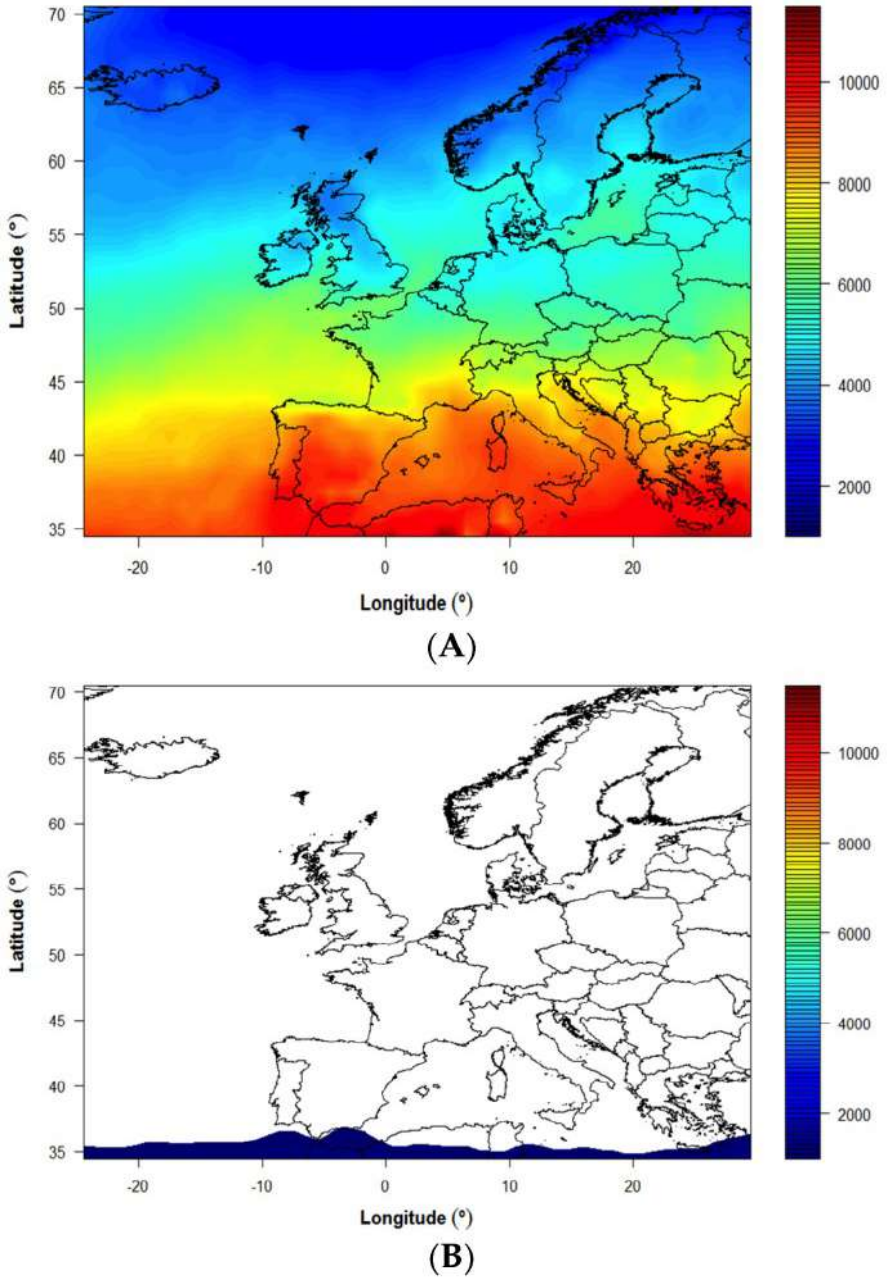
The major source of vitamin D is photosynthesis in the skin, and since 7-deoxychokesterol is abundant, presence of UVB-light is the limiting factor in this process. Absorption of UVB-light in the skin is dependent on distal and proximal factors (54, 55). Minimal erythemal dose (MED), the dose that causes slight erythema in the skin of an individual, is often used to measure sun-exposure in the individual. A consensus from reported studies is that a standardized MED after 24 hours results in 25-OHD-levels that can be compared with levels observed after oral intake of 10 000- 25 000 International Units (IU) of vitamin D (88). Maximum absorption of energy from UVB-light per time unit is achieved at shortest possible distance from the sun and with minimal absorbing factors between the sun and epidermal cells (54, 55, 88). The absorption relies on both external and individual factors.

Distant factors: The effect of latitude, altitude time of day and weather conditions have been studied in an experimental setting and several clinical studies have been performed, with measurement of 25-OHD after standardized sun exposure (55). Using meteorological data., modelled mean yearly UVB-doses for different latitudes have been calculated. "Vitamin D winter" has been coined as a phrase used to describe months with mean monthly UVB-doses below 1000 Jm^{-2}, when there is no vitamin D synthesis in the skin even under sunny weather conditions (89). At 60 Nº (Oslo), estimated vitamin D winter is six months (89), Fig 5. The variability of sun exposure over seasons explains seasonal variability in 25-OHD (90).

Individual factors: With age, skin becomes thinner and has lower amounts of 7-dehydrocholesterol with elders therefor in the need for more sun exposure than younger individuals to produce the same amount of 25-OHD (91). Still, adequate amounts of vitamin D can be attained in elderly individuals solely through sun exposure (91). A number of individual factors further impact bioavailability of active vitamin D, amongst them: Melanin

that acts as a natural sunscreen and markedly lowers vitamin D production in the skin, increased sequestration of vitamin D in body fat in obese patients, impaired renal and liver function, diseases causes impaired uptake of vitamin D from the intestines as well as genetic variability (54, 55).

Figure 5: Mean monthly modeled UVB doses effective for pre-vitamin D3 synthesis (Jm−2) across Europe for June (A) and December (B), based on average of data from years 2003–2012. Scale begins 1000 Jm−2 .



O'Neill CM, et al. Seasonal Changes in Vitamin D-Effective UVB Availability in Europe and Associations with Population Serum 25-Hydroxyvitamin D. *Nutrients*. 2016;8(9):533. reprinted with permission under the terms of the CC BY

1.4.3 Oral intake and 25-OHD

1.4.3.1 Dietary recommendations

The amount of vitamin D in foodstuffs and supplementary products is presented a microgram (µg) or international units (IU), with 0,025 µg = 1 IU or 40 IU = 1 µg. Given the need for vitamin D for optimal health and the difficulty to maintain optimal levels of 25-OHD individuals solely through sun exposure, guidelines regarding desired nutritional intake of vitamin D and guidelines regarding supplementation in risk groups have been developed. Sweden collaborates with the other Nordic Countries in updating Nordic Nutrition Recommendations for vitamin D (92, 93). They conclude that in absence of sun exposure, dietary intake of 15 ug or 600 IU/day is needed to maintain 25-OHD > 50 nmol/L all year round in Nordic countries (92). Dietary recommendations are then modified based on assumptions of sun exposure in different groups on population level. Fish, meat and fortified dairy products are the most important dietary sources of vitamin D, with vegetabilic sources adding a smaller amount to the total oral intake. Supplements are proposed for risk groups, Table 3 (94).

Table 3 Swedish recommendations for vitamin D supplementation

Population	**Recommended daily supplementary vitamin D dose**
Adults/general population	10 µg/400 IU
Infants and children < 2 years	10 µg/400 IU
Children and adults who don't eat fortified products	10 µg/400 IU
Children and adults who don't eat fish or fortified products	10 µg/400 IU
Children and adults who are not exposed to sun during summer	10 µg/400 IU
Children and adults who are not exposed to sun during summer AND do not eat fish or fortified products	20 µg/800 IU
> 75 years (all)	20 µg/800 IU

Adapted from Brugård Konde, å. (2018). "Råd om D-vitamintillskott till riskgrupper." Livsmedelsverkets rapportserie (nr 21).

1.4.3.2 Vitamin D supplementation

Vitamin D supplements can contain both vitamin D_2 and vitamin D_3 and is offered as either powdered tablets or oil drops or capsules containing oil. Intake of 40 IU vitamin D_3 is assumed to raise 25-OHD concentration by approximately 1 nmol/L, and thus daily intake of 1500–1600 IU/day raises 25-OHD concentrations with 37.5–40 nmol/L (84).

Detremin and Divisun are the two preparations of vitamin D_3 produced according to Good Manufacturing Practice (GMP) that are presently available in Swedish pharmacies.

Divisun is a powdered vitamin D_3 tablet, with 800 IU vitamin D_3 per tablet. Determin is vitamin D_3 oil. When trials included in this thesis were planned and conducted, one drop of Detremin contained 500 IU. The manufacturer has since changed the concentration, and now one drop contains 800 IU of vitamin D_3.

1.4.3.3 Risk of vitamin D intoxication

It is not possible to attain toxic levels of 25-OHD solely through sun exposure or oral intake of regular foodstuffs. Vitamin D supplementation in excessive doses can however increase 25-OHD above 250 nmol/L and thus cause hypercalcemia, dehydration and incerase the risk of forming kidney stones (95). Supplementation with daily doses of up to 10 000 IU for five months are however considered safe (84). Our group has studied the effect of 4000 IU/day for three months in several clinical trials, with no patients reaching toxic 25-OHD-levels (96, 97).

1.4.3.4 Vitamin D_3 tablets versus vitamin D_3 oil

Data comparing efficacy of vitamin D_3 tablets and oil is scarce. A prospective, randomized study in a small cohort of healthy adults in Norway receiving 400 IU of vitamin D/day for one month showed a mean increase in 25-OHD of 36 nmol/L (95% CI 31–41) in participants taking multivitamin tablets. Participants taking oil capsules had a mean increase in 25-OHD of 32 nmol/L (95% CI 27–37) (98, 99).

Efficacy of vitamin D_3 tablets and oil has also been studied in patients with cystic fibrosis. Fat malabsorption caused by pancreatic insufficiency is common in these patients and raises the suspicion that intestinal uptake of oil preparations may be poor. So far, studies have however not shown a clinically significant difference in effect between preparations in patients with this diagnosis (100-102).

1.4.3.5 25-OHD-levels in healthy populations in Sweden

Observational data on 25-OHD from healthy populations vary due to the geographical setting as well as to cultural and individual factors (54, 103). In European data from 2016, 12% of Europeans have 25-OHD < 30nmol/ (103). Data on Swedish cohorts have been included in two systematic reviews with partly overlapping cohorts (104, 105). The mean or median 25OHD-levels in these cohorts were all well above 50 nmol/L (104), range 65 to 95 nmol/L (105). In a recent study on Swedish adults (n=268, aged 18–80 years, data mostly collected during summer), the mean 25-OHD concentration in women was 65 nmol/L with 21% of women having levels below 50 nmol/L. In men, the mean concentration was 62 nmol/L (24% <50 nmol/L) (94).

1.4.3.6 25-OHD and patients with advanced or metastatic cancer

Low serum levels of 25-OHD are observed more frequently amongst patients with advanced or metastatic cancer compared to healthy individuals (106-109). In a Swedish cohort of palliative cancer patients, median 25-OHD was 40nmol/L (range 8-154 mol/L) (110), and in all screened patient in the 'Palliative D'-study 51nmol/L (range 8-95 nmol/L) (111).

1.5 ASSOCIATION BETWEEN 25-OHD LEVELS AND PAIN, INFECTIONS, FATIGUE AND QOL

1.5.1 25-OHD and pain-related conditions

A systematic review and meta-analysis of observational studies investigating the relationship between pain-related conditions and 25-OHD pooled data from 81 studies with more than 50 000 participants (112). Patients with chronic widespread pain, arthritis and muscle pain had significantly lower 25-OHD-levels compared to controls (mean difference in 25-OHD ranged between 7.8 and 12.3 nmol/L). There was no difference between subjects and controls regarding headache or migraine.

The large Vitamin D Assessment Study (VIDAL) from New Zealand reported no association between 25-OHD and self-reported chronic pain in baseline data from 837 patients recruited from general practices, neither in the unadjusted nor in a model adjusted for demographic and lifestyle factors, prescription of analgesics and vitamin D, body mass index and medical history (113).

Recently, a retrospective cohort study with more than 14 000 subjects investigated the association of 25-OHD-levels and postoperative opioid after elective surgery. In a multivariate analysis, vitamin D-depleted patients used opioids for a longer period and at higher doses compared to vitamin D-sufficient subjects (114).

1.5.2 25-OHD and infections

A systematic review of association of vitamin D deficiency and infections in healthy adults identified eight observational studies including more than 15 000 mostly American and European adults. Results were inconclusive regarding association between low levels of 25-OHD and frequency and severity of infections, but supported an association between longer duration of infection and lower 25-OHD (115).

A review on observational cohort studies with data from almost 10 000 patients treated in intensive care units showed a significant increase in the relative risk for infection and sepsis with almost 1.5 if 25-OHD was lower than 50 nmol/L compared to > 50 nmol/L (116).

According to the results from a retrospective study on more than 2 100 patients investigating the association between 25-OHD and development of hospital-acquired bloodstream infections, the risk for positive blood cultures within 48 hours after hospital admission for patients with 25-OHD < 25 nmol/L was twice that of patients with 25-OHD > 75 nmol/L (117).

1.5.3 25-OHD - fatigue and QoL

Observational studies investigating the correlation between 25-OHD and fatigue have been performed in various cohorts, with conflicting results. In patients with early rheumatoid arthritis, fibromyalgia and inflammatory bowel disease, 25-OHD did not correlate with fatigue scores (118-120). In patients followed for two years after completing curative treatment for colorectal cancer, higher concentrations of 25-OHD were however associated with better global quality of life and less fatigue (121).

The literature on QoL and vitamin D status in patients with advanced or metastatic cancer is scarce. Some, but not all, studies have shown an association between 25-OHD insufficiency and increased fatigue. No study has yet shown an association between 25-OHD and QoL in these patients.

Fatigue: In a study on100 consecutive cancer patients with moderate to severe fatigue assessed with ESAS, no correlation between low levels of 25-OHD and self-assessed fatigue was observed (107). In contrast, a Spanish study with 30 mostly male patients with advanced cancer and insufficient levels of 25-OHD, showed that higher levels of 25-OHD correlated with absence of fatigue and improved well-being measured with the Functional Assessment of Cancer Therapy (FACT) questionnaire (122). In baseline data from 530 patients screened for the 'Palliative-D' study, more severe fatigue correlated with lower 25-OHD-levels in men but not in women using one self-assessment instrument (EORTC QLQ-15-PAL), but not another (ESAS) (111).

QoL: An observational study at our center failed to establish an association between 25-OHD and patient reported QoL according to the ESAS-scale in patients with advanced cancer (110). Neither could the Spanish study cited above establish a correlation between QoL assessed with EORTC-QLQ-C15-PAL and 25-OHD (122).

1.6 EFFECTS OF VITAMIN D SUPPLEMENTATION

1.6.1 Vitamin D supplementation and pain

1.6.1.1 Vitamin D supplementation and non-cancer pain

Many studies have been performed studying the effect of vitamin D supplementation on both cancer and non-cancer pain. Results remain inconclusive. A Cochrane analysis reviewed vitamin D for the treatment of chronic painful conditions in adults (123). The report included ten studies with 811 participants up until February 2015 and stated that vitamin D was not better than placebo in any chronic painful condition (low quality evidence due to methodological problems). In contrast, a systematic review published some months later reported a significant difference between supplemented and non-supplemented groups regarding mean change in pain score (-0.57, p=0,007) in studies where pain was assessed both at baseline and at final follow-up (122). There was no difference between groups if pain was assessed only at the final follow up (122).

After these reviews, several systematic reviews on specific chronic pain conditions have been published. Two different authors pooling data from studies of vitamin D supplementation in patients with fibromyalgia state that it is difficult to draw conclusions regarding a causal relationship between vitamin D supplementation and pain in fibromyalgia patients due to small samples and unreliable control groups (124, 125). Reviews on vitamin D supplementation in chronic back pain arrived at the same conclusion (126, 127).

However, a recent systematic review on patients with diabetic peripheral neuropathy included four studies with 364 patients and reported that an improvement in self assessed pain was observed in the supplementation group, irrespective of baseline or change in 25-OHD (128).

A randomized controlled trial (RCT), in which more than 5000 community dwelling participants were prescribed 10 000 IU of vitamin D or placebo (1:1) for 3.3 years showed that use of both opioid and non-opioid pain medication as well as self-assessed pain were similar between groups (129).

1.6.1.2 Vitamin D supplementation and pain in cancer patients

Vitamin D supplementation has been studied in breast cancer patients (curative, non-metastatic) who receive adjuvant aromatase inhibitors, with the well-known side effect of aromatase-inhibitor-associated musculoskeletal symptoms. The hypothesis was that vitamin D supplementation could prevent these side effects. However, several prospective studies have failed to show a positive effect in this setting (130-133).

Effect of vitamin D supplementation in palliative cancer patients regarding pain has barely been studied, and trials have in some cases involved very small cohorts (134, 135). A trial enrolling breast cancer patients with skeletal metastases (n=40) studied the effect of 10 000 IU vitamin D daily for four months in addition to bisphosphonates and calcium. This intervention resulted in a significant reduction in number of sites with pain, but not in pain intensity (136).

1.6.2 Vitamin D supplementation and infections

A large review published in 2017 based on individual data from more than 11 000 patients in 25 RCTss showed that vitamin D supplementation reduced the number of respiratory tract infections (137).

Supplementation with vitamin D in an intensive Care setting did not result in fewer infections or improvement in other clinical outcomes according to a review analysing data from 16 trials with very heterogeneous cohorts (138).

To our knowledge, no other group has previously studied effects of vitamin D treatment on infections in palliative cancer patients.

1.6.3 Vitamin D supplementation, fatigue and QoL

A review of QoL-outcomes using validated instruments that included both diseased and healthy cohorts, as well as 25-OHD-replete subjects and a broad range of supplementation schemes, reported no overall effect of vitamin D supplementation on self-assessed QoL (139).

Very little data has been published on the effect of vitamin D supplementation on fatigue and QoL in patients with advanced or metastatic cancer. In an Indian study on a small cohort of patients with advanced oral cancer receiving chemoradiotherapy, with 75% of patients 25-OHD-deficient at baseline, vitamin D supplementation with 1000 IU/day significantly improved QoL in the intervention group compared to controls (140).

A study protocol for the VIDAFACT study, a RCT primarily investigating the effect of vitamin D supplementation on quality of life, was published in 2014, but the results remain to be presented (141). Effects of vitamin D supplementation on fatigue is also the primary outcome in an ongoing, large German study on non-metastatic colorectal cancer (142).

2 RESEARCH AIMS

The aims of this thesis are:

I: To study the effect of antibiotics in end-of-life cancer patients if an infection is suspected.

II and III: To study the effect of vitamin D supplementation in cancer patients with advanced or metastatic disease regarding pain, infections, fatigue and Quality of life.

IV: To test the hypothesis that equivalent doses of vitamin D_3 administered as tablets and in oil are equally effective in raising 25-OHD, and to analyse possible associations between type of vitamin D preparation and antibiotic use.

3 MATERIALS AND METHODS

	Study I	Study II	Study III	Study IV
Design	Retrospective Cohort	Prospective Matched case-control	Prospective Randomized, double blind	Post-hoc analysis of a cohort with prospectively collected data
Site	Single center	Single center	Multi-center	Single center
Time period	April 2014-April 2016	Sept 2015-June 2016	Nov 2017-June 2020	March 2013-October 0213
Patients	Cancer (palliative)	Cancer (palliative)	Cancer (palliative)	Immunodeficient
N=	160	39 + 39	244	206
25-OHD-level[a]	NA/< 75 nmol/L	< 75 nmol/L	< 50 nmol/L	< 75 nmol/L
Intervention	NA	Vitamin D3 4000 IU/day	Vitamin D3 4000 IU/day	Vitamin D3 1500 IU or 1600 IU/day
Intervention period	NA	12 weeks	12 weeks	12 months
Outcome *Assed as*	Effect of antibiotic use in end-of-life	Pain, infections, QoL, 25-OHD	Pain, infections, QoL, 25-OHD	25-OHD, infections
25-OHD	NA	25-OHD (nmol/L)	25-OHD (nmol/L)	25-OHD (nmol/L)
Infections	Recorded effects of antibiotic use in patients' charts	Number of days on antibiotics during the past 30 days	Number of days on antibiotics during the past 30 days	Any or no antibiotic use Number of prescriptions Number of days on antibiotics/year
Pain	NA	Fentanyl µg/hour	Fentanyl µg/hour	NA
QoL	NA	QoL (ESAS)	QoL (ESAS + EORTC QLQ-15-PAL)	NA
Fatigue	NA	NA	Fatigue (ESAS + EORTC QLQ-15-PAL)	NA
Data analysis	Fisher's exact test Mann-Whitney U Chi^2	Paired t-test McNemar's test Wilcoxon matched pairs test Fixed effects linear regression	Fixed effects linear regression Multivariate linear regression Kaplan-Meier	Fisher's exact test Mann-Whitney U Wilcoxon matched pairs test Mc Nemar's

3.1 STUDY DESIGN

3.1.1 Study I

In Study I, we performed a single centre retrospective review of medical records of patients included in one previous cross sectional and in one ongoing intervention study with prospectively collected data on vitamin D in a palliative setting.

3.1.2 Study II

In study II, we performed a single centre, non-randomized prospective intervention study with vitamin D supplementation and assessed differences between the intervention group and the control group regarding pain, infections and QoL. Study II was a pilot study, and the results from this study were used for planning and for power-calculation of study III.

3.1.3 Study III

In study III, we performed a multi-centre randomized, double-blind placebo-controlled trial (RCT) investigating the effects of vitamin D supplementation versus placebo on pain, infections, fatigue and QoL. The study protocol has been published earlier (143).

3.1.4 Study IV

In study IV we performed a post-hoc analysis of prospectively collected data from a single-centre, non-randomized parallel group study. The original study was designed as a confirmatory study set out to evaluate the real-world effect of newly implemented guidelines regarding vitamin D supplementation on infections (144) in immunodeficient patients. Participants were prescribed either of two different types of vitamin D_3 formulation, and we re-analysed the data-set to investigate differences in outcome attributable to type of drug formulation.

3.2 PARTICIPANTS

3.2.1 Study I

In study I, we collected data from deceased patients who had been recruited consecutively to an observational, cross-sectional study at ASIH Stockholm Södra from April 2014 investigating a possible association of vitamin D status and pain, infections and QoL in patients with advanced or metastatic cancer (110). We also included some patients from the cohort recruited in study II. In these two cohorts combined, 163 patients were deceased at the end of April 2016, and 160 patients were included in the final analysis. Three patients were lost to follow-up.

3.2.2 Study II

In study II, we consecutively included patients aged 18 and above, with a diagnosis of cancer (any type of cancer) and considered to be in a palliative phase of their disease trajectory. Patients were admitted to the ASIH Stockholm Södra advanced home care team or palliative ward between September 2015 and June 2016. Expected survival time was more than month and 25-OHD < 75 nmol/L. Study subjects were matched with untreated controls from the previous observational study from ASIH Stockholm Södra regarding age (+/- 10 years), sex, survival time (1-3 months, 3-6 months or more than 6 months after inclusion), type of cancer, 25-OHD at baseline and number of days in the study (110).

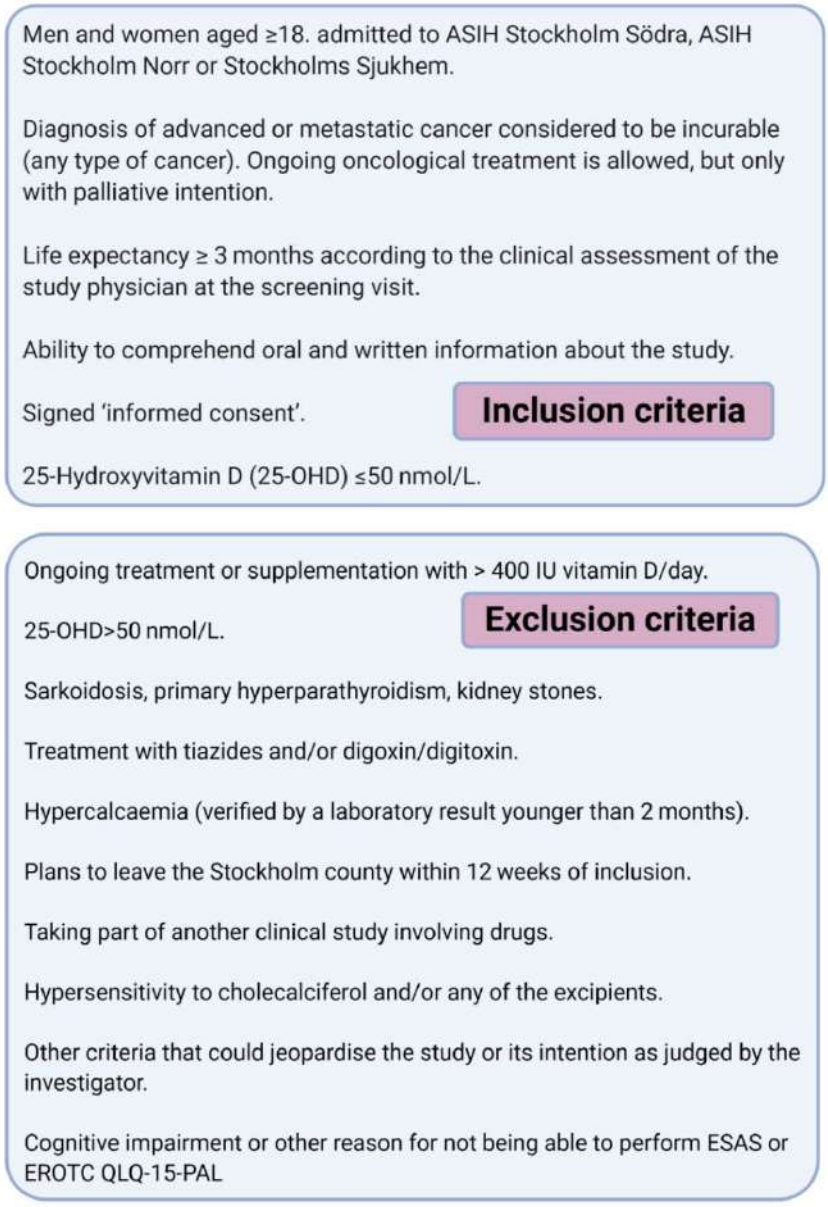
3.2.3 Study III

In study III, we screened patients who were admitted to ASIH Stockholm Södra (from November 2017), From Stockholms Sjukhem (from January 2018) and from ASIH Stockholm Norr (from January 2019) up until March 2020. In Figure 1 (p 2), the geographical areas covered by each of these facilities are presented - ASIH Stockholm Södra area 3 and 4, Stockholms Sjukhem area 5 and 6 and the two participating teams from ASIH Stockholm Norr parts of area 6, as well as area 7 and 8.

Due to the Covid-19 pandemic, inclusion stopped early. As in both the observational study (110) and the pilot study (study II), all patients had advanced or metastatic cancer of any type, and were aged 18 or above. Two inclusion criteria differed from both earlier studies – life expectancy should be at least three months instead of at least one month, and upper 25-OHD limit was 50 nmol/L instead of 75 nmol/L. Although pain assessed as fentanyl dose/hour was the primary outcome in the study, patients who were not on current medication with opioids could be included. At first, treatment with any dose of vitamin D was an exclusion criterion. After a protocol amendment early in the trial, daily intake of up to 400 IU of vitamin D was allowed.

A list of inclusion and exclusion criteria as anticipated at study start in November 2017 has been published previously (143). An updated list of inclusion and exclusion criteria after amendments to the original protocol is presented in Figure 5.

Fig 5. Final version of inclusion and exclusion criteria in study III



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3.2.4 Study IV

In study IV, 277 immunodeficient patients with the single inclusion criterion of 25-OHD < 75 nmol/L were recruited from the Immunodeficiency Unit at Karolinska University Hospital between March and October 2013. Exclusion criteria were ongoing treatment with vitamin D, as well as a diagnosis of tuberculosis or sarcoidosis or any other reason for not being considered suitable for participating in a trial.

3.3 DATA COLLECTION AND STUDY PROCEDURES

	Study I	Study II	Study III	Study IV
Demographic data	Age, sex	Age, sex	Age, sex	Age, sex
Disease specific information	Type of cancer	Type of cancer	Type of cancer	Type of immune insufficiency Immunoglobulin use
Lab				
CRP	*Last measurement*	*Baseline, 12 weeks*	*Screening, 4, 8, 12 weeks*	*NA*
Albumin	*Last measurement*	*Baseline, 12 weeks*	*Screening, 4, 8, 12 weeks*	*NA*
25-OHD	*Last measurement*	*Baseline, 12 weeks*	*Screening, 4, 8, 12 weeks*	*Baseline, first follow up (3-5 months)*
Electrolytes	*NA*	*Baseline, 12 weeks*	*Screening, 4, 8, 12 weeks*	*NA*
Antibiotic use	Number of days Type of antibiotic Indication Assessed effect	% of days during last 30 days	Number of days during last 30 days	Number of days Number of prescriptions Antibiotic use yes/no
Time of assessment	*Last 14 days*	*Baseline, 4, 8, 12 weeks*	*Screening, baseline, 4, 8, 12 weeks*	*12 months before/after start of intervention*
Opioid use	NA	Fentanyl dose (µg/hour)	NA	NA
Time of assessment	(not applicable)	*Baseline, 4, 8, 12 weeks*	*Screening, baseline, 4, 8, 12 weeks*	
Fatigue	NA	NA	ESAS EORTC QLQ-15-PAL	NA
Time of assessment			*Screening, 4, 8, 12 weeks*	
QoL	NA	ESAS	ESAS EORTC QLQ-15-PAL	NA
Time of assessment		*Baseline, 4, 8, 12 weeks*	*Screening, 4, 8, 12 weeks*	
Cultures	Culture yes/no Pos culture yes/no Type of pathogen	No	No	No
Adverse effects	*Retrospective review of patients' records*	*Monthly*	*Monthly*	*At planned outpatient visits*
Survival time	NA	Days in the study > 90 days survival	Overall survival	NA

3.3.1 Study I

3.3.1.1 Prospectively collected data in the original study cohort (previously published, not part of this thesis)

Patients fulfilling inclusion criteria were invited to participate by the patient's responsible physician and written informed consent was obtained from those who wanted to participate in the study. After inclusion

- 25-OHD, albumin and CRP were measured in a serum sample
- Information on
    - Age, sex, type of cancer
    - Antibiotic use during the last three months prior to inclusion
    - Current opioid dose translated to fentanyl dose/hour
    - QoL measured with ESAS

  were retrieved from the participant's medical records.

Data on survival time was collected at time of analysis of the collected data.

Detailed information on methods used in this study has been published previously (110).

3.3.1.2 Additional data collected in the retrospective analysis (Study I)

For the restrospective analysis of the effect of antibiotics in the late palliative phase we accessed patients' records once more, to collect detailed information on infections in end-of-life. We identified deceased patients with antibiotic treatment for at least one day during the last week of life.

Details on data retrieved from medical records is displayed in the overview of collected data on page 29. No measurement of CRP or Albumin was older than three weeks. Only measurements of 25-OHD collected within 60 days before death were used. Indication for antibiotic treatment was defined as one of the following: Treatment of or pervention of sepsis, treatment of urinary, respiratory, gastrointestinal or dermal infections.

Effect of antibiotic use was assessed independently by two physicians and included all recorded information regarding evaluation of antibiotic treatment (resolution of fever, fatigue, resolution of local symptoms, statement that the patient's condition had improved/worsened, remained stable etc). If the two physicians had different opinion regarding effect of antibiotics in the individual case, the discussed the case and reached a consensus.

3.3.1.3 Measurement of 25-OHD

The levels of 25-OHD in serum were analyzed by chemiluminescence immunoassay (CLIA) on a LIAISON-instrument (DiaSorin Inc, Stillwater, MN, USA,) with a detectable

range of 7.5–175 nmol/L, CV 2–5% at the Department of Clinical Chemistry, Karolinska University Hospital.

3.3.2 Study II

After study I, patients at ASIH Stockholm Södra were routinely screened for vitamin D deficiency. Patients with 25-OHD < 75 nmol/L were invited to participate in study II by their responsible physician. The baseline lab values from the intervention group in this study were thus taken as a routine screening procedure and were accessed by the study physician after written informed consent had been obtained. Details on data retrieved from medical records from the recruited patients is displayed in the overview of collected data on page 29. The conversion table we used for translating other types of opioids to fentanyl is presented in Table 3. Data on survival was collected by accessing the patients chart once again after death or at time of data analysis.

Table 3. Opioid dose equivalent/conversion guide

Morphine/Ketobemidone (mg)				**Oxycodone (mg)**				**Hydromorphone (mg)**				**Fenta-nyl (µg/h)**
Daily po	Extra po	Daily iv/sc	Extra iv/sc	Daily po	Extra po	Daily iv/sc	Extra iv/sc	Daily po	Extra po	Daily iv/sc	Extra iv/sc	Trans-dermal
20	2,5-5	7-10	1-2	10	1-2	7	1					12
40	5-10	15-20	2-5	20	2-5	15	2	4-8	1.3	2-4	≤1	12
60	10	20-30	3-5	30	5	20	3	8-12	2.6	4-6	≤1	25
80	10-15	30-40	5-7	40	5-10	30	5	12-16	2.6	6-8	1	25
100	15	35-50	6-8	50	5-10	35	6	14-20	2.6-3,9	7-10	1-2	37
120	20	45-60	7-10	60	10	45	7	18-24	2,6-3.9	9-12	2	50
160	25	60-80	10-15	80	10-15	60	10	24-32	3.9-5.2	12-16	2-3	50
220	40	80-110	15-20	110	20	80	10-15	32-44	5.2-7.8	16-22	3-4	75
320	55	120-160	20-25	160	25	120	20	48-64	7.8-10.4	24-32	4-6	100
400	70	150-200	25-35	200	35	150	25	60-80	10.4-13	30-40	5-7	125
500	85	185-250	30-40	250	40	185	30	74-100	12-17*	37-50	6-9	150
580	100*	215-290	35-50	290	50	215	35	86-116	14-19*	43-58	7-10	175
680	115*	255-340	45-55	340	55	255	45	102-136	17-23*	51-68	9-12	200
760	125*	285-380	50-65	380	65	285	50	114-152	19-25*	57-76	10-13	225
860	145*	320-430	55-70	430	70	320	55	128-172	21-29*	64-86	11-15	250
940	155*	350-470	60-80	470	80	350	60	140-188	23-31*	70-94	12-16	275
1040	175*	390-520	65-85	520	85	390	65	156-208	26-35*	78-104	13-18	300

*Order in form of capsules . Extra dose is 1/6 of daily intake. Po: peroral, sc: subcutaneous, iv:intravenously

3.3.2.1 Edmonton Symptom Assessment Scale

At ASIH Stockholm Södra, an unrevised version of ESAS is used in clinical routine, with "Pain during the last 24 hours" as an optional question, and with an extra question asking the patient to assess quality of life on the same 11-point NRS scale as the other questions (Figure 6). ESAS is routinely used for biweekly symptom assessment in the advanced home care unit, and weekly in the palliative ward. The results can be accessed and monitored over time in patients' electronic medical records. In our assessment of fatigue, we used the single item "tiredness".

Figure 6. ESAS used at ASIH Stockholm Södra

Ingen smärta No pain	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara smärta Worst possible pain
Smärta under de senaste 24 timmarna	0	1	2	3	4	5	6	7	8	9	10	Pain during the last 24 hours
Ej orkeslös/kraftlös No tiredness	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara orkeslöshet/kraftlöshet Worst possible tiredness
Inget illamående No nausea	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara illamående Worst possible nausea
Ingen nedstämdhet No depression	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara nedstämdhet Worst possible depression
Ingen oro/ångest No anxiety	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara oro/ångest Worst possible anxiety
Ingen trötthet/dåsighet No drowsiness	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara trötthet/dåsighet Worst possible drowsiness
Bästa möjliga aptit Best appetite	0	1	2	3	4	5	6	7	8	9	10	Sämsta möjliga aptit Worst possible appetite
Bästa möjliga välbefinnande Best feeling of well-being	0	1	2	3	4	5	6	7	8	9	10	Sämsta tänkbara välbefinnande Worst possible well-being
Ingen andfåddhet No shortness of breath	0	1	2	3	4	5	6	7	8	9	10	Värsta tänkbara andfåddhet Worst possible shortness of breath
Bästa möjliga livskvalitet	0	1	2	3	4	5	6	7	8	9	10	Sämsta tänkbara livskvalitet Worst quality of life

3.3.3 Study III

The results from Study II were used to plan Study III. The power calculation was performed by a statistician with clinical input from the study group. A sample size of 190 patients resulted in an estimated power of 81.6%. Attrition rate was expected to be 25%, and the estimated sample size thus 254 (127 per arm).

As displayed in the overview on page 29, collected data in Study III adheres well to the pilot study. However.

- Antibiotic use was assessed as number of days on antibiotics during the past 30 days (instead of % of days)
- EORTC QLQ-C15-PAL (Fig 7) was added as self assessment instrument at screening and at end-of-study.
- Samples for biobanking were collected at screening and at end-of-study.
- In Study III, data on oioid and antibiotic use was collected both at screening and at baseline (in the pilot study there was no screening visit).
- In Study III, 25-OHD after 12 weeks was only accessed by the safety physician until the study was unblinded.

Eligible patients were identified by the study team in collaboration with physicians responsible for the specialized palliative home-based facilities and palliative wards. The study team then contacted patients, mostly over phone, and gave oral information about the study. If patients were interested, we sent written information to their home addresses and contacted them again a few days later. If they were still interested in study participation after having read the written information, we planned for a visit by the study team.

Most often, this visit took place in patients' homes, but sometimes at the ASIH office. During this visit, we repeated oral information, answered questions regarding the study, signed written informed consent and performed screening procedures if the patient fulfilled all inclusion and no exclusion criteria. Patients recruited from the palliative ward received oral and written information and signed written informed consent during their stay at the ward.

When results from the lab test arrived, we called the screened patient and informed about test results. If 25-OHD was ≤ 50 nmol/L and kidney function and S-Calcium levels were within accepted range, the patient was offered study drug. If the patient accepted, we randomized the patient and arranged for delivery of the study drug to the patient within seven days.

At baseline, we planned for study visits every four weeks, but these could be rescheduled +/- seven days if needed. At 4, 8 and 12 weeks, nurses drew blood samples and recorded ESAS-results in patients' medical records as part of the regular weekly visit to the patient. At 12 weeks, the nurse also collected the EORTC QLQ-15-PAL form. When lab results (electrolytes and Creatinine) arrived, a member of the study team called the patient, informed about the lab results, assessed adverse events and asked about compliance. Deranged lab results were passed on to the patient's responsible physician.

Compliance was also assessed by inspection of returned bottles with study drug after collecting these at the final study visit.

ESAS questionnaires routinely used at Stockholms Sjukhem and ASIH Stockholm Norr differed from the ones used at ASIH Stockholm Södra, since both ASIH Stockholm Norr and Stockholms sjukhem used ESAS-r. Still, the "tiredness-question" and QoL were included in questionnaires from all facilities, and thus data from ESAS-questionnaries could be collected as specified in the trial protocol.

In EORTC QLQ-C15-PAL, fatigue scale consist of responses to the question 'Have you felt weak'' (Question 7) and "Were you tired' (Question 11). In our analysis we only incorporated the answer to Question 11.

Figure 7. EORTC QLQ-C15-PAL

EORTC QLQ-C15-PAL (version 1)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ☐☐☐☐
Your birthdate (Day, Month, Year): ☐☐☐☐☐☐☐☐
Today's date (Day, Month, Year): ☐☐☐☐☐☐☐☐

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
2. Do you need to stay in bed or a chair during the day?	1	2	3	4
3. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
4. Were you short of breath?	1	2	3	4
5. Have you had pain?	1	2	3	4
6. Have you had trouble sleeping?	1	2	3	4
7. Have you felt weak?	1	2	3	4
8. Have you lacked appetite?	1	2	3	4
9. Have you felt nauseated?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
10. Have you been constipated?	1	2	3	4
11. Were you tired?	1	2	3	4
12. Did pain interfere with your daily activities?	1	2	3	4
13. Did you feel tense?	1	2	3	4
14. Did you feel depressed?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

15. How would you rate your overall quality of life during the past week?

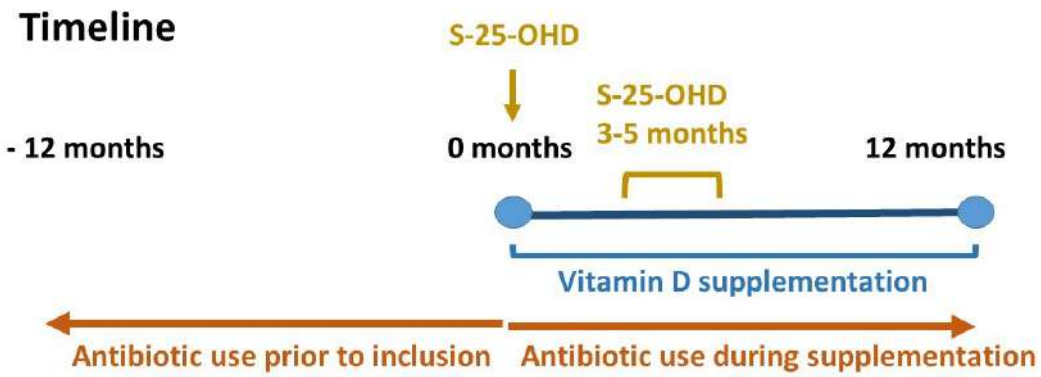
1	2	3	4	5	6	7
Very poor						Excellent

3.3.4 Study IV

Patients were recruited at planned visits to the Immunodeficiency unit at Karolinska University Hospital Huddinge. After written informed consent was obtained, data on age, sex, IgG replacement therapy and type of Immuno-insufficiency were retrieved from patients' medical records. According to study plan, 25-OHD was to be measured at baseline and at every visit to the outpatient clinic during the following year (4 planned visits in most cases). However, 25-OHD was not measured regularly after the first follow-up visit, and therefore only the first measurement of 25-OHD after initiation of vitamin D supplementation was used in this post-hoc analysis.

Data on prescription of oral antibiotics, both number of prescriptions and number of days on antibiotics, was retrieved from the Swedish Prescribed Drug Registry for a period of twelve months before and after start of intervention, Figure 8.

Figure 8



Helde Frankling, M.; Norlin, A.-C.; Hansen, S.; Wahren Borgström, E.; Bergman, P.; Björkhem-Bergman, L. Are Vitamin D_3 Tablets and Oil Drops Equally Effective in Raising S-25-Hydroxyvitamin D Concentrations? A Post-Hoc Analysis of an Observational Study on Immunodeficient Patients. *Nutrients* **2020**, *12*, 1230. Reprinted with permission under open access Crative Common CC BY license.

3.4 INTERVENTION, STUDY II, III AND IV

In study II, patients were offered a bottle of vitamin D_3 (Detremin), 20 000 IU/ml and 500 IU/drop, and prescribed 8 drops or 4000 IU/day for 12 weeks.

In study III, patients were offered study drug. They were randomly assigned to vitamin D_3 drops (Detremin), 20 000 IU/ml and 500 IU/drop, at a dose of 4000 IU/day, or placebo (1:1). Eight drops of study drug were prescribed to each patient for 12 weeks. Placebo consisted of Miglyoil oil, the same type of oil that was used for dissolving vitamin D_3. Both vitamin D_3 oil and placebo were dispensed in identical bottles that were sequentially numbered.

In study IV, patients were prescribed vitamin D_3 tablets (Divisun), 800 IU per tablet, 2 tablets/day with a total dose of 1600 IU/day, or vitamin D_3 oil (Detremin), 20 000 IU/ml, 500 IU/drop, with 3 drops or 1500 IU/day for 12 months.

3.5 DATA ANALYSES

Statistical methods used in the papers included in this thesis are listed in the overview on page 28. Baseline patient characteristics were analysed using standard descriptive statistics. Data analysis in study II and III, including power-calculation for study III (p 32), were performed by statisticians.

Software

In study I, statistical analyses were performed using Graph Pad Prism. In study II, statistical analyses were performed using Stata v. 13.1., and in study III Stat v. 15. Data analysis in study IV was performed in SPSS version 26.

Comparisons

In study I, Mann Whitney U was used to compare demography parameters and laboratory results. Chi^2 was used to compare dichotomous variables such as effect of antibiotics and whether a culture was taken or not in patients with different types of cancer. When analysing the distribution of patients who had effect of antibiotic treatment in relation to type of infection, Fischer's exact test was used.

In Study II, comparison of baseline characteristics for treated patients and untreated controls was made with paired t-test for continuous variables, McNemar's test for binary variables and Wilcoxon signed rank test for number of days in the study. The paired t-test was also used to compare vitamin D levels after interventions with baseline values. Crude fixed effects linear regression, where the fixed effects took the matched structure of the data into account, was used in comparisons of the change from baseline in opioid dose, infections and quality of life between cases and controls. Bootstrap were used to calculate 95% confidence intervals for the mean differences. Since there was an imbalance concerning CRP between the groups, data on CRP were log transformed, and we included a model adjusted for this imbalance in the analysis.

In Study III, the main analysis was performed using linear regression adjusting for baseline values and treatment arm including all patients who completed all 12 weeks (per protocol). Bootstrap methods were used to estimate confidence intervals. Adjusted models included age, sex, colectomy and oncological treatment at inclusion. The Kaplan-Meier method was used for survival analysis. Fixed effects regression was used to analyze the time varying effect of randomization on pain and antibiotic use.

Study IV

In comparison of baseline characteristics, Fishers exact test was used for dichotomous variables and Mann Whitney U for continuous variables. Comparison of continuous variables before and after intervention was performed thorough Wilcoxon matched pairs signed rank test in both main and subgroup analysis. The variable "no antibiotic use" was analyzed using McNemar's test. Mann Whitney U was used to calculate the difference in mean change in S-25-OHD between groups.

3.6 ETHICS APPROVAL

Ethical approval was obtained from the Regional Ethical Review Board in Stockholm (dnr 2013/2244-31/3, 2014/455-31, 2015/776-31 and 2017/405-31/1), and 'Palliative-D is also approved by the Swedish Medical Products Agency (EudraCT:2017-000268-14) and registered at Clinicaltrial.gov: NCT03038516

4 RESULTS, DISCUSSION AND FUTURE PERSPECTIVES

In this section, I will introduce our results in the context of challenges in palliative care research, and more specifically in the planning, conduction of and analysing data from clinical trials. The results-section starts with flow-charts from the prospective studies (II, III and IV). These are followed by presentation, comparison, and discussion of results from the four included papers based on outcomes (25-OHD, pain, infections, fatigue and QoL), and suggestions for further research based on our results. Finally, I will return to a discussion on the studies presented in this thesis in the context of literature discussing optimization of clinical trials in palliative medicine.

4.1 GENERAL CONSIDERATIONS REGARDING CLINICAL TRIALS IN PALLIATIVE SETTINGS

Design of clinical studies involving patients with a limited remaining life span requires careful planning. To minimize discomfort, trials involving patients in a palliative setting should aim at minimizing side effects of intervention, discomfort from procedures and negative psychological reactions. In addition, variability in symptom burden, individualized rather than standardized pharmacological interventions and comorbidities can skew results and pose questions regarding validity and generalizability of trial outcomes. In this setting, failing to reach recruitment goals through low inclusion and/or high dropout rates as well as through use of unsuited methods can further compromise validity of trial results.

4.2 CHALLENGES FACING RESEARCHES CONDUCTING INTERVENTION TRIALS IN PALLIATIVE CARE

4.2.1 Large drop-out rates - the challenge of prognosticating remaining life span in patients with advanced cancer

Attrition due clinical deterioration and death is often underestimated in the planning of clinical trials in palliative care. Medical professionals tend to overestimate remaining lifespan of patients with advanced or metastatic cancer (145, 146). A review on methodological characteristics and quality of reporting of clinical trials in palliative care stated that only 21/57 studies that presented a sample size calculation had reached inclusion goal (147).

In order to help professionals assess remaining life span in patients in palliative care, several prognostic tools have been developed. The patient's physical functioning is the factor that affects prognostic scores most. No prognostic tool is yet widely used (in contrast to oncology clinics), and discussion regarding best practice in this field continues (147-149). A study on 478 patients with median survival of 4.3 months showed that a combination of standardized assessment of functional performance status (Eastern Cooperative Oncology Group performance status, ECOG-PS) and a prognostic score based on CRP and Albumin (modified Glasgow Prognostic Score, mGPS) was superior to other methods in predicting survival at both one and three months (150). However, Clinician Predicted Surival, CPS, was third respectively fourth best in predicting survival. The author argues that this combination of

prognostic tools should be implemented in routine palliative care, since it is easy to use and aligns with practices in oncology clinics.

Another approach is to use tests developed for other clinical settings in patients with advanced cancer, such as the Shock Index developed for Emergency Departments (151). In cases where patients with advanced cancer were assessed in an emergency setting, this was a useful tool in predicting 60-day mortality. Yet another approach is the development of machine learning techniques using historical data from larger cohorts. In a recent validation study, an algorithm could distinguish between high versus low risk of dying of within six months in cancer patients visiting an outpatient oncology department (152).

4.2.2 Low inclusion rates - patients, health care professionals and next-of-kins perceptions of participation in clinical palliative care trials

Another difficulty in reaching planned inclusion goals in randomized trials in palliative care is the difficulty of recruiting patients. Clinicians and next of kin can act as concerned gatekeepers, afraid of adding to a frail patient's burden (153).

A perspective on this is offered in a qualitative study involving ten different Swedish palliative home care facilities (154). Two major difficulties were identified; the communication of the RCT-design to patients and family caregivers and the contradiction between the regular palliative care approach of offering patients all possible support and the withholding of intervention in the comparator group in a randomized trial. Learning over time eased the process, but other types of studies than RCTs were also proposed by health care professionals in the palliative care setting, in order to increase inclusion rates.

In a structured review of studies on dying patients' perspectives on participation in research (not limited to RCT:), "desire to help" was identified as the dominant theme. This desire included desire to help others, to help oneself and to help research at large or the researcher (155). Results from a Swedish questionnaire-based study is in line with this, stating that cancer patients' main motives for participating in oncological RCTs were personal hope for cure and altruism. However, in the studied cohort only few patients were treated with palliative intention (156).

4.2.3 Statistical challenges - analysing results from trials in palliative care setting with high drop-out rates due to deterioration or death

The large drop-out rate in longitudinal randomized trials in patients in a palliative care setting raises ethical questions, since many randomized patients were asked to participate in a clinical trial in their last weeks of life. Collected data should therefore be analysed in an optimal way.

A large dropout rate increases the risk of falsely rejecting the null hypothesis. In the case of a clinical trial comparing effect of a drug with placebo, this would mean that a statistically significant difference in effect between drug and placebo would be a false positive finding, a type I error. To minimize the risk for type I errors when analysing results from RCTs, all

patients who were randomized are included in the final analysis (intention to treat, ITT). In an ITT analysis, imputation techniques compensate for missing data from patients who no longer participate in the trial.

Another approach of analysing data is to do the analysis 'per protocol', and only include patients who complete the trial and from whom data has been collected on all pre-specified data collection points. This approach increases the risk of type II error, or not detecting a difference between groups, when a difference truly exists (false negative). It also increases the risk of missing information on adverse events.

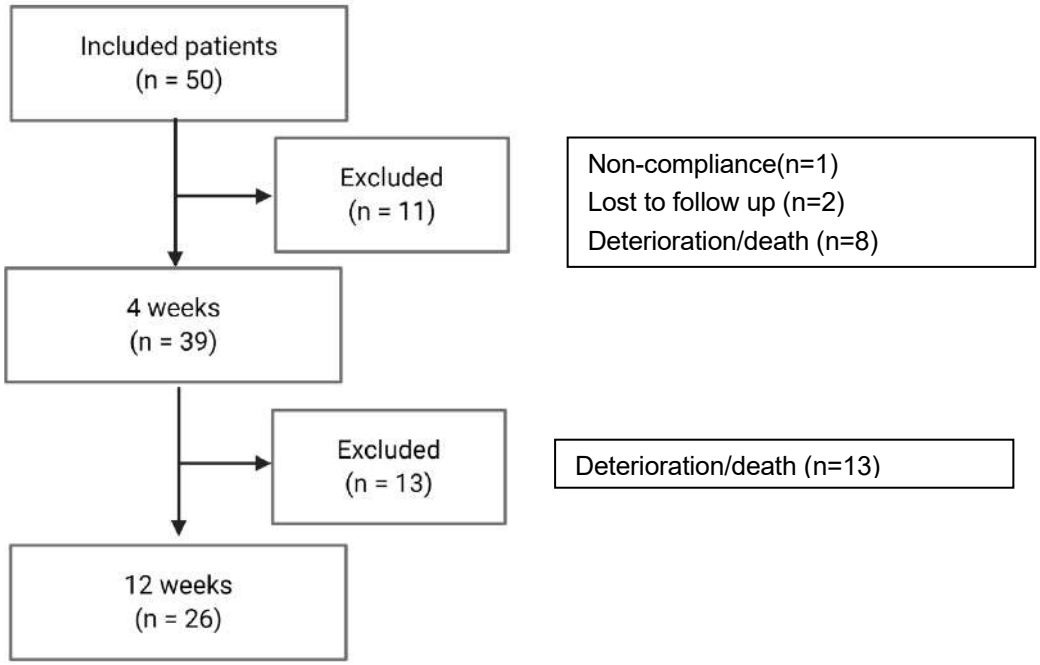
Currow et al argue that in a palliative care setting, non-completion of patients in RCTs is caused by disease progression rather than intervention. This creates a systematic bias away from the true effect (157). This is presented as especially troublesome, since RCTs in palliative care often are small in numbers. Reaching statistical power is therefore already a challenge. ITT with imputation is not an optimal technique in this setting, since data from deteriorating patients dilutes the effect of intervention, also reducing power. The authors therefore propose a different approach 'on a continuum between ITT and per protocol analysis', where data from deceased patients should not be included in the analysis of the primary outcome, given that

- It is prespecified in the original protocol
- Deterioration is undebatably due to disease progression
- An independent data Monitoring Committee also identifies the deterioration as such
- Dropout is shown in a CONSORT flow-chart.

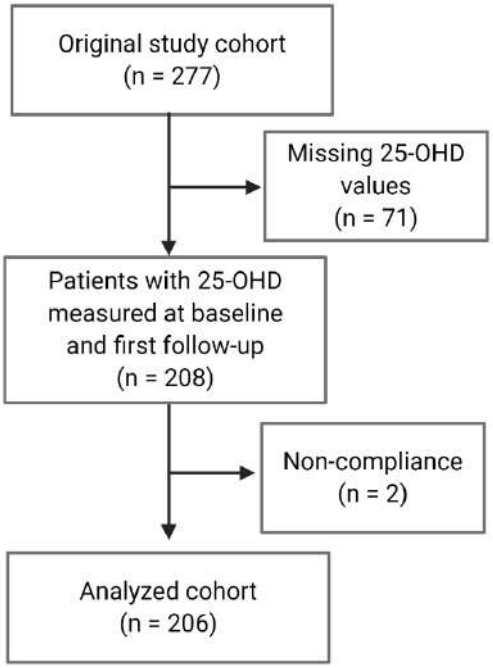
To aid the monitoring committee, functional status of patients should be assessed with a validated instrument (157).

4.3 FLOWCHARTS OF RECTRUITED AND EXCLUDED PATIENTS STUDY II, III, IV

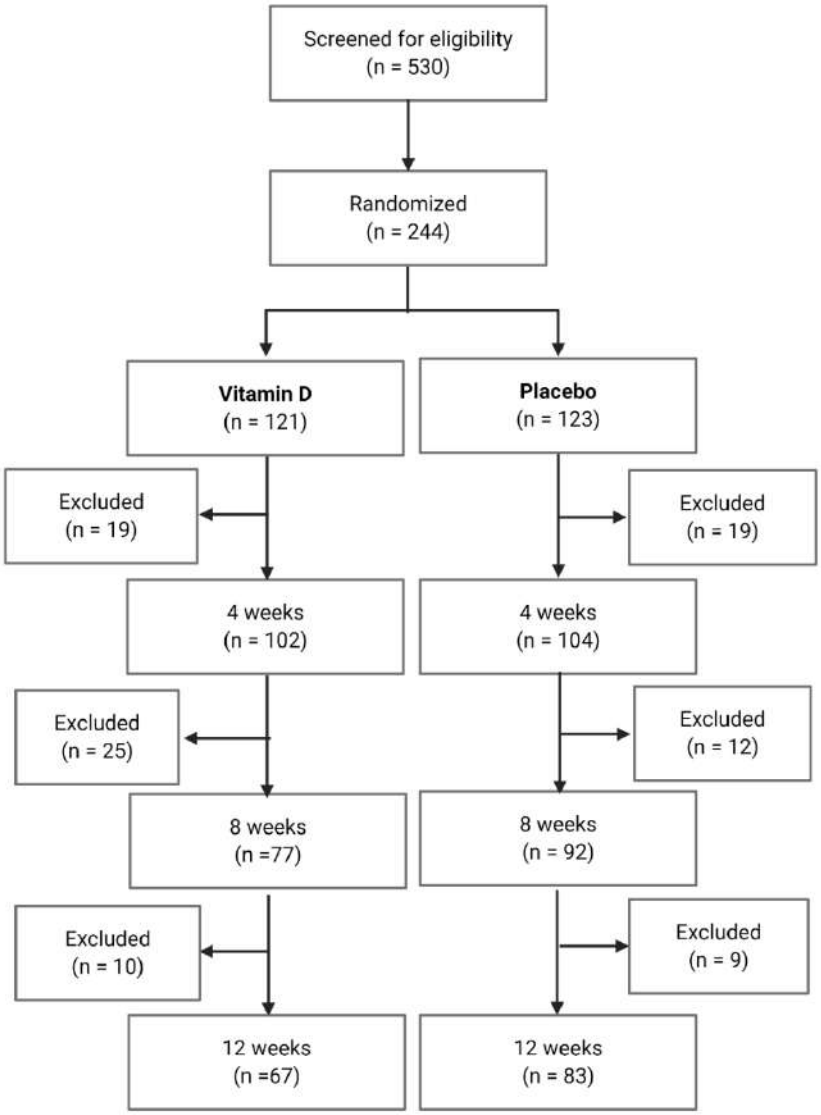
4.3.1 Flowchart Study II



4.3.2 Flowchart Study IV



4.3.3 Flowchart study III



Reasons for exclusion between screening and randomization (n=286):

n= 268 with 25-OHD > 50 nmol/L, n=7 due to deterioration/death, n=3 declined study drug, n=3 with hypercalcemia, n=4 with low eGFR

Reasons for exclusion in placebo group:

0-4 weeks (n=19): n=10 due to deterioration or death, n=2 declined further participation, n=1 lacked compliance, n=1 developed hypercalcemia and n=1 had side effects (GI).

4-8 weeks (n=12): n=10 due to deterioration or death, n=1 developed hypercalcemia, n=1 due to new medication (thiazides)

8-12 weeks (n=9): n=5 due to deterioration or death, n=2 declined further participation, n=1 lacked compliance, n=1 due to low eGFR

Reasons for exclusion in the vitamin D group:

0-4 weeks (n=19): n=12 due to deterioration or death, n=5 declined further participation, n=2 lacked compliance, n=2 were prescribed vitamin D outside study, n=1 due to prolonged hospital stay, n=1 other

4-8 weeks (n=25): n=18 due to deterioration or death, n=7 declined further participation

8-12 weeks (n=10): n=6 due to deterioration or death, n=2 due to hypercalcemia, n=2 due to prolonged hospital stay

4.4 25-OHD

4.4.1 25-OHD in the studied cohorts – overview of baseline data and results (study I, II, III, IV)

	I	II	III Screening cohort	III Vitamin D	IV Oil drops	IV Tablets
Type of outcome		Secondary	Secondary	Secondary	Primary	Primary
n=	123[a]	39	530	121	69	137
Baseline 25-OHD	36[d] (8-133)	33[c] (±26)	51[b] (37-67)	38[b] (28-45)	55[b] (36-63)	57[b] (40-69)
n=		23		67	69	137
IU/day	NA	4000	NA	4000	1600	1500
25-OHD after intervention	NA	73 (±31)	NA	81[c] (±26)	86[b] (68-104)	87[b] (73-98)
Increase in 25-OHD		40[c]	NA	42[b] (36 – 49)	31[b]	33[b]
Type of supplementation	NA	Detremin	NA	Detremin	Detremin	Divisun
Duration of intervention	NA	12 weeks	NA	12 weeks	12-20 weeks	12-20 weeks

[a] patients who had their 25-OHD-levels measured within 60 days before death were included in the analysis

[b] median values and IQR, [c] mean values and standard deviation or CI, [d] median values and range

NA:Not applicable

Comparison of the efficacy of vitamin D3 oil and powdered tablets in raising 25-OHD (results from study IV) In study IV, the primary endpoint was to study change in 25-OHD in immunodeficient patients supplemented with vitamin D3 oil drops (1500 IU/day) or powdered tablets (1600 IU/day) for 3–5 months. The original study was a purely academic evaluation of new guidelines in a real-world setting. So as not to favor any of the two companies offering vitamin D3 produced according to GMP on the Swedish market, the intention was to prescribe oil drops and powdered tablets to every other patient who visited the outpatient clinic. However, patients preferred tablets over oil, and twice as many patients used tablets (n=137) compared to oil drops (n=69).

A majority (n=129, 63%) of included patients were women and median age was 56 years (range 19–88). There were no significant differences between groups at baseline.

Median 25-OHD increased significantly in both groups, from 55 to 86 nmol/L (difference 31 nmol/L) in the group taking oral drops and from 53 to 87 nmol/L (difference 33 nmol/L) in

the group taking powdered tablets. The difference in increase in 25-OHD between groups from baseline to follow-up was not significant (Mann–Whitney U test, p = 0.77), Fig 9.

4.4.2 Comparison of the efficacy of vitamin D3 oil and powdered tablets in raising 25-OHD

In study IV, the primary endpoint was to study change in 25-OHD in immunodeficient patients supplemented with vitamin D3 oil drops (1500 IU/day) or powdered tablets (1600 IU/day) for 3–5 months. The original study was a purely academic evaluation of new guidelines in a real-world setting. So as not to favor any of the two companies offering vitamin D3 produced according to GMP on the Swedish market, the intention was to prescribe oil drops and powdered tablets to every other patient who visited the outpatient clinic. However, patients preferred tablets over oil, and twice as many patients used tablets (n=137) compared to oil drops (n=69).

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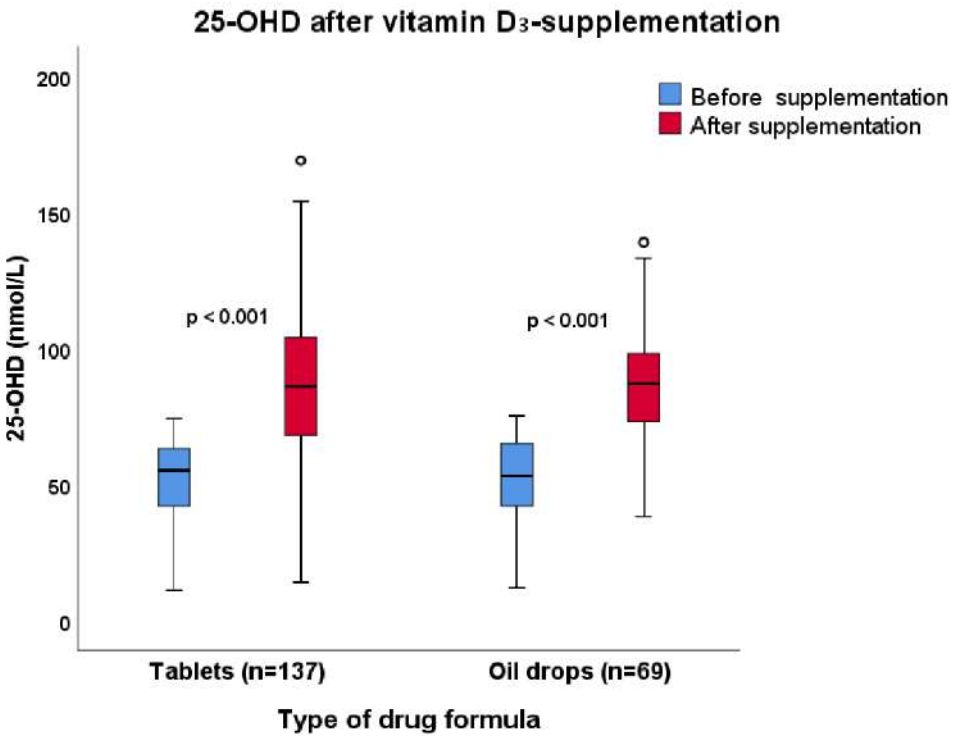
Figure 9



Helde Frankling et al, Are Vitamin D_3 Tablets and Oil Drops Equally Effective in Raising S-25-Hydroxyvitamin D Concentrations? A Post-Hoc Analysis of an Observational Study on Immunodeficient Patients. *Nutrients* **2020**, *12*, 1230. Reprinted with permission under open access Crative Common CC BY license.

4.4.3 25-OHD in the studied cohorts – discussion

4.4.3.1 Comparison of baseline 25-OHD

In study I and II, with partly overlapping cohorts, mean 25-OHD was lower than in the RCT (study III). I would argue that this is due to the fact that many patients in study III were recruited to the study shortly after referral to advanced palliative home care, whereas the majority of patients in study I or II were included during a stay in a palliative ward, possibly later in their disease trajectory and with more severe disease.

Levels of albumin and CRP, known to be strong prognostic markers for survival in cancer patients, differ between the cohorts in study II and III, supporting this notion. In study II, mean albumin levels were 28 (±6) g/L in the vitamin D treated cohort and 27 (±7) g/L in controls. CRP was 47 (±76) mg/L and 67 (±75) mg/L respectively. In study III, median Albumin was slightly higher, 30 g/L (IQR 26-34) and CRP levels much lower (9 mg/mL (IQR 3-31)). In baseline values for the screened cohort for study III, Albumin and CRP levels were comparable to the randomized cohort, but median 25-OHD was 51 nmol/L (111).

When comparing with studies on 25-OHD levels in advanced cancer patients in other cohorts, our results land "somewhere in the middle". In an Australian study from a latitude with more sun-exposure compared to Stockholm, mean 25-OHD in outpatients was 60 nmol/L and in inpatients 50 nmol/L(109). In a US cohort, 70% of patients had 25-OHD-levels < 75 nmol/L, and 46% < 50 nmol/L (107). A Turkish material presents much lower median levels at 30 nmol/L (106).

Study IV, with a cohort of non-cancer patients, had higher levels of 25-OHD at baseline, but still not sufficient for optimal immune functioning.

4.4.3.2 Comparison of 25-OHD levels after supplementation

The increase in 25-OHD after supplementation was slightly above 30 nmol/L with vitamin D_3 doses of 15-1600 IU/day, and around 40 nmol/L with 4000 IU/day. This is lower than the anticipated increase according to the Institute of Medicine, who conclude that from aggregated data, 40 IU vitamin D_3 is assumed to raise 25-OHD concentration by approximately 1 nmol/L, and thus daily intake of 1500–1600 IU/day should raise 25-OHD concentrations with 37.5–40 nmol/L and 4000 IU/day with 100 nmol/L (84).

4.4.3.3 Differences in 25-OHD levels after supplementation –compliance issues or other explanations?

Increase in 25-OHD after three months of vitamin D treatment were similar in the pilot study (**study II)** and the RCT **(study III),** indicating similar levels of compliance, although all bottles were not returned for visual inspection of remaining study drug. Interestingly, we observed a large increase in 25-OHD levels in some patients in the placebo group in the RCT as well, which may be attributable to increased oral intake or sun exposure.

In study IV, two patients with sharp drops in 25-OHD over time were excluded from analysis, since they most likely did not take the prescribed supplement. The mean increase of 25-OHD in the cohort was 31–34 nmol/L, slightly lower than anticipated. Since this was a real-life confirmation study, patients were not regularly asked about adherence to prescribed supplementation, and it is therefore possible that compliance was somewhat lower than in intervention studies.

In study II we had two patients with suspected impaired uptake of vitamin D. One participant, meticulous about his medication, had undetectable levels of 25-OHD both before and after intervention, most likely due to impaired uptake through intestinal mucosa caused by inflammatory bowel disease and short bowel syndrome after numerous operations. Another participant, a woman with GI-cancer, experienced decrease in 25-OHD from 62 nmol/L to 32 nmol/L. She was given a second bottle of Detremin after 2 months, indicating compliance. Both patients were included in the final analysis.

In contrast, there was no difference in 25-OHD levels between patients who had and who had not undergone bowel resections that could possibly reduce uptake of vitamin D in study III. This finding from study III also contradicts previously published results, where patients with GI cancers or having undergone GI-surgery had lower levels of 25-OHD (106, 107).

4.4.4 25-OHD in the studied cohorts – future perspectives

The analysis plan for study III did not include analysis of seasonal variation of vitamin D baseline levels or analysis of 25-OHD after supplementation in relation to season during which intervention took place. Since, to our knowledge, no one has investigated seasonal variations in 25-OHD in palliative cancer patients, such a post-hoc analysis would be relevant.

4.5 VITAMIN D AND OPIOID USE IN PATIENTS WITH ADVANCED CANCER

4.5.1 Results from a pilot study and an RCT (study II, III)

4.5.1.1 Study II

Opioid dose. The mean fentanyl dose in vitamin D treated patients was 31, 37 and 22 μg/h at baseline, after one and three months, respectively. Corresponding values for untreated controls was 43, 95 and 117 μg/h, Figure 10 A. Mean change in opioid dose from baseline was a decrease of 6 μg/h in the intervention group and an increase of 85 μg/h in controls. After three months, the mean difference in change in opioid dose between the groups was 91 μg/h (± 56-140) in the unadjusted and 120 μg/h (±49-185) in the adjusted model.

In the treatment group, 36% of patients (n = 14) reduced their daily opioid dose during the follow up period. Eight of these patients were on active oncological treatment when recruited to the study. Amongst controls, one patient (receiving palliative chemotherapy), reduced his or her opioid dose.

Number of patients on opioids

In the treatment group, 28% of patients (n = 11) were not treated with opioids at baseline, and these remained opioid-free throughout intervention, although only seven could be evaluated after three months. Seven patients (18%) stopped taking opioids during the study period (fentanyl dose at baseline 12–25 µg/h).

In the control group, 12 patients were not on opioids at baseline, six of these were still not prescribed opioids after one month, and five patients were opioid free after three months.

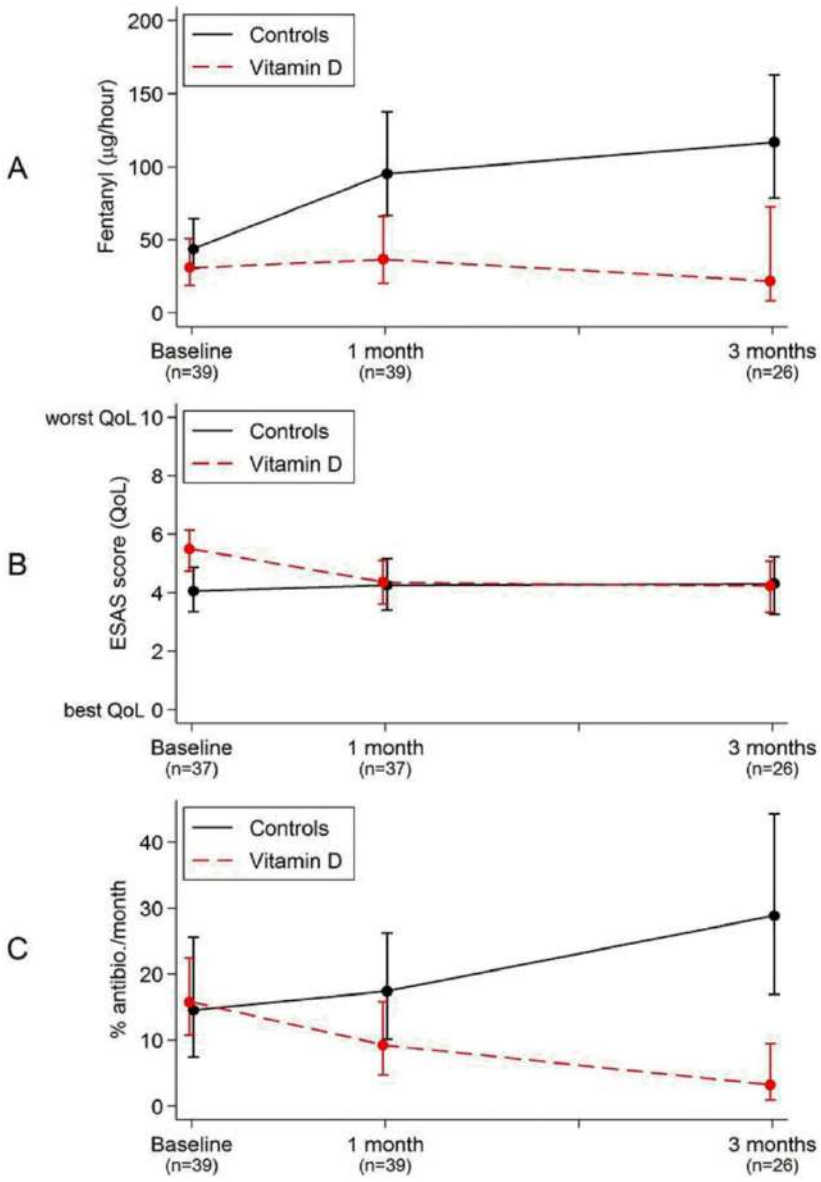
4.5.1.2 Study III

Opioid dose Data on opioid doses were not normally distributed, and thus medians instead of mean values are used. At baseline, median fentanyl dose was 0 (IQR 0-37) in the vitamin D group. A mean reduction in opioid dose of a little more than 6 µg/h from baseline to end of study was observed in the vitamin D group, which is in line with the results from study II. Opioid doses in the placebo group remained stable, with mean fentanyl dose in the placebo group with 24 (±56) ug/h at screening and 25 (±60) ug/h at three months.

Number of patients on opioids

Analysis of number of patients on opioids was not prespecified in the study protocol

Figure 10, A, B and C. Differences in opioid dose (µg fentanyl/h) (A), Quality of Life (according to ESAS-assessment) (B) and antibiotic consumption (% of days with antibiotic the month before) (C) between 39 vitamin D treated palliative cancer patients and 39 matched controls. Points show mean values and bars show 95%CI.



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Helde-Frankling M, Höijer J, Bergqvist J, Björkhem-Bergman L. Vitamin D supplementation to palliative cancer patients shows positive effects on pain and infections-Results from a matched case-control study. PLoS One. 2017 Aug 31;12(8):e0184208. Reprinted with permission.

4.5.2 Results regarding opioid use in study II and III – discussion

4.5.2.1 Does difference in opioid use between study II and study III reflect difference in disease trajectory in the cohorts?

In our pilot study, a significant effect of vitamin D supplementation was observed already after one month, with an increasing difference between groups over time. After twelve weeks, we saw a significant difference between groups in the RCT as well, but at a much smaller scale. However, the difference between groups in the pilot study was mainly due to a fast increase in fentanyl dose in controls. In the RCT, opioid use remained constant in the placebo group throughout the intervention period.

We argue that the discrepancy between the pilot study and the RCT regarding fentanyl use in the control group can be explained by the fact that patients and controls in the pilot study were mainly recruited from a hospice ward rather than from the advanced home care teams. Patients in the hospice ward are in either in a later phase of their disease trajectory and/or have more severe symptoms and thus use larger doses of opioids. This is supported by survival time in the different cohorts. In study II, 15/50 or 30% of patients recruited to the intervention group were alive after 6 months (unpublished data). In study III, 50% of randomized patients were alive after 6 months, with no significant difference between vitamin D and placebo groups.

In the Canadian study mentioned in the Background section, opioid use increased in all quintiles during the last weeks of life (14), (Fig 2, p 3). In our studies, more patients in study III compared to study II would be in the earlier stages of their disease trajectory (further left on the x-axis in the study) and vice versa.

4.5.2.2 Was fentanyl ug/hour a wise choice of primary outcome measure?

Fentanyl ug/hour is not the most common outcome measure in literature on palliative care or pharmacological treatment of pain. This outcome measure was however chosen already in the association study (110) preceding the pilot study (study II) and the RCT (study III). Results from these studies can therefore be readily compared with each other. The main reason for choosing fentanyl ug/hour rather than MEDD, was that many patients recruited to the association study were prescribed fentanyl patches, and this outcome measure thus reflected clinical practice at the unit. Using fentanyl dose/hour as outcome measure also kept calculated opioid conversions to a minimum. This helped maintain accuracy regarding primary outcome, since every opioid conversion introduces some extent of inaccuracy.

4.5.2.3 Is assessment of opioid dose once a month a wise choice of collecting data on primary outcome?

Or rather – why did we not use cumulative opioid dose during a certain period of time, for example during the past week? The answer to that is that our clinical setting, nurses visit patients weekly and assess pain and other symptoms. During these visits, short-acting opioids are dispensed for use in the coming week. If patients have increasing pain and have used more extra opioid doses for breakthrough pain during the past week, the nurse consults a palliative care physician, and the dose of long-acting opioid is readily adjusted. Thus, we think that measurement of opioid dose in our clinical setting reflects actual opioid doses well. For patients at the palliative ward, opioid doses are adjusted daily, if needed. Pain assessed with ESAS did not differ between groups. We argue that this is due to continuous adjustment of pain medication. Pain assessed with ESAS would thus not have constituted an appropriate outcome measure.

4.5.3 Results regarding opioid use in study II and III – future perspectives

In study III we only adjust for oncologic treatment at screening when analysing outcomes. Pain and opioid use over time can however be influenced by changes in oncologic treatment during the intervention period. A post-hoc analysis with more detailed data on all oncologic treatment would therefore be of interest. It would also be of interest to see whether or not a post-hoc analysis of the effect of vitamin D supplementation on opioids could detect a difference in effect in patients with a survival of 0-3 months, 3-6 months and more than 6 months, given that the pilot study with more diseased patients detected a larger difference between groups than the RCT. A post hoc analysis regarding possible differences in effect between men and women would also be interesting, especially since there was a difference in association between fatigue and 25-OHD in the screened cohort (111).

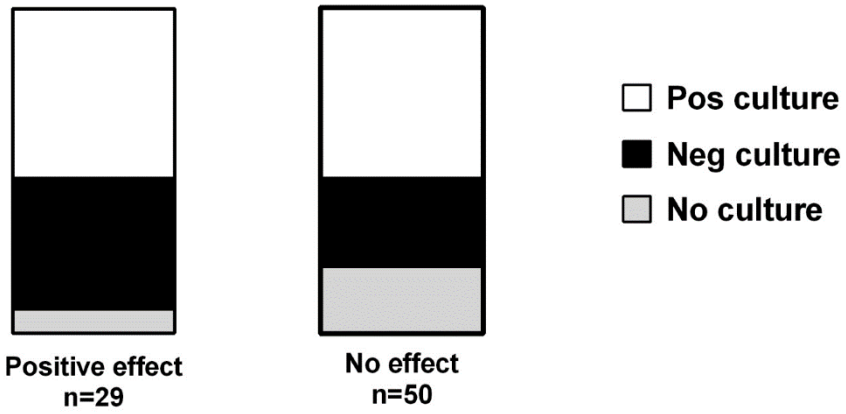
4.6 ANTIBIOTIC USE IN END OF LIFE CANCER PATIENTS & VITAMIN D AND ANTIBIOTIC USE IN PATIENTS WITH ADVANCED CANCER

4.6.1 Antibiotic use in the late palliative phase – results from study I

In study I, we studied a cohort of 160 deceased cancer patients, in which 57% were women, and median age was 71 (range 18-95). Almost half of the patients, n=79, had been treated with antibiotics during the last week in life, with slightly more than three quarters of patients had received intravenously administered antibiotics. Ceftriaxone, piperacillin/tazobactam and cefotaxime were the most frequently used types of antibiotics. Cultures were taken in n=67 patients, and 41 of these returned a positive answer. The most frequently detected pathogen was S aureus, with positive cultures from blood, urine, skin, sputum, nasopharynx, and synovial fluid In most cases, S Aureus was possibly a colonizer rather than an agent causing a clinically relevant infection. Two E coli- cultures had acquired antibiotic resistance, Beta-Lactamase Extended Spectrum (ESBL), but no Methicillin Resistant Staphylococcus Aureus (MRSA) were found. Two patients experienced diahorrea and one patient nausea attributable to antibiotic use. Frequency of adverse events was 4%.

There was no difference between treated and untreated patients regarding sex or age, but the mean CRP level was significantly higher in patients treated with antibiotics (124 versus 44 mg/L, $p< 0,001$). Symptom relief was documented in n= 29 (37%) of patients treated with antibiotics. In the remaining 63% of cases there was no effect of antibiotic use, or effect was not assessed and documented in medical records (=unknown effect). Type of cancer did not predict use of or effect of antibiotics. Positive bacterial cultures were evenly distributed (50%) between patients who had documented effect of antibiotics and those who had not, Fig 11.

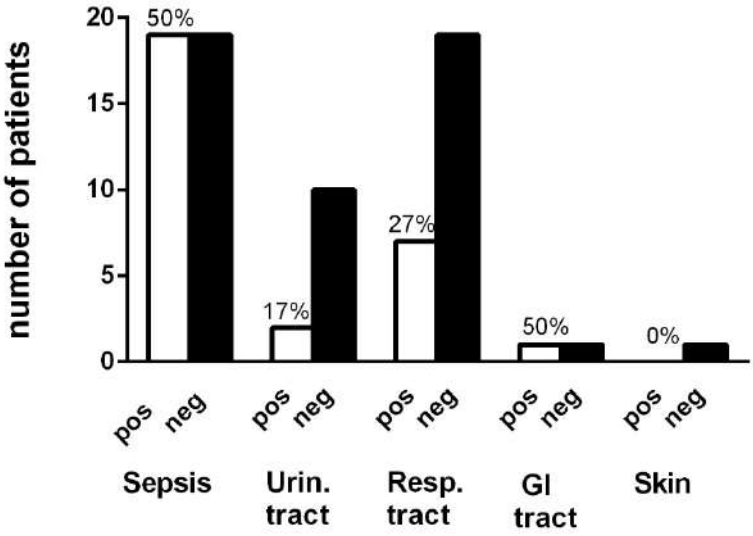
Figure 11



Helde-Frankling et al (2016). Antibiotic Treatment in End-of-Life Cancer Patients-A Retrospective Observational Study at a Palliative Care Center in Sweden. *Cancers*, *8*(9), 84. Reprinted with permission.

In manifest or suspected sepsis, 50% of patients had documented effect of antibiotic treatment. In respiratory tract infections or GI-infections, effect of antibiotic use was less evident (Fig 12).

Figure 12. Effect of antibiotics at different infection indications in end-of-life cancer patients



Helde-Frankling et al (2016). Antibiotic Treatment in End-of-Life Cancer Patients-A Retrospective Observational Study at a Palliative Care Center in Sweden. *Cancers*, *8*(9), 84. Reprinted with permission.

4.6.2 25-OHD-levels, vitamin D supplementation and antibiotic use – results from study I, II, III, IV

4.6.2.1 Study 1

In the retrospective cohort study on antibiotic use in the late palliative phase, there was no association between antibiotic use in the last week of life (yes or no) and 25-OHD in the 123/160 patients who had 25-OHD measured at most 60 days prior to death (p=0,20). Neither was there an association between 25-OHD and effect of antibiotic treatment (yes/no), p=0,32.

Seventeen patients had 25-OHD > 75 nmol/L, considered optimal levels for a well-functioning immune system. One third of these patients (n=6) were treated with antibiotics, compared to 55% for those with lower vitamin D-levels. Among the six patients with sufficient 25-OHD-levels, four responded to antibiotics. The reverse proportion was shown regarding effect of antibiotics in patients with 25-OHD < 75 nmol/L. These differences between groups were not statistically significant (p=0.19 and p = 0.17 respectively).

4.6.2.2 Study II

In baseline data for study II, mean 25-OHD-level was 33 nmol/L (±26) and 38 nmol/L (±18) in the intervention group and controls respectively (p=0,34). At baseline, mean proportion of days an antibiotics during the past month was 16% (± 19%), equivalent to 4,8 (± 5,7) days in a 30-day period, in the intervention group, and 15% (± 27%) equivalent to 4,5 (±8,1) days amongst controls. After three months, mean proportion of number of days on antibiotics during the past month differed significantly between the vitamin D supplemented group and controls, with less usage in supplemented patients, -26% (95% CI -41%–(-12%)), Fig 10 C

4.6.2.3 Study III

In baseline data for study III, mean 25-OHD was 36 nmol/L (±10 nmol/L) in n=244 patients, with very similar values in the vitamin D and placebo groups. Mean number of days on antibiotics during the past month was 2.7 (± 5.6), in the entire cohort. After intervention, mean 25-OHD in the treatment group was 82 (±26), and mean number of days on antibiotics 2.8 (± 6.5). Thus, an increase in 25-OHD through supplementation did not affect use of antibiotics in the longitudinal analysis. In comparison with the placebo group, there was no significant difference regarding mean change in antibiotic use (median values) during the past 30 days between groups and any data collection point (4, 8, 12 weeks).

4.6.2.4 Study IV

The effect of vitamin D_3 oil drops versus powdered tablets on antibiotic use was studied using three different parameters (158). The 12-month period prior to start of intervention was compared to the 12-month long intervention period. Oil drops were more efficient in all three parameters:

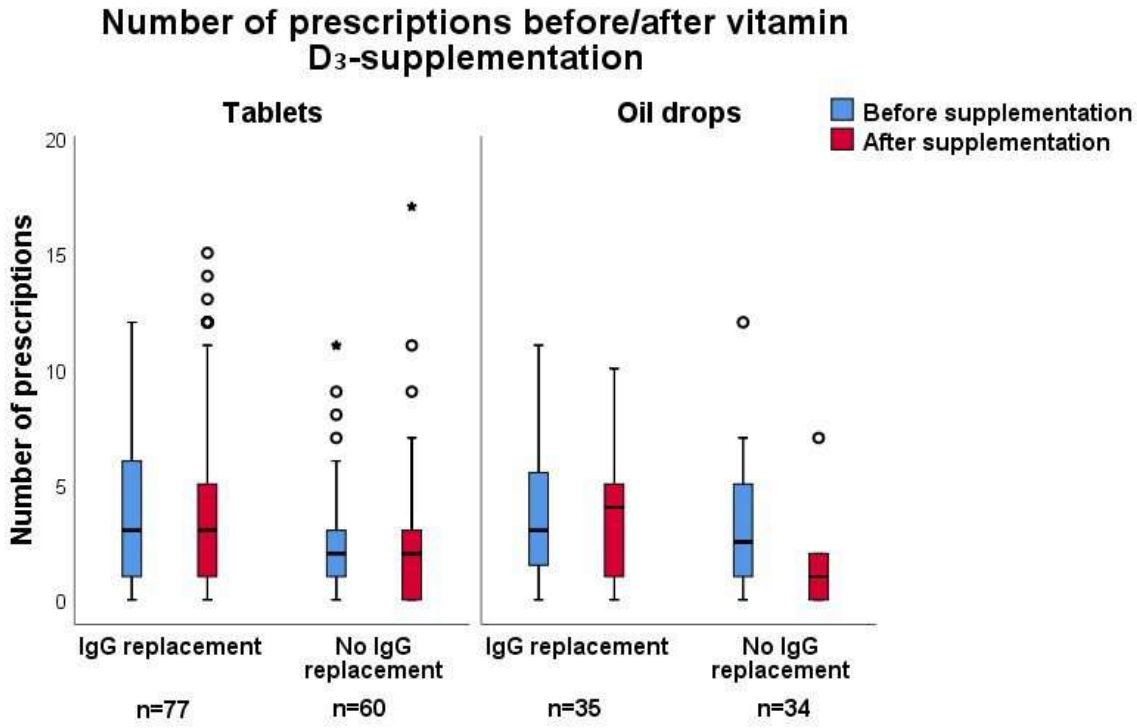
1. In the oil drops group, the number of patients who did not use antibiotics at all increased from 10 to 21 ($p = 0.03$). An increase in “antibiotic free” patients was

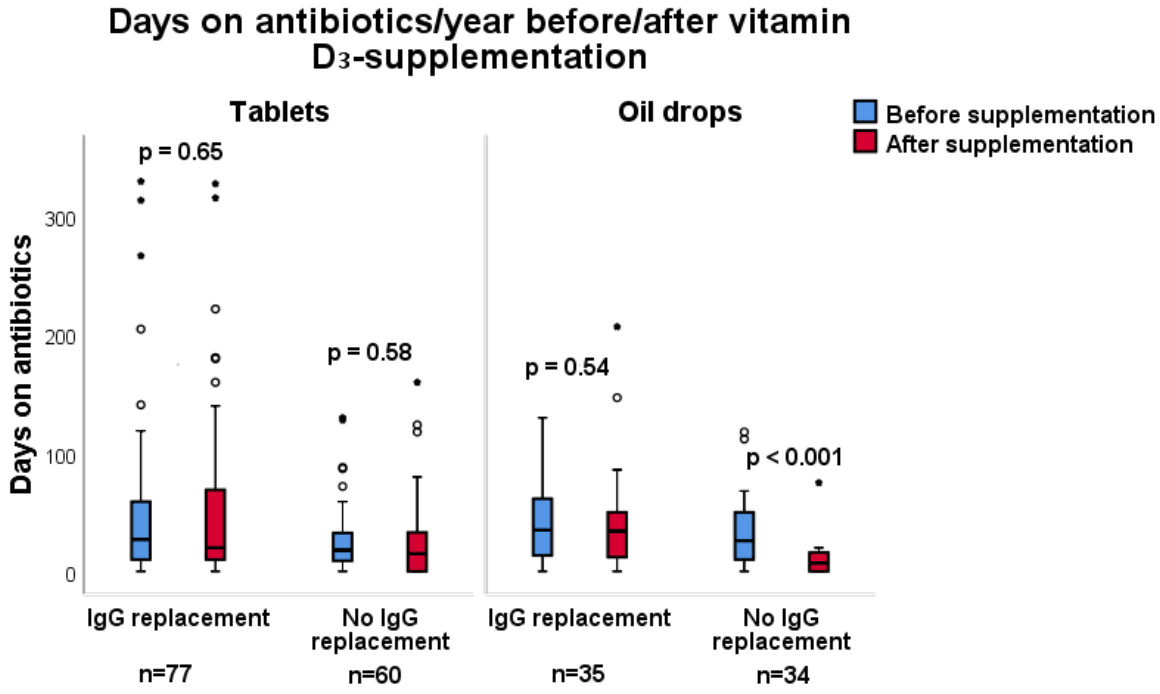
observed in the tablet-group as well, from $n = 22$ to $n = 32$, though this chage was not statistically significant ($p = 0.13$).

2. In the oil drops group, the median number of prescriptions of antibiotics dropped from 3 (IQR 1-5) to 2 (IQR 0-4), $p = 0.003$. In the tablets group, there was no significant change in number of presciptions.
3. In the oil drops group, the number of days on antibotics dropped from 27 (IQR 10-52) to 15 (IQR 7-46), $p = 0.003$, and in the tablet group there was no significant change in days on antibiotics.

In the original study, a subgroup analysis comparing patients with immunodeficiency receiving IgG replacement therapy was compared to those who were not. When repeating this subgroup analysis in study IV, we could see that the entire effect on antibiotic use was found in patients who were not on IgG replacement therapy (n=34). Median number of prescriptions decreased from 3.1 to 1 (p< 0,001), and median number of days on antibiotics from 26 to 7 (p< 0,001), Fig 13.

Figure 13. Effect of vitamin D supplementation using oild drops versus tablets regarding number of prescriptions and number of days on antibiotics in immunodeficient patients with or without IgG replacement therapy.





Helde Frankling M et al, Are Vitamin D3 Tablets and Oil Drops Equally Effective in Raising S-25-Hydroxyvitamin D Concentrations? A Post-Hoc Analysis of an Observational Study on Immunodeficient Patients. Nutrients. 2020;12(5):1230. Reprinted with permission

4.6.3 Antibiotic use in End-of-Life cancer patients and vitamin D and antibiotic use in advanced cancer patients – Discussion

4.6.3.1 Developments in the field 'Antibiotic use in End-of-life cancer patients' since we published our study

As study I was planned and conducted, there was scarce evidence upon which to base treatment decisions regarding antibiotic use in end-of-life cancer patients, and no written guidelines had been published (159). Similar to our retrospective study, most published materials reflect single-centre experiences and show us that intensity of care and the frequency with which cancer patients in end of life receive antibiotic treatment differs greatly between different parts of the world and over time (20-22, 160-163). It has been discussed that cultural aspects as well as individual preferences of patients, families or physicians may influence treatment decisions (164-166).

In 2012, the Infections Society of America published guidelines for antimicrobial stewardships programs, proposing coordinated interventions designed to improve and measure the appropriate use of antibiotics (167). In in 2016 recommendations for implementing these programs were offered (168). Instead of focusing on a specific group of patients, these programs stress the importance of appropriate use of antibiotics given local organization of health care, antibiotic resistance and other factors that may influence antibiotic use in a specific setting. These initiatives also stress the importance of education.

Based on these initiatives, antibiotic use in end-of-life cancer patients has been much discussed in recent years, but still without reaching a consensus regarding best practice (25, 169-171). A survey from 2018 showed that in the US, 64% of hospitals monitored antibiotic use in end-of-life care, but that clinician still sought more information on how to make clinical decisions in this setting (172).

4.6.3.2 Aspects on antibiotic use in End-of-life not discussed in the original publication (study I)

In contrast to some other experiences (21, 173), the reason for initiating treatment was recorded in all patients in our cohort.

Malignancy itself may cause fever and elevated CRP-levels (165), thus mimicking infection and presenting a common clinical problem. This fact may partly explain why we saw a positive effect in antibiotic use in only 50% of patients with positive cultures.

A narrative review from 2019 argues that parenteral administration may be less burdensome than peroral administration in end-of-life cancer patients, due to difficulties swallowing caused by thrush, mucositis, or other underlying conditions (174). This supports the frequent use of intravenous administration of antibiotics (77%) in our cohort.

4.6.3.3 Effect of Vitamin D supplementation and antibiotic use in patients with advanced or metastatic cancer – why did we see an effect in study II and not in study III?

In the pilot study (study II), we observed a significant difference between vitamin D - supplemented patients and controls after three months, achieved through both reduction of antibiotic use in the treatment group, and increased use in controls over time (Fig 10 C). This observation could not be reproduced in our randomized, placebo-controlled trial (study III). However, after three months the difference between groups was increasing, and we remain curious regarding possible differences between groups if the intervention period had been four or five months instead.

Since study III was underpowered due to higher attrition rate than expected and only 55% of patient randomized to the treatment arm could be assessed after 12 weeks, there is a risk of type II error, meaning that we might not detect a significant difference between the groups regarding this outcome.

4.6.3.4 Why would vitamin D supplementation using oil drops be more efficient than tablets? And why is there an effect on antibiotic use in immunodeficient patients NOT receiving IgG replacement therapy?

It is possible that this finding could have a mechanistic basis, since oil emulsions might be better absorbed in the gut than tablets (175). Consequently, oil preparations could reach the liver faster and cause a more efficient downstream effect on target cells. In the original publication (144), authors hypothesized that IgG supplementation is the most potent

immunomodulatory treatment available for these patients and that additional immune-active interventions could not boost the immune system further.

4.7 VITAMIN D AND PATIENT-RECORDED OUTCOMES – FATIGUE AND QOL

4.7.1 QoL (study II and III)

In the cross-sectional observation study in our advanced palliative home care facility preceding study II and III, we did not observe an association between 25-OHD-levels and QoL (110), a result in line with other cohorts of advanced cancer patients (122). Still, data on both association between 25-OHD and HRQoL and effects of vitamin D supplementation in advanced cancer patients is scarce, and we decided to add HRQoL as an outcome measure to our studies to explore this subject further.

In study II, QoL was assessed as the last question in the 11-point numeric scale ESAS questionnaire used at ASIH Stockholm Södra, with the verbal anchor "best possible QoL" at 0, and "worst possible QoL" at 10. At baseline, the vitamin D-treated group assessed QoL as 5.5 (±2) and controls as 4.1 (±2), and the difference in QoL between groups (p=0,02) was significant. After four weeks this difference had disappeared, due to a small but significant increase in QoL in the vitamin treated patients (-1.4, CI -2.6- (-0.21)). When assessed again after twelve weeks, QoL remained stable in both groups (Fig 10 B, p 50).

The significant result regarding HRQoL from study II encouraged us to include this PROM as a secondary outcome in study III as well. We decided to add EORTC QLQ-C15-PAL as an assessment tool, since this is an instrument specifically used cancer patients that has also been validated in Swedish (in contrast to the assessment of HRQoL using ESAS). In study III, QoL was regularly assessed with the ESAS instrument, but also with EORTC QLQ-C15-PAL at screening and after 12 weeks. In EORTC QLQ-C15-PAL the scale is reversed compared to ESAS, with the verbal anchor "very poor" at one and "excellent" at seven on a seven-grade numeric scale. There was no significant difference between groups at baseline using either instrument (median ESAS score 4 (IQR 2-6), median EORTC QLQ-15-PAL 4 (3-5)), and neither (but very close to) was there a significant change in QoL from baseline to last follow-up at twelve weeks.

4.7.2 Fatigue (Study III)

In study III, we added fatigue as an outcome measure. While planning our study, positive results on fatigue after vitamin D supplementation in an observational study in similar patient cohort had been observed in a clinical trial (122). We were curious as to whether this result could be reproduced in a randomized controlled trial. We also hypothesized that change in opioid dose and in frequency and severity of infections might influence assessment of fatigue more than the broader construct of global HRQoL.

To assess fatigue, we used 1. the "tiredness-question" from ESAS, catching the patients feeling of fatigue at the moment, and 2. Question 11 from EORTC QLQ-C15-PAL asking the

patient to describe the level of tiredness during the past week. This deviates from the EORTC scoring manual, which states that fatigue is a construct of question 7 and question 11.

At baseline, there was no difference between groups regarding median levels of fatigue, irrespective of instrument. After twelve weeks, there was a significant difference in mean change in fatigue from baseline between vitamin D-supplemented patients and the placebo group, with lower fatigue scores in supplemented patients (– 1,12 (CI -1,86 – (-0,36)), when fatigue was assessed with ESAS.

Study III was not powered for subgroup analyses, but we did an exploratory analysis of sex differences. This analysis showed that vitamin D supplementation reduced fatigue in men ($p<0.001$) but not in women. No difference in mean change of scores from baseline was seen when fatigue was assessed with EORTC QLQ-C15-PAL. However, the choice of using only Q11 for assessing fatigue is in concordance with our recently published study on fatigue in the baseline data from the Palliative-D cohort (111).

4.7.3 Results in context, methodological considerations and future perspectives

4.7.3.1 Fatigue.

The result in study III regarding fatigue is in accordance with the positive association between lower levels of 25-OHD and more severe fatigue found in baseline material from the entire screening-cohort (111). So is the difference in effect in men and in women.

There are several possible explanations for the effect of vitamin D supplementation on fatigue: a lower opioid dose may contribute, but so may a less pronounced inflammatory immune response (69, 71, 73). A Cochrane analysis of evidence-based interventions to reduce fatigue in palliative care published in 2015 concluded that so far, evidence for pharmacological interventions was weak (176). Two study protocols for RCTss studying the effect of vitamin D supplementation on fatigue in cancer patients as a primary endpoint have been published, but results are yet to be presented (141, 142).

4.7.3.2 HRQoL

We did not see an effect on QoL, which is understandable since effect on fatigue was small, and since QoL is a larger construct.

4.7.3.3 Using two different instruments in study III made our results more difficult to interpret. Could we have handled this differently?

ESAS: In an attempt to adhere as closely as possible to regular care practices, and to be able to compare result from study II and study III with earlier trials at our facility, we chose ESAS as symptom measurement instrument, and decided to use QoL-assessment on this eleven-point numerical scale in our assessment of QoL in study II and III. This decision was made although measurement of QoL using this method is not well established in literature. We believe that there is a historical background for the present use of QoL measurement with

ESAS in our facility, since assessment of QoL on a visual analogue scale (VAS-scale) in advanced cancer patients method has been used in two previous studies in Sweden (177, 178).

1. In small Swedish cohort (n=35) of patients with advanced or metastatic cancer, ESAS total score and ESAS assessments for single symptoms were correlated to QoL on a visual analogue scale (VAS-scale). There were significant correlations for all parameters, indicating that the most distressing symptom affected assessed QoL. The authors argue that a simple VAS for QoL may be adequate in clinical routine in palliative care, since assessment tools have to be kept simple in patients with low performance status (177).
2. In a cohort of more than 400 palliative cancer patients, ESAS and VAS-QoL were used together. In this study, well-being correlated with Symptom Distress Score (combined score of separate scale items), and authors argue that "well-being" was a better word to use compared to QoL (178).

EORTC-QLQ-C15-PAL: In study III, we added EORTCQLQ-C15-PAL as an instrument to assess fatigue and QoL, since this a disease-specific, widely used instrument in cancer patients that has also been validated in Swedish cohorts. Using a validated instrument would make our results easier to compare with other experiences.

Correlation between ESAS and EORTC QLQ 15-PAL

The two assessment instruments cover different time frames – ESAS offers a snapshot of current symptom burden and EORTC QLQ1-C5-PAL allows the patient to review intensity of symptoms and QoL during the past week. When writing the trial protocol for study III in 2016, we were not aware of any direct comparisons between instruments. Since then, two studies on this subject have been published (179, 180).

In a Norwegian cohort of n=54 cancer patients receiving palliative care, recruited from an outpatient oncology clinic, the test-retest stability in assessments was higher in EORTC QLQ-C15-PAL compared to ESAS (180). In this study, median score of "feeling of well-being" assessed with ESAS was 1 (IQR 0-3) and median score of EORTC QLQ-C15-PAL QoL 5 (IQR 4-6). At inclusion, fatigue assessed with "the tiredness-question" in ESAS was 2 (IQR 0-5), corresponding with "a little" or "2" (IQR2-3) in EORTC QLQ-C15-PAL (180).

In a Croatian study from 2018, there were however significant correlations between corresponding items in the two instruments in an inpatient hospice setting (179).

Conclusion and future perspectives

Given our clinical setting, where more frequent assessment using EORTC QLQ-C15-PAL would have posed a practical problem, I think we would have ended up planning the study similarly today. However, a post-hoc analysis comparing the instruments in our relatively large cohort would be of interest. In this analysis it would also be interesting to see if there is

a difference in how "feeling of well-being" and QoL assessed with ESAS correspond with QoL assessed with EORTC-QLQ-C15- PAL.

4.8 OPTIMIZING CLINICAL TRIALS IN PALLIATIVE MEDICINE – LESSONS LEARNED FROM STUDY III

As outlined in the introduction to the Results-section in this thesis, a clinical researcher in palliative care faces several methodological challenges: high attrition rates and low inclusion rates, as well as difficulties in statistical interpretation of results are some of them. The heterogeneity in palliative care cohorts also creates difficulties regarding results internal validity (to infer cause and effect), generalizability and applicability of results. In palliative care facilities with few and infrequent clinical studies, lack of research infrastructure and familiarity with research methods may pose problems.

4.8.1 Internal validity and generalizability

Research in Palliative medicine has some similarities with research in public health interventions. Rychetnik discusses three dimensions that evidence needs to respond to: 1. strength of evidence (study design, methodological quality, and statistical precision), 2. the magnitude of measured effect and the relevance of measured effect to the implementation context and 3. transferability of results, since outcomes in trials depend on social, organizational and political settings (181). This is true for research in palliative care as well and might partly explain why some positive results regarding palliative care interventions have been difficult to reproduce in other settings (182).

The problem of generalizability in palliative care research is discussed in detail by Currow et al (183). Most importantly, clinical terminology in palliative care is not standardized. One example is the term "hospice", that describes different types of care in the United States compared to Europe. Further, heterogenous cohorts regarding both diagnoses and different contexts due to cultural and organizational differences make it difficult to know if an intervention in one setting could be applied with similar results in another. The authors propose that five domains are described in a systematical form when planning and reporting clinical trials in a palliative care setting: Professional, service, national/state health, social policy, and research factors (183).

4.8.2 Lessons learned in other palliative care research settings

A direct approach to help researchers optimize clinical trials in palliative care was put forward by The Australian Palliative Care Clinical Studies Collaborative. They published their reflections on trials in palliative care after a national workshop where fourteen clinical trials (12 RCTs) were presented, (184). Their reflections adhere well to our experience.

4.8.3 What did we do well, and what could we have done better regarding study III?

In Table 4, I have tried to synthesize lessons learnt from other palliative care researchers with our experiences in study III (157, 183, 184). The table is adapted from The Australian Palliative Care Clinical Studies Collaborative (184), with some issues that are not of importance in our setting deleted and issues such as analysis plan and standardized reporting added.

A strength in study III is the comprehensiveness of data, with very little missing data for individual participants until they reached end of intervention or were excluded due to deterioration. This, we argue, adds to internal validity. So does the fact that patients were accrued in the same region, with similar use of both pain medication and antibiotics in the three participating HPCs. The fact that we included patients with all types of cancer, as well as patients with varying remaining lifespan, renders good generalizability regarding our results in palliative care cancer patients. The fact that we have not collected data on socioeconomic factors, however, makes generalizability more difficult. A post-hoc analysis could help us with this.

Oncological treatment effects may bias our results and have a negative effect on internal validity, but randomization should have compensated for this. A post-hoc analysis with in-depth characterization of oncological treatment can help us address this issue. Further, we have not considered use of other types of pain medication than opioids. Regarding this issue as well, we hope that randomization has managed to balance groups.

Table 4. Study III in comparison with suggested conduction of RCTs in palliative care

Study design	Proposed success factors/best practice	Study III		Comment
Protocol	Minimize burden on participants and clinical staff		✓	Excellent cooperation with team nurses
	Align protocol with standard clinical practice	ESAS, assessment at regular nurse visits	✓	Flexible assessment +/- 7 days proved to be a success factor
Participants	Keep inclusion and exclusion criteria as broad as possible		✓	Intake of a small amount of vitamin D was allowed after amendment
	Ensure eligibility criteria can be applied uniformly across sites		✓	
Sample size	Allow for an attrition rate of 25-40% (mostly unrelated to the intervention in trial)	Attrition rate of > 50% in treatment and >30% in placebo arm	✗	Due to high drop-out rate + Covid pandemic that stopped inclusion early
Baseline	Include sociodemographic data		✗	Can be added in a post-hoc analysis
	Standardized physical functioning assessment	Not included in protocol	✗	Would have increased generalizability
Outcome measurement	Time primary endpoints to occur as soon as clinical benefit is likely	3 month follow up, based on evidence from previous trials	✓	
Analysis plan	Modified ITT	According to analysis plan: both ITT and per protocol analysis	✓	An independent monitoring committee would have been wise.
Study conduct	Work with ethics committees		✓	Constructive cooperation with ethics committee regarding written patient information
	Provide support and coordination from a central office	Karolinska Trial Alliance were contracted for help with initial study protocol and biobank	✓	More regional expertise and cooperation regarding palliative are trials would be beneficial in future trials
	Initiate studies as soon as practicable after ethics approval	We started as soon as approvals and study drug were available.	✓	
	Develop links with all disciplines able to refer	We planned for recruitment of patients through the patient's responsible physician.	✓	Could not be generally implemented. Instead, the study team approached patients after consulting with the responsible physician.
	Promote routine screening of inclusion criteria	Initially, we tried to promote screening of patients when they were admitted to palliative care.	✓	This sometimes worked, but not always. Instead, the study team screened all admitted patients regularly
Reporting of study	Detailed and standardized information on the palliative care service provided		✓	We have included this in the supplementary material of the submitted manuscript

5 CONCLUSION

In conclusion, the papers in this thesis

- Support the notion that antibiotic use in end-of-life relieves symptoms, most notably symptoms of sepsis, and gives few side effects.
- Show that vitamin D may reduce opioid use and fatigue in cancer patients in a palliative setting. The effect size is small but clinically relevant, and may be underestimated due to metodological issues.
- Show that vitamin D in doses of 4000 IU/day are safe and well tolerable in this cohort or severely diseased cancer patients.
- Show that vitamin D oil drops are equally effective as powdered tablets in raising 25-OHD, but that further research is warranted to investigate if these two types of preparations have different effects on antibiotic use or not.
- Show that large RCTs can be conducted in palliative care

6 FUTURE RESEARCH

6.1 POST HOC ANALYSES

The material from study III that warrants further study, and we have identified several post-hoc analyses that would be interesting to perform, some of them already addressed in section 4

- An analysis of seasonal variation of 25-OHD baseline levels and 25-OHD after-intervention levels.
- An analysis of oncologic treatment during the interventions period in relation to outcome.
- A subgroup analysis only comprising patients who were prescribed opioids at baseline.
- A subgroup analysis of antibiotic use in patients who were prescribed antibiotics during the last 30 days prior to study-inclusion
- An analysis of effect of vitamin D supplementation on outcome meassues in relation to survival (0-3, 3-6, > 6 months).
- An analysis of association between socioeconomic factors and outcome measures.

These post-hoc analyses would all be conducted in smaller cohorts, and thus hypothesis-generating.

Caritha Klasson is also further investigating sex differences in fatigue and QoL in association with 25-OHD based on data from study III, and is conducting a qualitative study on the perceptions of professionals and patients regarding participation in an RCT in palliative care as part of her thesis project.

Further, a comparison of ESAS and EORTC QLQ-C15-PAL in this cohort would be interesting.

6.2 IDEAS FOR FURTHER STUDIES IN THE FIELD

The results from study II and III also encourages further exploration of the effect of vitamin D supplementation to cancer patients. A new RCT that includes only patients on opioids, preferably recruited already at the oncology unit, would hopefully help us better answer questions regarding the role of vitamin D in cancer pain. Further, pilot studies on more specific groups of cancer patients could identify new target populations. Could correction of vitamin D deficiency help patients with side effects of curative radiotherapy, for example.

To conclude, the results from this thesis suggest that correction of vitamin D deficiency may be of benefit to cancer patients in Palliative Care, that the treatment is safe and well-tolerable and the field warrant further research in the coming years.

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