DISSERTATION

Diagnosis of Vitamin D Deficiency in Critical Care

submitted by

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DECLARATION

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz".

May 16th, 2020 Dr.med.univ. Paul Zajic eh.

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Paul Zajic and Karin Amrein originally designed the study; Stefan Heschl, Petra Srekl-Filzmaier, Michael Schörghuber, Viktoria Weixler and Paul Zajic performed patient screening, information and inclusion; Sieglinde Zelzer performed laboratory measurements; Tatjana Stojakovic provided support in laboratory data extrication; Paul Zajic performed statistical analyses; Paul Zajic wrote the manuscript. All authors approved of the publication ahead of submission and gave consent to the use of their contributions in the above publication and this dissertation.

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ABBREVIATIONS AND DEFINITIONS

1α,25(OH)$_2$D.....1α,25-dihydroxy-vitamin D_3

25(OH)D.....25-hydroxy-vitamin D_3

ASA.....American Society of Anesthesiologists

AVR.....aortic valve replacement

CABG.....coronary artery bypass grafting

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CCU.....critical care unit

CICU.....cardiac intensive care unit

CPB.....cardio-pulmonary bypass

ECLIA.....electrochemiluminescence assay

EDTA.....ethylenediaminetetraacetic acid

FGF23.....fibroblast growth factor 23

HDU.....high dependency unit

HIV.....human immune-deficiency virus

ICU.....intensive care unit

IMC.....intermediate care unit

IQR.....interquartile range

ITU.....intensive treatment unit

IUPAC.....International Union of Pure and Applied Chemistry

LoB.....limit of blank

LoD.....limit of detection

LoQ.....limit of quantitiation

MICU.....medical intensive care unit

NICU.....neonatal intensive care unit

PACU.....post-anaesthesia care unit

PTH.....parathyroid hormone

UV-B.....ultraviolet B light

VDBP.....vitamin D binding protein

VDR.....vitamin D receptor

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ZUSAMMENFASSUNG AUF DEUTSCH

Einführung: Ein Vitamin D-Mangel kommt oft vor bei kritisch kranken Patientinnen und Patienten, jedoch gibt es unzureichende Daten, wie akute Erkrankungen und deren Behandlung Vitamin D-Spiegel beeinflussen. Diese Studie untersuchte den Einfluss perioperativer Flüssigkeitsgabe und Inflammation auf 25-Hydroxy-Vitamin D-Werte [25(OH)D] und 1,25-Dihydroxy-Vitamin D-Werte [$1,25(OH)_2D$] mittels eines rasch verfügbaren Chemilumineszenz-Assays (ECLIA, IDS-iSYS) und der Flüssigchromatographie mit Massenspektrometrie-Kopplung (LCS-MS/MS) zur Diagnose des Vitamin D-Mangels perioperativ und während der Intensivtherapie.

Material und Methoden: Es wurde eine prospektive Pilot-Beobachtungsstudie bei erwachsenen Patientinnen und Patienten, welche sich herzchirurgischen Eingriffen an Herz-Lungen-Maschinen (HLM) unterzogen, durchgeführt. Präoperativ (t1), nach dem Abgehen von der HLM (t2) und bei der Aufnahme an der Intensivstation (t3) gewonnene Blutproben wurden in einer „perioperativen" Kohorte analysiert, weitere Proben vom ersten (t4) und zweiten (t5) postoperativen Tag in der „intensivmedizinischen" Kohorte.

Ergebnisse – Resultate: 66 Patientinnen und Patienten wurden in die perioperative Kohorte eingeschlossen, 26 davon in die intensivmedizinische. Flüssigkeitsladung durch die HLM führte zu einer medianen Reduktion von 25(OH)D um 23% ($p<0.001$) zwischen t1 und t2 mit langsamer Erholung bis t5, während $1,25(OH)_2D$-Spiegel um etwa 55% ($p<0.001$) fielen, ohne Tendenz zur Rückkehr zum Ausgang. Der mittlere Unterschied zwischen den 25(OH)D-Messungen durch ECLIA und LC-MS/MS war 4.8 ng/ml (±5,7). Pearson's Korrelations-koeffizient für diese Messwerte war 0.73 ($p<0,001$). LC-MS/MS-Messergebnisse wurden nicht durch das inaktive C3-Epimer beeinflusst.

Diskussion: Vitamin D kann als „negatives Akut-Phase-Reagens" angesehen werden; die Serum-Messwerte werden signifikant durch Flüssigkeitsgabe und Inflammation beeinflusst. 25(OH)D-Messungen durch Chemilumineszenz-Assays können merklich von denen durch LC-MS/MS abweichen; diese kann dagegen als der Goldstandard in der Diagnose in diesem Patientinnen- und Patientenkollektiv angesehen werden. Striktere Definitionen des Vitamin D-Mangels als in der Allgemeinbevölkerung, z.B. 25(OH)D-Werte kleiner als 12 ng/ml, könnten besser geeignet sein, um Vitamin D-Mangel mit niedriger Falsch-Positiv-Rate bei kritisch Kranken zu diagnostizieren.

ABSTRACT IN ENGLISH

Introduction: Vitamin D deficiency is common in critically ill patients; yet there is uncertainty how acute illness and its treatment affect levels and how vitamin D should best be measured in critically ill patients. This study therefore sought to assess the influence of fluid loading and inflammation on 25-hydroxy-vitamin D [25(OH)D] and 1,25-diyhydroxy-vitamin D [1,25$(OH)_2$D] levels using a readily available chemiluminescence assay (ECLIA, IDS-iSYS) and the presumed gold standard of liquid chromatography / mass spectrometry (LC-MS/MS) for diagnosis of vitamin D deficiency perioperatively and during intensive care.

Materials and Methods: A prospective, observational pilot study in adult patients undergoing cardiovascular surgery on cardiopulmonary bypass (CPB) was conducted. Blood samples drawn at preoperative baseline (t1), after weaning from CPB (t2) and at intensive care unit (ICU) admission (t3) were analysed in a "perioperative phase" cohort, further samples were collected on the first (t4) and second (t5) postoperative day and analysed in a "critical care phase" patient cohort.

Results – Findings: 66 patients were included in the "perioperative phase", 26 of these were included in the "critical care phase". Fluid loading by CPB led to a median 25(OH)D reduction of 23% ($p<0.001$) between t1 and t2 with recovery towards t5, while 1,25$(OH)_2$D levels were reduced by 55% ($p<0.001$) and did not recover during the observation period. Mean difference between 25(OH)D measurements by ECLIA and LC-MS/MS was 4.8 ng/ml (±5.7). Pearson's r for correlation of these measurements was 0.73 ($p<0.001$). LC-MS/MS results for 25(OH)D were not influenced by the inactive C3-epimer.

Discussion: Vitamin D can indeed be considered a "negative acute-phase reactant"; its levels are significantly altered by fluid loading and inflammation. 25(OH)D measurements using chemiluminescence assays may be notably different from LC-MS/MS, which can be considered the gold standard for measurements in this patient group. Stricter definitions of vitamin D deficiency than in the normal population, such as serum levels of 25(OH)D below 12 ng/ml, may be better suited for the diagnosis of vitamin D deficiency in critically ill patients with a low false-positive rate.

1 INTRODUCTION

1.1 Background on vitamin D

Vitamin D is the term used both colloquially and scientifically to describe several similar steroid compounds of great relevance to almost all mammals, especially humans. Although vitamin D is the denominator most commonly used ever since the conception of the term "vitamin", it is not an adequate representation of the biochemical properties and physiological actions of these substances.

The concept of a "vitamin" in general and the term itself have been conceived in the early 20th century. This has happened following the isolation of substances that have been shown to cure previously incurable diseases thought to be caused by deficiencies in the uptake and availability of substances that the human body could not produce on its own. The Polish scientist Casimir Funk has first proposed the idea of a vitamin, a word he has derived from the Latin word *vita*, meaning life, and *amine*, a chemical structure derived from ammonia (NH_3) he believed to be present in all of these vitally important substances [2].

Vitamin D meets none of the aforementioned criteria. It is neither non-producible by the human body nor is it an amine. These shortcomings have been already known to scientists of their time. Most notably, the American biochemist Elmer McCollum has suggested not to use the term "vitamin" and instead has proposed a classification of (at the time) unknown water-soluble and fat-soluble substances following the Roman alphabet [3]. The term vitamin has prevailed nevertheless; it has simply been merged with this alphabetical classification.

Elmer McCollum himself found proof of the existence of the substance later to be called vitamin D in experiments with rats suffering from rickets [4]. The letter D was chosen simply because the compound found was the fourth so-called vitamin classified at the time [5]. Its biochemical properties, synthesis and metabolism as well as known physiological actions are described in the following sections to provide sufficient background information.

1.1.1 Biochemical structure and properties

Vitamin D encompasses a group of fat-soluble substances that are of *secosteroid* structure. These structures are derivates of the basic steroid structure (**Figure 1**), in which one of the basic

ring structures has been broken (the Latin word *secare* literally means to cut). In vitamin D, bonds of the B-ring are broken.

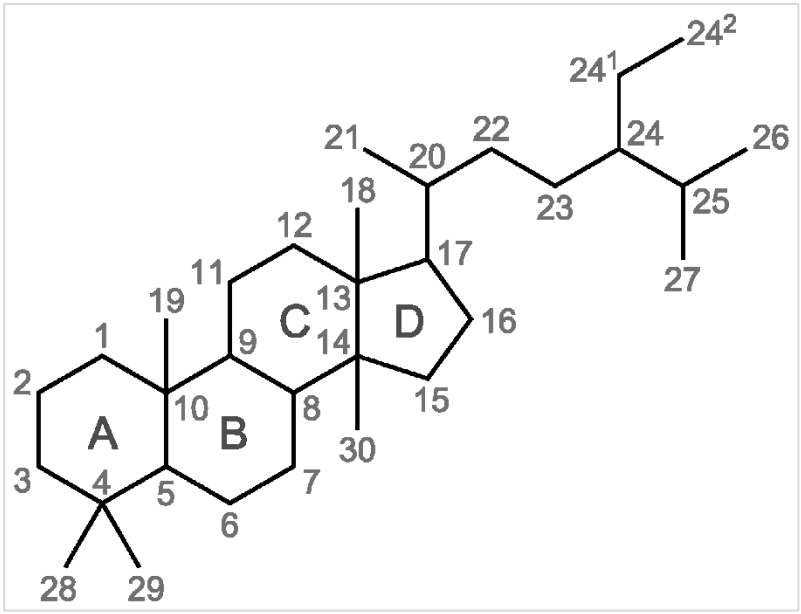

Figure 1 Basic structure of steroids consisting of three six-member cyclohexane rings (rings A, B and C) and one five-member cyclopentane ring (ring D). Lettering and numbering according to the International Union of Pure and Applied Chemistry (IUPAC). Image in the public domain.

Vitamin D exists in several forms. Generally speaking, the name "vitamin D" refers to all substances in this vitamin group that confer similar biological actions; specific subtypes are identified by subscript numbering (**Table 1**). In humans, cholecalciferol (vitamin D_3) is physiologically produced; "vitamin D", "vitamin D_3" and "cholecalciferol" will thus be used exchangeably within this dissertation.

Name	Chemical compounds
Vitamin D_1	ergocalciferol + lumisterol, 1:1 mixture
Vitamin D_2	ergocalciferol
Vitamin D_3	cholecalciferol
Vitamin D_4	22-dihydroergocalciferol
Vitamin D_5	sitocalciferol

Table 1 Classification of vitamin D subtypes and corresponding chemical compounds

1.1.2 Biosynthesis, metabolism and breakdown

Vitamin D is synthesised primarily in the skin in all mammals including humans [6]. The basic compound 7-dehydrocholesterol is photolyzed by ultraviolet-B light (UV-B light) during exposure to sunlight [7]. The resulting compound, precholecalciferol (previtamin D_3) is thermodynamically unstable and thus undergoes spontaneous isomerisation to cholecalciferol (vitamin D_3). Vitamin D is bound to its transport protein, vitamin D binding protein (VDBP), in the blood for distribution within the body (**Figure 2**).

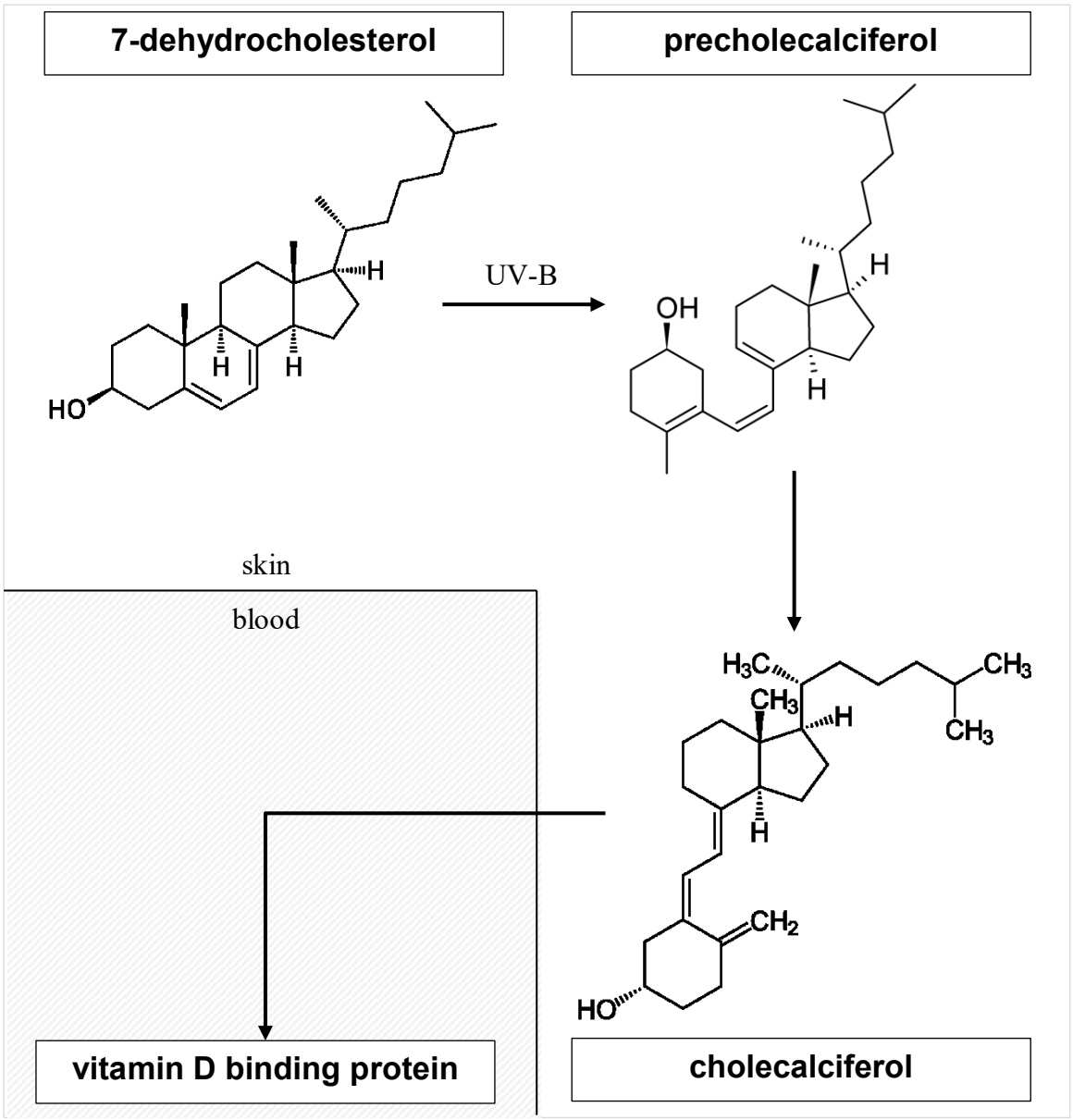

Figure 2 Vitamin D synthesis pathway from 7-dehydrocholesterol to cholecalciferol in human skin and transportation by Vitamin D Binding Protein (VDBP). Images in the public domain.

Vitamin D_3 in itself is not biologically active. After binding to VDBP, it is transported mainly to the liver where it is hydroxylated to 25-hydroxycholecalciferol (calcifediol, simplified as 25(OH)D in this dissertation) under the influence of cytochrome P450 (CYP) 2R1 (or cholecalciferol 25-hydroxylase) [8]. 25(OH)D is a prehormone still and mainly relevant as a storage form of vitamin D in the human body (**Figure 3**).

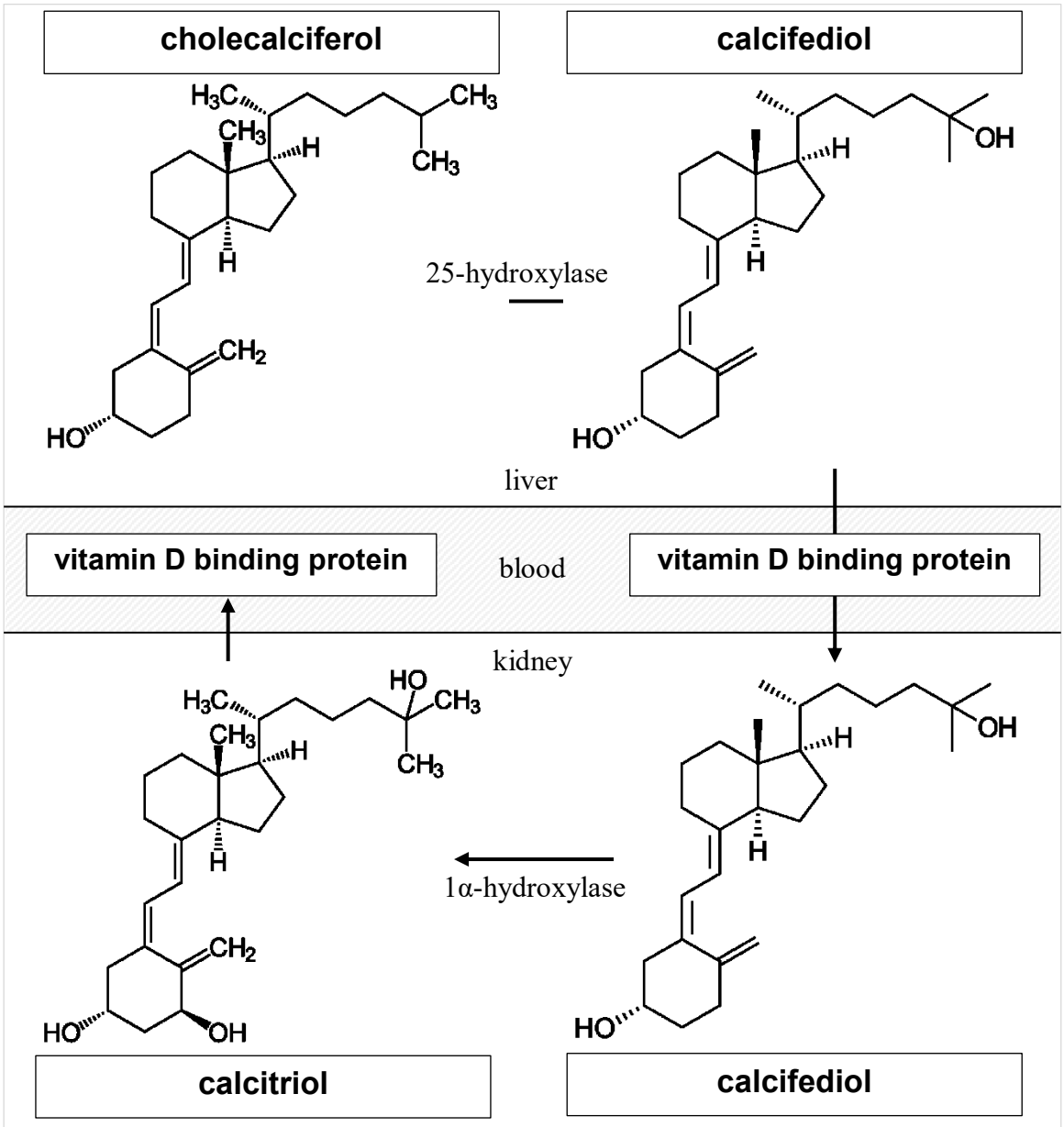

Figure 3 Vitamin D hydroxylation pathway; upper part: 25-hydroxylation from cholecalciferol to calcifediol, lower part: 1-alpha-hydroxylation from calcifediol to calcitriol. Images in the public domain.

It is only after a further hydroxylation that 1,25-dihydroxycholecalciferol (calcitriol, simplified as $1,25(OH)_2D$ in this dissertation) is produced under the action of CYP 27B1 (or 25-hydroxycholecalciferol 1-alpha-hydroxylase) [8]. This reaction occurs mostly in the kidneys, although other cells have been found to express 1-alpha-hydroxylase and are therefore capable of producing calcitriol [9]. Both 25(OH)D and $1,25(OH)_2D$ are also bound to VDBP for transportation purposes in the blood (**Figure 3**).

Both 25(OH)D and $1,25(OH)_2D$ can be further hydroxylated in position 24 via the action of CYP24A1 (or 24-hydroxlase). This mechanism most likely serves to avoid the accumulation of calcifediol and calcitriol and thus avoid toxicity. However, the resulting compounds – $24,25(OH)_2D$ and $1,24,25(OH)_3D$ – have affinity for the vitamin D receptor and may be relevant for the overall function of the vitamin D hormone system [10].

Other breakdown pathways can lead to epimerisation of 25(OH)D and $1,25(OH)_2D$ [11]. These epimers may amount to a significant proportion of overall vitamin D concentrations in humans, although conflicting results were presented in different age groups and various physiological states [12–14]. While $1\alpha,25(OH)_2$-3-epi-vitamin D_3 is a potent suppressor of PTH secretion and can therefore be considered biologically active [15,16], the physiological role of 25(OH)-3-epi-vitamin D_3 is remains uncertain [17].

1.1.3 Physiological action and regulation

Actions of the steroid hormone vitamin D are mediated by a trans-acting transcriptional regulatory factor similar to steroid and thyroid hormone receptors called vitamin D receptor (VDR) [18]. VDRs are expressed in almost all organs of the human body [19]. $1,25(OH)_2D$ is the most effective ligand at this receptor and is therefore considered the compound that actually conveys the physiologic action of the vitamin D system.

The best and longest known actions of vitamin D – now usually referred to as "classical actions" – concern calcium homeostasis and bone health [20]. VDR activation by $1,25(OH)_2D$ leads to increased intestinal calcium absorption, increased calcium reabsorption in the kidneys and increased bone resorption. The latter two functions require parathyroid hormone (PTH) as a co-factor, which in turn up-regulates calcitriol formation. As a negative-feedback loop, higher levels of circulating $1,25(OH)_2D$ suppress PTH synthesis in the parathyroid glands [21].

Research over the last decade has unravelled several more effects of vitamin D, which by in large can be explained by the ubiquitous presence of the VDR in most human body tissues. These effects that extend beyond calcium homeostasis and bone metabolism are nowadays usually referred to as “non-classical actions” [22]. These non-classical effects can be further – albeit somewhat artificially - categorised into regulation of hormone secretion, regulation of immune function and regulation of cellular proliferation and differentiation [23].

As a regulator of hormone secretion, vitamin D has its known effects on PTH secretion as described previously. Furthermore, $1,25(OH)_2D$ stimulates the secretion of insulin in pancreatic ß-cells, although the underlying mechanism is not yet fully understood [24,25]. Vitamin D also stimulates the production of FGF23 (fibroblast growth factor 23) in osteoblasts and osteocytes, again following an incompletely understood pathway [26,27].

FGF23 itself has profound effects on renal tubular reabsorption of phosphate independent of PTH and can in turn reduce circulating levels of 1, $25(OH)_2D$ [28]. In patients suffering from end-stage renal disease, FGF23 levels have been found to be closely correlated with mortality [29–31]. However, vitamin D supplementation has not convincingly been shown to alter FGF23 levels in vivo [32].

Vitamin D is a regulator of both innate and adaptive immune function. Human leukocytes express VDRs as binding sites for calcitriol [33]; activated macrophages express 1-alpha-hydroxylase and are therefore capable of producing calcitriol themselves [34]. Vitamin D moderates innate immune function by inducing the synthesis of cathelicidin, an antimicrobially active peptide [35–37]. In the adaptive immune system, $1,25(OH)_2D$ can inhibit both B-cell differentiation [38] and T-cell proliferation [39].

The effects of vitamin D on cellular proliferation and differentiation are most prominent in the skin. Not only does vitamin D synthesis take place in epithelial cells, keratinocytes are also capable of metabolism to its active form [40]. Vitamin D modulates differentiation proliferation of keratinocytes [41] and hair follicle cycling [42]. These postulated antiproliferative and prodifferentiating effects of vitamin D probably extend to other cell types as well; modulatory effects of vitamin D have been implicated in the expression of cell-cycle inhibitors [43] and adhesion molecules [44,45].

1.2 Assessment of vitamin D status and deficiency states

Taking into account the plethora of effects vitamin D metabolites exert physiologically, it appears obvious that an adequate vitamin D status is vital for the normal function of several organs and processes within the human body. A lack in vitamin D stores (hypovitaminosis D) may convey several pathophysiologic sequelae [46] (**Figure 4**).

1.2.1 Unfavourable effects of vitamin D deficiency

Diseases due to inadequate vitamin D status have long been known, even before vitamin D had been isolated and described as a compound. Failure to exert its "classical" actions leads to severe disturbance of calcium and phosphate metabolism and therefore to severe perturbance of bone health, termed "rickets" in children and "osteomalacia" in adults [47].

This is due to insufficient uptake of both calcium and phosphorus in the intestines and subsequently ensuing hyperparathyroidism [18]. Besides increased reabsorption of calcium in the renal tubular system and stimulation of renal $1,25(OH)_2D$ synthesis, PTH mediates osteoblast activation. Activated osteoblasts propagate the transformation of pre-osteoclasts into osteoclasts, which dissolve the bone's collagen matrix. The clinical correlate is rickets in infants and children [48,49] and osteomalacia in adults [50,51].

Insufficient vitamin D status also contributes to osteoporosis and fragility fractures [52,53]. It may also be associated with general muscle weakness [54,55] and an increased incidence of falls [56,57], especially in older people, thereby further increasing the risk of fractures.

Higher vitamin D intake is negatively associated with the development of type 1 diabetes [58,59]. Similarly, vitamin D levels may also have a role in glycaemic control and the development of type 2 diabetes [60]. However, randomised controlled trials up to this date have failed to demonstrate beneficial effects of vitamin D supplementation on glycaemic control, insulin resistance or prevention of type 2 diabetes in general [61,62].

Inadequate vitamin D status is associated with increased risks of acquiring seasonal viral infections and may be effective in their prevention. [63–66]. This notion has most recently been supported by a systematic review and meta-analysis of individual participant data on vitamin D supplementation for the prevention of acute respiratory tract infections [67]. In 10,933 individuals, vitamin D supplementation has been found to significantly reduce the risk of

respiratory infections (adjusted OR 0.88, 95% CI 0.81 - 0.96), with the strongest effect in severe vitamin D deficiency.

Hypovitaminosis D may also be associated with severe infections; patients infected with human immune deficiency virus (HIV) [68,69] as well as well as severe bacterial infections including tuberculosis [70,71] were found to more often have inadequate vitamin D levels than healthy subjects.

An association between the prevalence of allergies, especially in infants and children, and vitamin D intake and home latitude has also been described in several studies [72–74]. This effect is however doubted by some authors and has not been uniformly reproduced in all studies [75]. Vitamin D deficiency has also been implicated in other autoimmune diseases such as systemic lupus erythematosus (SLE) [76,77] and rheumatoid arthritis [78].

Due to its modulatory effects on gene expression, vitamin D status has repeatedly been linked to the occurrence of malignancies. There is now plenty of evidence to support the hypothesis that low vitamin D levels are associated with colorectal cancer and colorectal adenomas [79,80], some evidence for an association with breast cancer [81,82] and suggestions for such a link with prostate cancer [83,84]. Increasing vitamin D intake may reduce the occurrence of these cancers [85], although the evidence is conflicting [86].

Vitamin D deficiency may also affect the cardiovascular system. Several studies associated inadequate vitamin D status with cardiovascular diseases such as arterial hypertension [87], coronary artery disease [88] and heart failure [89]. Moreover, mortality due to major cardiovascular events [89,90], myocardial infarction [91] and stroke [92] has been demonstrated to be higher in patients with low vitamin D levels compared to those with higher levels. Prospective randomised controlled trials and meta-analyses of these have repeatedly failed to demonstrate benefit of vitamin D supplementation for these endpoints [93–96].

Diseases of the central nervous system may also be a consequence of insufficient vitamin D status. First and foremost, the development of multiple sclerosis (MS) has been associated with hypovitaminosis D [97,98]. Similar associations have been demonstrated for psychiatric disorders, especially schizophrenia [99].

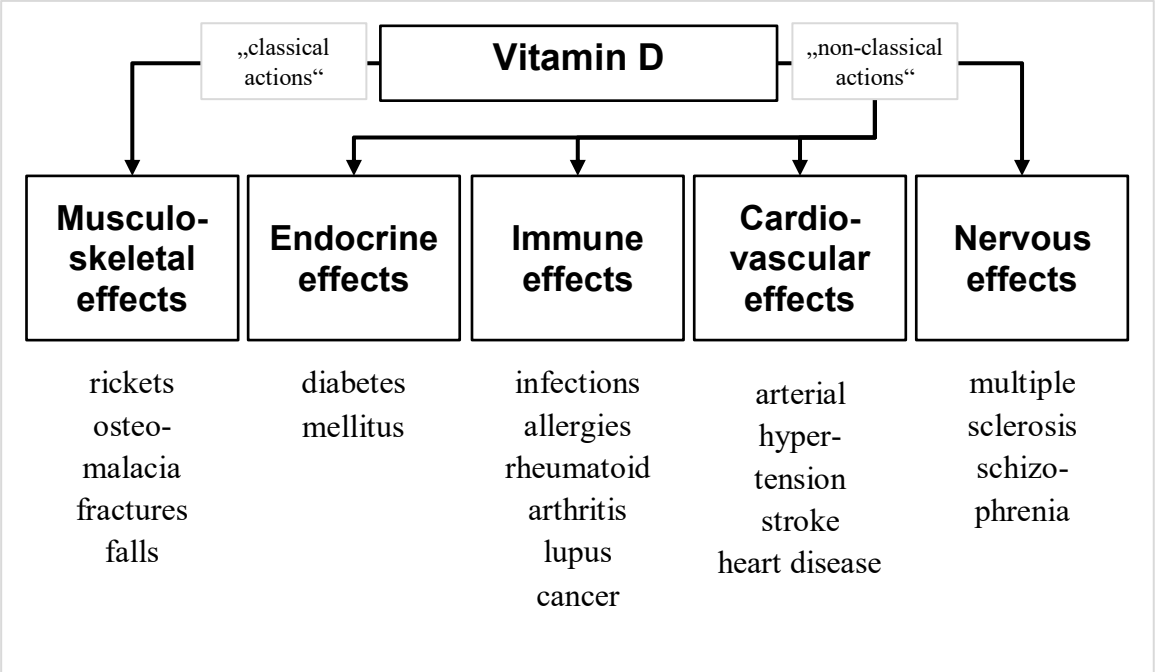

Figure 4 Postulated unfavourable effects of insufficient vitamin D status (hypovitaminosis D).

Taken together it is evident that an adequate vitamin D status is important for overall health. This is reflected in findings that hypovitaminosis D by and large is associated with increased risk of all-cause mortality in various large patient cohorts [100–102].

It remains unclear, however, whether vitamin D supplementation can influence risk of mortality in the general population: a Cochrane Systematic Review of 56 randomised trials with 95,286 participants published in 2014 has concluded that vitamin D supplementation can decrease mortality (RR 0.97, 95% CI 0.94 - 0.99, p=0.02, I^2=0%) [103], while such a benefit has not been demonstrated in a meta-analysis of 52 trials including 75,454 participants from 2019 (RR 0.98, 95% CI 0.95 - 1.02, I^2=0%) [104].

Exact assessment of vitamin D status seem paramount, as interventions are more likely to be beneficial when applied to patients with more severe deficiency states [105].

1.2.2 Definition and classification of vitamin D status

Because of its rather complex biosynthesis and metabolism in the body, assessment of vitamin D status is not straightforward. While $1,25(OH)_2D$ represents the actually biologically active metabolite of the vitamin D system, its measurement is believed to add little to the

clinical assessment of vitamin D status [46] due to its short half-life of only a few hours and its regulation within a narrow range [106].

By convention, 25(OH)D is measured in the serum to assess vitamin D status, as it is better suited to represent body storages of vitamin D than calcitriol levels do [46]. Although 25(OH)D measurements have found their way into clinical practice guidelines for various disease states, a definitive consensus on what constitutes adequate vitamin D status, terminology of different stages of vitamin D status and adequate cut-off values for 25(OH)D does not exist to this day.

This uncertainty is exemplified in **Table 2**, **Table 3** and **Table 4** by various proposed categories of vitamin D status and their corresponding cut-off values:

Category	**25(OH)D [nmol/l]**	**25(OH)D [ng/ml]**
Deficiency	<25	<10
Insufficiency	25 – 49	10.0 – 19.9
Hypovitaminosis D / suboptimal supply	50.0 - 74.9	20.0 – 29.9
Adequacy	75 - 372	30 – 149
Intoxication	>372	>149

Table 2 Categories of vitamin D status and their corresponding 25(OH)D cut-off values as proposed by Zittermann et al. in ref. [107]

Category	**25(OH)D [nmol/L]**	**25(OH)D [ng/ml]**
Deficiency	≤50	≤20
Insufficiency	51-74	21-29
Sufficiency	75-250	30-100

Table 3 Categories of vitamin D status and their corresponding 25(OH)D cut-off values as proposed by the Endocrine Society in ref. [108]

Category	**25(OH)D [nmol/L]**	**25(OH)D [ng/ml]**
Deficiency	<30	<12
Inadequate	30 - <50	12 - <20
Adequate	≥50	≥20
Potential adverse effects	>125	>50

Table 4 Categories of vitamin D status and their corresponding 25(OH)D cut-off values as proposed by the Institute of Medicine in ref. [109]

This variation is not solely, but mostly due to differences in the respective authors' approaches to defining an adequate vitamin D status. A research committee of the Institute of Medicine (IOM) has conducted a thorough and systematic review of the available evidence on the topic [109]. They based their recommendations for categories and cut-off primarily on the occurrence of bone-related complications (rickets, osteomalacia) and secondarily on the suppression of PTH secretion [109]. Using this methodology, the committee concludes that vitamin D deficiency should be defined by 25(OH)D levels below 12 ng/ml and that levels of 20 ng/ml and above would suffice for 97.5% of the overall population.

In contrast, recommendations by a task-force for the Endocrine society [108] have been formulated based on the aforementioned factors as well, but other studies evaluating other aspects of "classical" vitamin D effects such as the mediation of intestinal calcium uptake have been factored in as well [110]. With this approach the task-force suggests that 25(OH)D levels of 20 ng/ml and below constitute vitamin D deficiency and levels between 21 ng/ml and 29 ng/ml should be considered as clinically relevant vitamin D insufficiency.

1.2.3 Measurement techniques

Several measurement methods for vitamin D and its metabolites – mostly 25(OH)D – exist and are listed and briefly described in the following:

Competitive protein binding assays: The first assays developed for the measurement have been based on competitive protein binding using vitamin D binding protein as the binder [111]. These assays recognise and measure both $25(OH)D_3$ and $25(OH)D_3$, which can be considered clinically useful. They do, however, also measure all other polar vitamin D metabolites, such as $24,25(OH)_2D$ and $25,26(OH)_2D$. Although usually of low contribution to overall vitamin D status, this measurement can lead to bias in vitamin D assessment.

Radioimmunoassays: The first antibody-based radioimmunoassay for the measurement 25(OH)D has been developed in 1985; the technology has been further developed and used ever since [111]. Similar to the older competitive protein binding assay, these assays detect both $25(OH)D_3$ and $25(OH)D_2$. They also affected by polar metabolites of the vitamin D axis, especially $24,25(OH)_2D$. Overestimation of vitamin D status is therefore just as possible as it is with competitive protein binding assays [112]. These assays can be automated and are therefore relatively simple to be operated; they are thus well suited for both everyday clinical practice

and clinical trials. Similar assays exist for the quantification of $1,25(OH)_2D$ if deemed necessary. First assays have also been based on the competitive protein binding principle. Vitamin D receptors derived first from chicken intestine [113] and bovine thymus [114] later on have been used as the binding protein. These assays have later been superseded by radioimmunoassays similar to those used for the measurement of 25(OH)D.

High-performance liquid chromatography: A method to separate 25(OH)D from other metabolites and thus abolish the inaccuracy of the above described assays, a technique of high-performance liquid chromatography (HPLC) has been developed and published in 1978 [115]. With this technique, the lipid component of plasma or serum is fractionated by adsorption and reversed-phase chromatography and the absorbance of vitamin D metabolites is measured at a wavelength of 254nm [115]. This method is very accurate, but has to be performed manually and is therefore perceived as being rather cumbersome.

Liquid chromatography – mass spectrometry: The coupling of liquid chromatography (for separation) with mass spectrometry / mass spectroscopy (for analysis), commonly referred to as LC-MS/MS, has become routinely available for both clinical and scientific purposes over the years. Several iterations of this technique are now used routinely for the assessment of vitamin D status. LC-MS/MS is well equipped to differentiate between $25(OH)D_3$ and $25(OH)D_2$, since there is a difference in molecular mass between these forms. It can also identify $24,25(OH)_2D$ and $25,26(OH)_2D$ reliably. Conversely, LC-MS/MS may be unable to distinguish 25(OH)D form its epimer 25(OH)-3-epi-vitamin D_3, which may lead to overestimation of vitamin D status in some populations [116]. Specific LC-MS/MS columns exist that allow for the differentiation of 25(OH)D from its epimer despite their identical mass [117].

LC-MS/MS measurements of 25(OH)D is now considered the gold standard in the assessment of vitamin D status [118]. However, there may be significant differences in measured vitamin D levels between different laboratories using this technique. In the United States, the Vitamin D Standardization Program (VDSP) has been initiated by the Office of Dietary Supplements (ODS) at the National Institute of Health (NIH) to equalise measurements across different laboratories [119]. It provides a reference measurement system that is comparable to the accurate and comparable to the National Institute of Standards and Technology (NIST) and Ghent Reference Measurement Procedures (RMP). Similarly, the Vitamin D External Quality

Assessment Scheme (DEQAS) has been incorporated in 1989 to ensure the analytical reliability of 25(OH)D and 1,25 1,25$(OH)_2$D assays [120]. DEQAS have issued a performance target and award certificates for participating laboratories achieving these goals annually [121].

Although LC-MS/MS is now considered the gold standard, the use of radioimmunoassays is still common, since these techniques are faster, less expensive and come with a lower workload for laboratory staff. Measurements using these assays usually correlate well with the gold standard in healthy subjects. However, significant deviations have been described in several patient populations such as pregnant women, patients with liver failure, haemodialysis patients and osteoporotic patients [122–124].

1.3 Background on critical care

Critical care – also referred to as *intensive care* – is a subspecialty of medicine that is tasked with the diagnosis and treatment of patients suffering from life-threatening diseases and injuries, now commonly referred to as the *critically ill* or *critically unwell*.

The practice of critical care includes sophisticated diagnostic methods, advanced monitoring techniques and invasive organ support interventions. It is nowadays carried out in specialised *intensive care units* (ICU), also referred to as *critical care units* (CCU) or *intensive treatment units* (ITU). These units may be responsible for the treatment of unselected critically ill patients or may be specialised in a specific area of expertise.

Examples are, but are not confined to, *medical intensive care units* (MICU), *cardiac intensive care units* (CICU), *paediatric intensive care units* (PICU) and *neonatal intensive care units* (NICU). Patients requiring close observation but a lower level of organ support may be treated in *intermediate care units* (IMC), *high-dependency units* (HDU) or *post-anaesthesia care units* (PACU) after operations and anaesthesia.

Patients may be admitted to ICU as emergencies due to acutely acquired illnesses or following severe injuries, after deterioration of their overall health during a hospital stay or after urgent surgical procedures. Planned admissions to ICU may occur following major surgery (e.g. major abdominal surgery, cardiac surgery, neurosurgery, …) or high-risk interventional procedures (e.g. percutaneous valve replacement, …).

1.3.1 Common conditions requiring critical care

The most common reasons for ICU admission in adults have been reported to be respiratory failure, acute myocardial infarction, intracranial haemorrhage or cerebral infarction, percutaneous cardiovascular procedures and severe sepsis [125].

In children, respiratory illnesses are the most common reason for ICU admission, either due to acutely acquired diseases or following hereditary respiratory disease, cardiac disease and neurologic disorders [126,127]. Neonates are admitted to NICUs when born preterm or due to serious medical or surgical complications after birth at term [128].

1.3.2 Physiologic derangements and therapeutic interventions

Following what is referred to as the ABC approach (Airway, Breathing, Circulation) immediate treatment in critically ill patients usually revolve around securing a patient's airway (e.g. by endotracheal intubation), support or take over the patient's breathing (e.g. by pressure support ventilation or mandatory ventilation) and to restore haemodynamic status (e.g. by the use of vasoactive drugs and the application of intravenous fluids).

Especially the latter can lead to significant changes in body fluid and ion homeostasis. This is particularly relevant when high volumes of intravenous fluids are applied over extended time periods. The most prominent example for the need of large volumes of fluids to be administered is resuscitation of patients suffering from sepsis. Current guidelines issued by the Surviving Sepsis Campaign suggest a fluid bolus of at least 30 ml/kg be applied over three hours in patients with signs of sepsis-induced organ hypoperfusion [129].

These high volumes of intravenous fluids – mostly crystalloid solutions such as normal saline or balanced crystalloid solutions – can lead to notable haemodilution and oedema. This may have further unfavourable downstream effects such as visceral swelling, increased intra-abdominal pressure and mesenteric vein compression leading to organ-hypoperfusion itself. As these circulatory disturbances may lead to further efforts of fluid resuscitation, a vicious cycle may ensue [130].

Although these unwanted side effects are well known, the need for fluid resuscitation occurs regularly in critical care. To limit the amount of fluid infused in critically ill patients and avoid negative sequalae, it has therefore been suggested to restrict the application of large fluid boluses to the short-term reversal of immediately life-threatening conditions and adjust the amounts of fluid infused continuously according to the degree of organ dysfunction and failure later on.

A proposed model to represent this approach is the ROSE model [131] (**Figure 5**, **Figure 6**). In this model, a proposed first hit – the acute condition that leads to a patient's critical illness – should be countered with a phase of fluid resuscitation (R). Second hits – immediate sequalae of the overall deranged physiology due to the first hit and its treatment – should not lead to any further attempts in fluid loading, but should encourage clinicians to fluid optimisation (O) instead. Following that, a phase of stabilisation (S) should ensue. If haemodilution and visceral

oedema lead to further organ failure, a third hit, fluid evacuation (E) instead of any further fluid resuscitation should be conducted to ameliorate the negative effects of fluid overload.

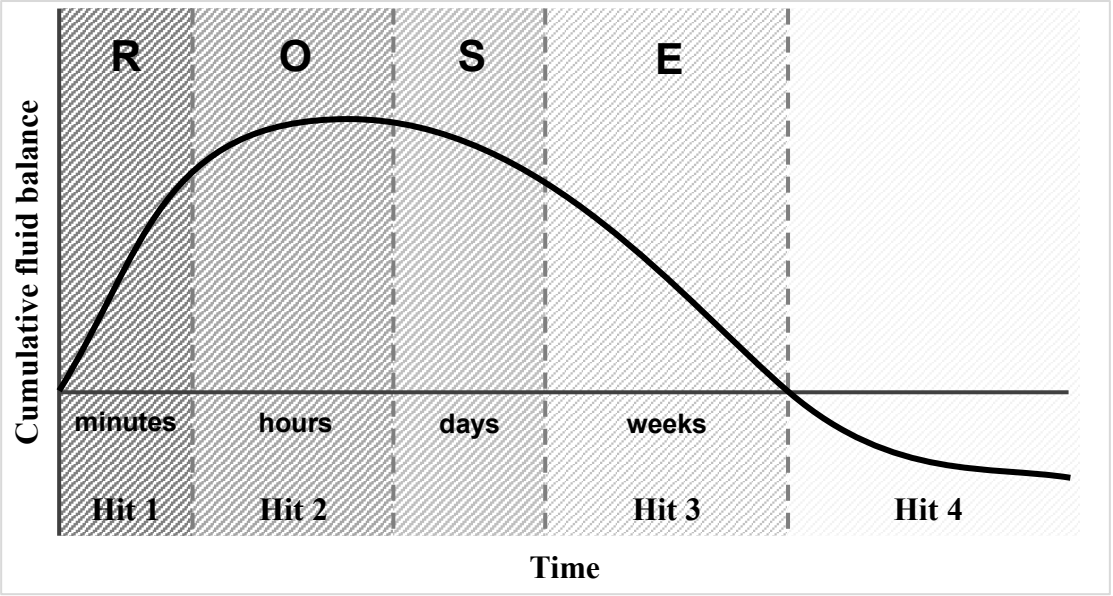

Figure 5 Cumulative fluid balance during resuscitation, optimisation, stabilisation and evacuation in the ROSE model as proposed in ref. [131]

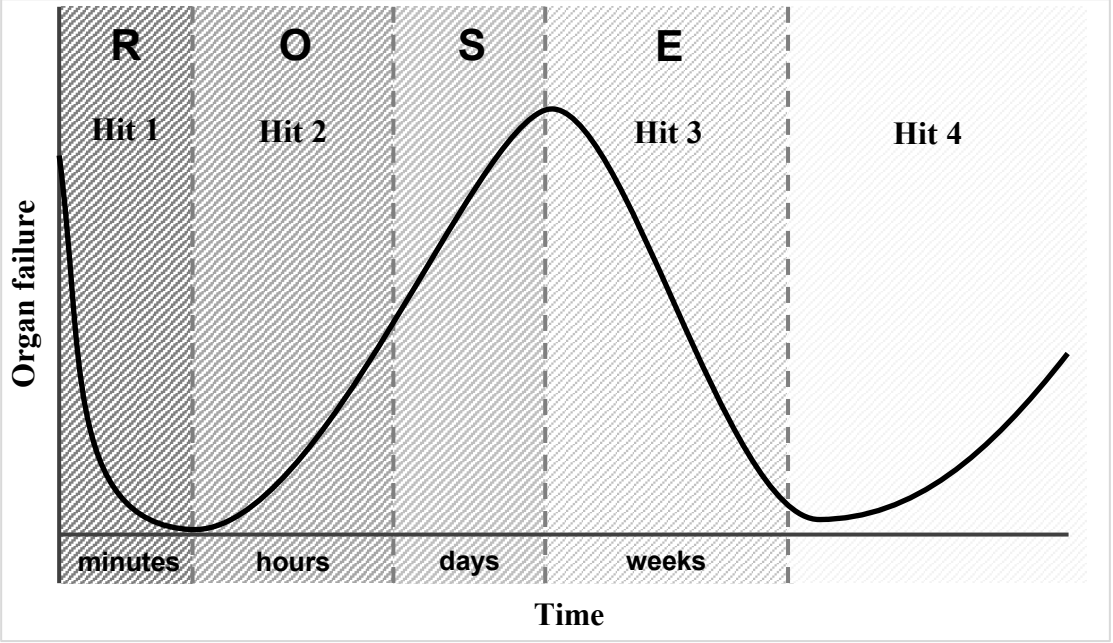

Figure 6 Degree of organ failure during resuscitation, optimisation, stabilisation and evacuation in the ROSE model as proposed in ref. [131]

1.4 Vitamin D in critical care

Because of its pleiotropic actions as a steroid hormone, vitamin D has increasingly received clinical and scientific interest in the area of critical care over the last two decades [132–134].

1.4.1 Epidemiology of vitamin D deficiency in critical illness

The prevalence of vitamin D deficiency is believed to be high in the world's overall population. Bearing in mind that the definition of vitamin D deficiency is not entirely consistent over the published literature, about 20% of the general population has been found to be vitamin D deficient even in the world's sunniest regions [135]. Austrian investigations put the prevalence of deficiency at about 25% of the overall population, with substantial differences across age groups and other subgroups [136].

Vitamin D deficiency is even more common in critically ill patients, who are almost universally deprived of sunlight by the need for critical care and typically were chronically ill before their acute illness. This hypothesis has first been supported with evidence in 2009 by Lee et al., who have reported vitamin D deficiency (defined as serum 25(OH)D < 12 ng/ml) in 38% of patients from intensive care units referred for endocrinologic evaluation [137]. In 2010, Lucidarme et al. have reported that 47% of critically ill patients are vitamin D deficient [138].

Building on this knowledge, results of cohort studies published in 2011 and 2012 have associated low vitamin D levels with increased rates of unfavourable outcomes including mortality; these finding have highlighted the importance of research in the field of vitamin D in critical care. Results from initial, mostly small studies have pointed towards increased rates of mortality [139,140] and sepsis [141] in patients with vitamin D deficiency. Is has to be noticed though, that other, smaller studies have not been able to demonstrate any association [142–145].

The largest retrospective studies on the topic in this time period have been published by Braun et al. Examining 2399 and 1325 patients, respectively, the authors have shown that pre-existing vitamin D deficiency (defined as serum 25(OH)D < 15 ng/ml before hospital admission) is associated with higher risks of 30-day mortality and blood-culture positivity [146,147]. The same author group has also found that vitamin D deficiency before hospitalisation is a significant predictor for acute kidney injury (AKI) in the intensive care unit [148]. Using the same dataset, is has later been demonstrated that vitamin D levels are also inversely associated

with hospital-acquired bloodstream infections [149] and duration of mechanical ventilation in post-surgical patients [150].

A retrospective Austrian study from 2014 in 655 critically ill patients with available 25(OH)D measurements has found a prevalence of vitamin D deficiency of up to 80% varying with season (**Figure 7**) and has also linked it to increased mortality, although it has failed to demonstrate any association with blood-culture positivity rate [151]. Around the same time, other smaller, but prospectively conducted studies also have found high prevalence of vitamin D deficiency in intensive care units and have also demonstrated significant associations with increased mortality rates [152,153].

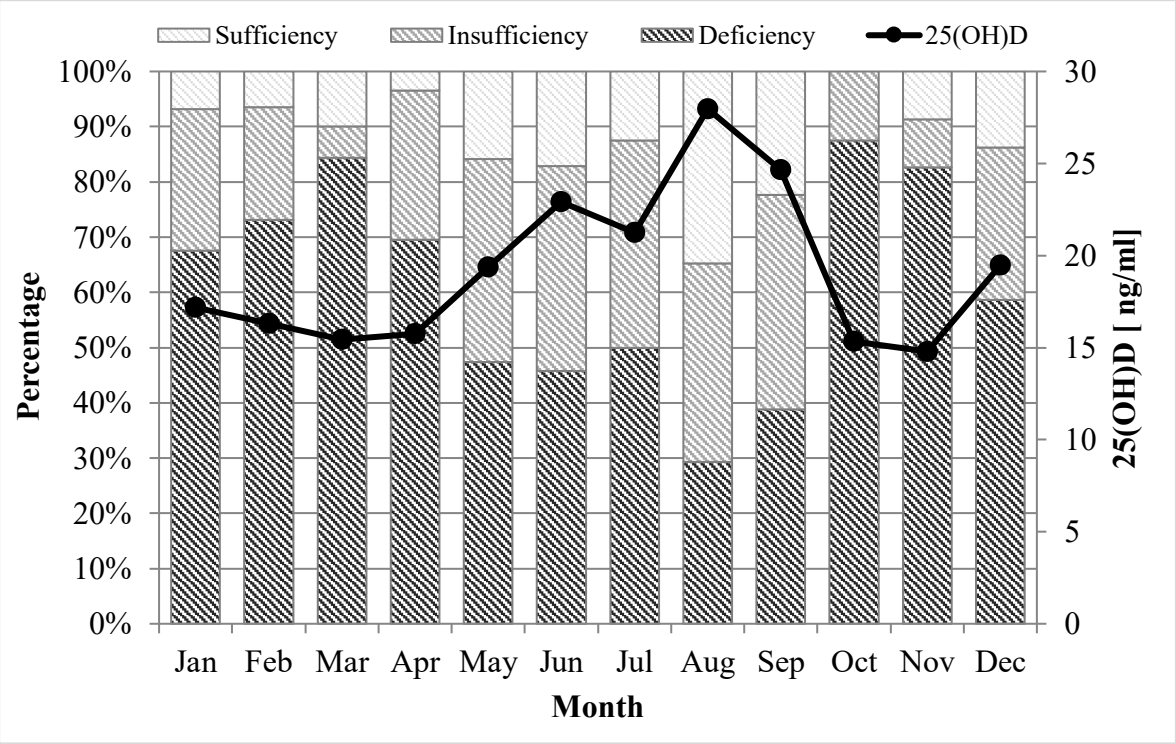

Figure 7 Percentages of critically ill patients with sufficient, insufficient and deficiency vitamin D status and mean 25(OH)D levels stratified by month of investigation. Reproduced using data from ref. [151] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); layout changed.

Two systematic reviews and meta-analyses on studies available at the time have been published in 2014. In the first, de Haan et al. have included fourteen studies involving 9,715 and conclude that vitamin D deficiency (defined as serum 25(OH)D < 20 ng/ml) is associated with an increased risk of in-hospital mortality (RR 1.79, 95% CI 1.49 to 2.16, p<0.001) [154]. In the

second, Zhang et al. have analysed seven cohort studies including 4,204 patients and have demonstrated that vitamin D deficiency is significantly associated with increased hospital mortality (OR 1.76, 95% CI 1.38 to 2.24, p<0.001) [155]. In 2017, McNally et al. have conducted a similar paediatric meta-analysis in seventeen studies concerning 2,783 PICU patients and have concluded, that vitamin D deficiency (using the same cut-off value) is associated with increased risk of death in critically ill children as well (OR 1.62, 95% CI 1.11–2.36, p=0.02) [156].

All in all, there is sufficient evidence to suggest that low vitamin D levels are clearly associated with increased rates of unfavourable outcomes in critically ill patients. Since association does not prove causation, however, clinical trials have become necessary to investigate and potentially prove a benefit of vitamin D supplementation in intensive care medicine.

1.4.2 Relevance of vitamin D as a treatment option in critical care

Supplementation of vitamin D is now considered good practice in critically ill patients [157]. This recommendation is largely based on expert consensus. This, in turn, has been derived from the previously described findings that hypovitaminosis D is associated with adverse outcomes in both the overall population and critically ill patients especially and that vitamin D supplementation has been shown to be safe in critical care.

Is has been shown though, that doses of vitamin D significantly larger than the usually recommended daily intakes are required in critically ill patients to correct vitamin D deficiency [158]. Application of vitamin D solely over time may also lead to drastically prolonged correction times in this patient population [159]. Supplementation schemes including bolus doses of up to 540,000 units of cholecalciferol have thus safely been used via enteral and parenteral routes to treat hypovitaminosis D in intensive care medicine.

In adults, a single-centre, randomised controlled, double-blind clinical trial entitled VITDAL-ICU has been conducted to investigate a potential benefit of such an aggressive correction scheme on patient-oriented outcomes in critical care [160]. In this study in 492 ICU patients, no benefit with regards to the primary endpoint (hospital length of stay) has been demonstrated in the intervention group (20.1, IQR 11.1-33.3 days for intervention vs. 19.3, IQR 11.1-34.9 days for placebo, p=0.98). Albeit an overall negative trial, some findings have highlighted

potential benefits of vitamin D supplementation as a treatment option in critical care and have sparked further interest in it.

Specifically, a trend towards reduced hospital mortality and 6-month mortality has been demonstrated (hospital mortality: 28.3%, 95% CI 22.6%-34.5% for intervention vs. 35.3%, 95% CI 29.2%-41.7% for placebo, p=0.18; 6-month mortality: 35.0%, 95% CI 29.0%-41.5% for intervention vs. 42.9%, 95% CI 36.5%-49.4% for placebo, p=0.09). In a subgroup of severely vitamin D deficient patients (25(OH)D<12 ng/ml), a significant reduction in hospital mortality has been demonstrated in the intervention group (28.6%, 95% CI 19.9%-38.6% for intervention vs. 46.1%, 95% CI 36.2%-56.2% for placebo, p=0.04).

Three systematic reviews and meta-analyses have been conducted on the subject matter. They have all tried to accumulate the relatively sparse evidence besides the VITDAL-ICU trial to give a more definitive answer as to whether high-dose vitamin D supplementation improves outcomes in critically ill patients. As the available evidence is limited and dominated by one single study, it has been questioned whether it is methodologically adequate to perform meta-analyses at that time point [161].

For this reason, it is of little surprise that the author groups have presented conflicting conclusions. In their meta-analysis, Langlois et al. conclude that vitamin D supplementation does not improve any patient-oriented outcomes (ICU mortality, hospital mortality, ICU length of stay, hospital length of stay) in critical illness [162]. In contrast, Putzu et al. have found vitamin D to significantly reduce mortality in critically ill patients (OR 0.70, 95% CI 0.50 - 0.98, p=0.04) [163]. Conversely again, Weng et al. have drawn the conclusion from their meta-analysis, that vitamin D supplementation does not significantly reduce hospital mortality (OR 0.81, 95% CI 0.63 to 1.04, p=0.10), but is associated with a reduction in hospital length of stay (mean difference -6.70 days, 95% CI -13.05 to -0.35) [164].

More recently, the VIOLET trial has sought to “assess the efficacy and safety of early administration of cholecalciferol in reducing mortality and morbidity” in 1,360 vitamin D deficient patients at “high risk for ARDS and mortality” in emergency departments, hospital wards, operating rooms and intensive care units [165]. The study was prematurely terminated for “futility” – originally it was planned to include 3000 patients.

Again, a high-dose cholecalciferol regime (540,000 units enterally) has been compared to placebo. No patient-oriented outcome benefit has been demonstrated; the primary endpoint of interest of 90-day all-cause, all-location mortality has been reported at 23.5% in the vitamin D group and 20.6% in the placebo group (p=0.26). Major criticism on the study protocol include the relatively high cut-off (20 ng/ml) and the use of only a megadose upfront without maintenance doses.

In summary, vitamin D treatment is a potentially valuable treatment option in critically ill patients, although beneficial effects have not been definitively proven yet. However, it has been argued that patient selection is crucial for both future studies and potential application in everyday clinical practice [166].

1.4.3 Uncertainties in vitamin D assessment in critical illness

Identifying patients who may benefit (most) from treatment is paramount before any intervention. Although vitamin D supplementation confers few side effects, it is unlikely that any benefit can be observed when patients with already acceptable vitamin D status are treated. Those who have suffer from relevant vitamin D deficiency must therefore be reliably identified. There is, however, debate about how this is best achieved in the setting of critical care [167].

Several factors related to deranged physiology during critical illness and medical interventions to counter these derangements may influence vitamin D levels. It is therefore possible that means and methods of definition and identification of hypovitaminosis D usually used in the overall population are not well suited to critically ill patients. The most relevant aspects to be considered are briefly summarised here:

Inflammatory response: It has been claimed that vitamin D constitutes a “negative acute-phase protein”. Contrary to “classic” acute phase proteins like C-reactive protein, fibrinogen, ferritin, haptoglobin and many others, levels of 25(OH)D may actually decrease during inflammation.

This claim has first been made and investigated by Waldron et al., who have analysed blood samples from non-critically ill patients undergoing knee arthroplasty [168]. The authors have drawn the conclusion that both 25(OH)D and VDBP drop in states of inflammation; hypovitaminosis D may therefore be more a sign of disease than its root cause. Inflammation

induced by relatively benign surgical procedures such as knee replacement surgery may obviously not be fully reflective of inflammatory states seen in critically ill patients.

Fluid loading and haemodilution: Large volumes of intravenous fluids may be used in the treatment of critically ill patients in intensive care units, especially during the initial resuscitation phase, as described in the ROSE model. The ensuing haemodilution may lead to seemingly low levels of vitamin D, when measurements are performed after fluid loading.

This hypothesis has first been examined in a small pilot study by Krishnan et al. [169]. In their study, the investigators have measured vitamin D levels at several timepoints before, during and after cardiac surgery on cardiopulmonary bypass. They conclude that vitamin D levels may drop up to 45% following a fluid bolus of approximately five litres in adults.

Assay validity: The validity of some methods of vitamin D measurement has been questioned in critically ill patients, similar to other selected patient populations, in whom significant deviations between the gold standard of LC-MS/MS and radioimmunoassays have been reported.

To investigate this matter, Rousseau et al. have analysed 25(OH)D of non-critically ill patients before and after systemic inflammation due to orthopaedic surgery with three immunoassays (Liaison, Diasorin; iSYS, IDS; Vidas, bioMerieux) and compared the results with LC-MS/MS [170]. The authors have concluded that LC-MS/MS seems to be superior to immunoassays and therefore the best option to measure 25(OH)D in critically ill patients. These results from relatively healthy young adults undergoing a single episode of elective minor surgery may also not necessarily be directly translatable to the setting of critical care, however.

On the contrary, LC-MS/MS may be more prone to overestimation of 25(OH)D levels, since the amount of 25(OH)-3-epi-vitamin D may be relatively higher compared to the overall adult population. Secondary laboratory analysis using LC-MS/MS of frozen blood samples collected during the VITDAL-ICU trial [160] suggest percentages of up to 10% in ICU patients [personal communication of unpublished data by Karin Amrein, principal investigator of the VITDAL-ICU trial].

1.5 Aim of this study

Based on the available knowledge on the matter at hand as well as existing uncertainties in the subject area as described in the introduction of this dissertation, the study underlying this dissertation aims to:

1) … confirm and quantify a possible decrease in 25(OH)D and 1,25$(OH)_2$D levels following acute fluid loading and systemic inflammation.
2) … assess a possible influence of inflammation on vitamin D levels at various timepoint of critical illness.
3) … evaluate which vitamin D levels are best suited to diagnose vitamin D deficiency preceding fluid loading and the inflammatory process.
4) … quantify the contribution of the biologically inactive C3 epimer to total 25(OH)D levels in critically ill patients and possible rates of overestimation.

1.5.1 Research questions

Derived from the underlying aims of this study, the following research questions were formulated to guide study design, protocol generation and research conduction:

RQ1 What vitamin D metabolite should be used to assess vitamin D status in critically ill patients?

RQ2 What vitamin D levels are to be expected in intensive care patients and how do they change over the course of critical illness and its treatment?

RQ3 Can possible changes in vitamin D status be predicted and can premorbid vitamin D levels be inferred from measurements performed later on?

RQ4 What cut-off values should be used to diagnose vitamin D deficiency in critically ill patients?

RQ5 Which measurement methods should be employed to assess vitamin D status in intensive care patients?

2 MATERIAL AND METHODS

2.1 Study design

This study was a prospective observational single-centre, one-arm, non-controlled study in adult patients undergoing major cardiovascular surgery and postoperative intensive care treatment. Blood samples were collected at different time points pre-, intra- and postoperatively and were analysed for vitamin D using a widely available, rapid and inexpensive radioimmunoassay and the gold standard LC-MS/MS from frozen samples. Information on baseline patient characteristics, treatments received, fluid balance and routine laboratory measurements as well as laboratory measurements concerning the vitamin D axis were also collected.

2.2 Study setting

This study was conducted at the University Medical Centre Graz, an academic teaching hospital with more than 1,500 beds in Graz, the capital of the southern Austrian province of Styria. Patients scheduled for major cardiac surgery requiring cardio-pulmonary bypass were screened, informed and included into this study. Routine care for these patients was provided by healthcare professionals of the Division of Cardiac Surgery, Department of Surgery, and the Division of Anaesthesiology for Cardiovascular Surgery (interim head: Prof Dr Ameli Yates) and Intensive Care Medicine, Department of Anaesthesiology and Intensive Care Medicine (head: Prof Dr Wolfgang Toller).

This setting was chosen primarily for three reasons: First, cardiac surgery requiring cardio-pulmonary bypass invariably leads to significant fluid loading and haemodilution and is therefore a suitable model for the resuscitation phase of intensive care fluid management as described in the ROSE model. Second, major cardiac surgery induces a state of systemic inflammation akin to many disease states in critical illness. Third, patients undergoing cardiac surgery mandatorily require intensive care treatment following their operation, but can be informed about the planned study and can provide informed consent ahead of ICU admission. In brevity, major cardiac surgery represents a plausible model for the physiologic changes in critical illness and the medical interventions usually performed, but allows for research according to Good Clinical Practice (GCP) at the same time.

Screening for study inclusion was conducted in patients planned for major cardiac surgery requiring cardio-pulmonary bypass who were deemed to be able to give informed consent well ahead of the surgical procedure. Screening and inclusion were carried out either in the anaesthetic preoperative clinic, the cardio-surgical normal ward or the cardio-surgical intensive care unit in patients who required preoperative optimisation (e.g. levosimendan infusion to increase cardiac inotropy preoperatively), as appropriate.

Sample collection was performed preoperatively before significant fluid loading, i.e. either in the normal ward, the cardio-surgical intensive care unit or the anaesthetic induction room. Intra- and postoperative samples were taken in the cardiosurgical operating theatre and the cardio-surgical intensive care unit.

Laboratory investigations were performed by two laboratories at the University Medical Centre Graz: routine laboratory measurements as well as analysis involving mass spectroscopy / mass spectrometry were provided by the Clinical Institute of Medical and Chemical Laboratory Diagnostics (chair: Prof Dr Markus Herrmann). Laboratory measurements concerning the vitamin D axis and other endocrine parameters were performed by the laboratory of the Division of Endocrinology and Diabetology, Department of Internal Medicine (laboratory director: Prof Dr Barbara Obermayer-Pietsch). This laboratory routinely participates in the DEQAS program.

2.3 Inclusion and exclusion criteria

2.3.1 Inclusion criteria

- Adult patients (age ≥ 18 years at screening for inclusion)
- Planned (i.e. scheduled and acute, non-emergency) cardiovascular surgery requiring cardio-pulmonary bypass (i.e. CABG and aortic valve replacement, …)
- High anticipated risk of staying in the ICU > 48 hours postoperatively
- Informed consent possible (both scheduled and acute, non-emergency operations)

2.3.2 Exclusion criteria

- Patients unable to give informed consent
- Planned mitral valve replacement (to avoid inclusion in a parallel study)

2.4 Study timepoints

The sample collection time points for this study were chosen with two aims in mind: First, to closely monitor possible changes in vitamin D status that may be induced by changes in inflammation and fluid status as proposed in the previously described ROSE model. Second, to resemble the common course of critical illness and intensive care treatment to make findings applicable for future clinical practice and research purposes.

One preoperative, one intraoperative and three postoperative measurement timepoints were predefined. Their definitions were described in **Table 5**, their relationship to the supposed changes in fluid balance were depicted in **Figure 8**.

Timepoint	Definition
t1	Preoperatively, either on the normal ward or at anaesthetic induction
t2	Intraoperatively, after disconnection from cardiopulmonary bypass
t3	Postoperatively, upon admission to the intensive care unit
t4	Postoperatively, in the morning of the first postoperative day
t5	Postoperatively, in the morning of the second postoperative day

Table 5 Definitions of study time points

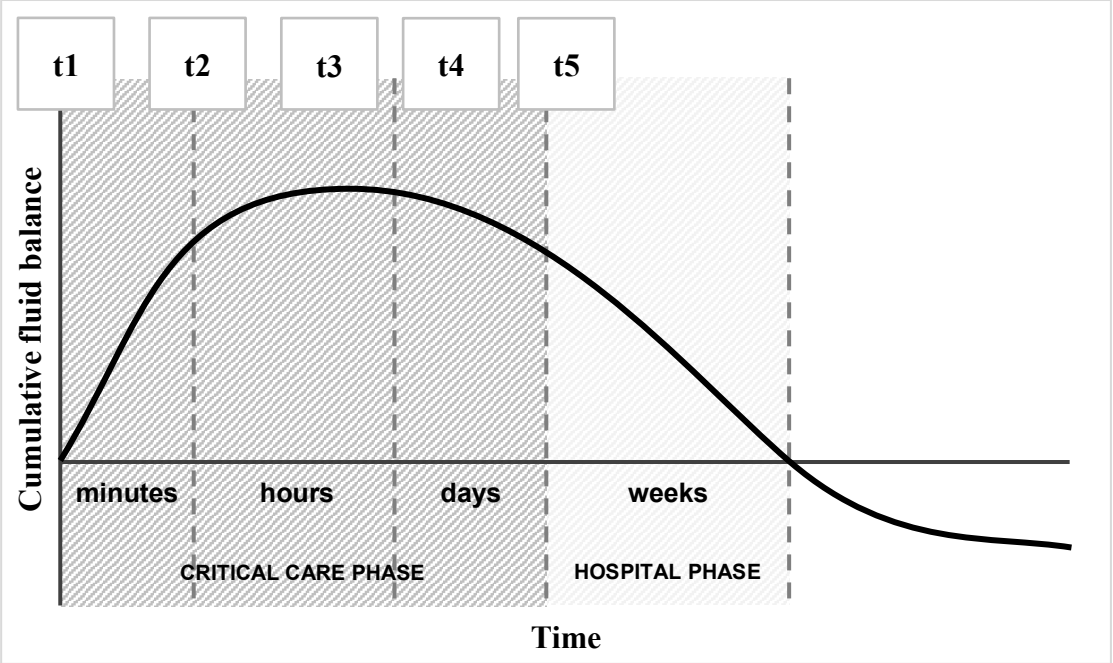

Figure 8 Relationship of study timepoints to expected degree of inflammation and fluid status

2.5 Study procedures

This was a non-interventional study, study-related procedures were therefore limited to sample collection, storage, analysis and documentation. The only invasive procedure to be performed for study purposes only was blood sample collection at the pre-defined time points. Care was taken to minimise any potential (albeit already improbable) harm by these procedures.

To do so, all study profiles to be processed by the Clinical Institute of Medical and Chemical Laboratory Diagnostics were designed in such a way that they would resemble the usual laboratory measurements performed in the intensive care units in the morning (MOF-TX profile). The results of these analyses were directly accessible by usual means for the treating physicians, thereby eliminating the need for double sampling. Additionally, sample collection was performed via vascular access already in place whenever possible to avoid unnecessary vascular puncture and associated discomfort.

2.5.1 Sample collection and storage

Predefined laboratory measurement sets identified by unique study codes were created by both the Clinical Institute of Medical and Chemical Laboratory Diagnostics (K codes) and the laboratory of the Division of Endocrinology and Diabetology (E codes) for all previously described study timepoints.

The codes used and their designated timepoints of use are listed in **Table 6**. Required blood collection tubes and collected volumes are listed in **Table 7** to **Table 10**. Laboratory parameters measured in the respective predefined sets are listed in **Table 11** to **Table 14**. All specified parameters have been measured, yet only those relevant to the stated research questions are presented throughout this thesis.

Timepoint	Study codes
t1	E94 + K119
t2	E95 + K119
t3	E95 + K119
t4	E95 + K119 or K120
t5	E94 + K119 or K120

Table 6 Study laboratory codes and respective timepoints of measurement

Blood samples were collected using vacuum PET plastic blood collection tubes (VACUETTE® system, Greiner Bio-One International, Kremsmünster, Austria) according to the clinical standard in the study centre. Blood was either collected by peripheral venepuncture or from already in-place arterial or central venous catheters using a dedicated blood-collection system.

To ensure collection of all required samples at the predefined timepoints and to simplify study conduction for all involved health care professionals, pre-sets were created for the different study timepoints. For pre-, intra- and immediately post-operative measurements (t1 to t3), bags containing all necessary blood collection tubes and pre-filled laboratory request sheets with specific study profile stickers were packed and used throughout the perioperative phase. For measurements in the intensive care unit (t4 to t5), pre-filled laboratory request sheets with specific study profile stickers were used in conjunction with blood collection tubes stocked at the respective units.

All samples were sent to the central laboratory, which was available 24 hours a day, 7 days a week, by routine methods (tube mail or courier, as appropriate). Those required to perform laboratory measurements included in the K-profiles were immediately processed in the central laboratory. Those intended for analysis as part of the E-profiles were stored on a cooling rack at the central laboratory and forwarded to the endocrinologic laboratory for processing and analysis on the next working day.

Once transferred to the endocrinologic laboratory, half of the sample volume was immediately used for measurements as defined in the E-profiles, while the other half was stored frozen at -70°C for later analysis by LC-MS/MS.

E94 study blood collection set

No of vials	Vial type	Volume per vial	Total volume
2	Serum	5ml	10ml
2	EDTA	6ml	12ml
			22ml

Table 7 Blood vials required for study profile E94

E95 study blood collection set

No of vials	Vial type	Volume per vial	Total volume
1	Serum	5ml	5ml
2	EDTA	6ml	12ml
			17ml

Table 8 Blood vials required for study profile E95

K119 study blood collection set

No of vials	Vial type	Volume per vial	Total volume
1	Serum	5ml	5ml
1	EDTA	6ml	6ml
1	Citrate	3ml	3ml
1	Lithium Heparin	8ml	8ml
			22ml

Table 9 Blood vials required for study profile K119

K120 study blood collection set

No of vials	Vial type	Volume per vial	Total volume
1	Serum	5ml	5nl
1	EDTA	6ml	6ml
1	Citrate	3ml	3ml
1	Lithium Heparin	8ml	8ml
			22ml

Table 10 Blood vials required for study profile K120

E94 study profile

Abbreviation	Name	Unit
25(OH)D	25-hydroxy-vitamin D	ng/ml
1,25(OH)$_2$D	1,25-dihydroxy-vitamin D	pmol/l
TSH	Thyroid-stimulating hormone	µU/ml
fT$_3$	Triiodothyronine	ng/l
fT$_4$	Thyroxine	ng/l
Prol	Prolactin	µg/l
Oest	Oestradiol	pg/ml
Test	Testosterone	ng/ml
ACTH	Adrenocorticotropic hormone	pmol/ml
Cort	Cortisol	nmol/l
HGH	Human growth hormone	ng/ml
FSH	Follicle-stimulating hormone	U/ml
LH	Luteinising hormone	U/l
IGF-1	Insulin-like growth factor 1	ng/ml
OC	Osteocalcin	ng/ml
PTH	Parathyroid hormone	pg/ml
ß-CTx	Beta cross laps	ng/ml

Table 11 Laboratory parameters measured in the E94 profile

E95 study profile

Abbreviation	Name	Unit
25(OH)D	25-hydroxy-vitamin D	ng/ml
1,25(OH)$_2$D	1,25-dihydroxy-vitamin D	pmol/l
OC	Osteocalcin	ng/ml
PTH	Parathyroid hormone	pg/ml
ß-CTx	Beta cross laps	ng/ml

Table 12 Laboratory parameters measured in the E95 profile

K119 study profile

Abbreviation	Name	Unit	Method
Na^+	Sodium	mmol/l	indirect potentiometry
K^+	Potassium	mmol/l	indirect potentiometry
Cl^-	Chloride	mmol/l	indirect potentiometry
PO_4^-	Phosphate	mg/dl	indirect potentiometry
Mg^{2+}	Magnesium	mmol/l	indirect potentiometry
Ca^{2+} total	Total calcium	mmol/l	photometry
Ca^{2+} ion	Ionised calcium	mmol/l	potentiometry
Crea	Creatinine	mg/dl	photometry
Urea	Urea	mg/dl	photometry
ALAT	Alanine-aminotransferase	U/l	photometry
ASAT	Aspartate-Aminotransferase	U/l	photometry
GGT	Gamma-glutamyl transferase	U/l	photometry
AP	Alkaline phosphatase	U/l	photometry
Bili total	Total bilirubin	mg/dl	photometry
TP	Total protein	g/dl	photometry
Alb	Albumin	g/dl	photometry
CRP	C-reactive protein	mg/l	immunoturbidimetry
NT-pro-BNP	N-terminal brain natriuretic peptide	pg/ml	electrochemiluminescence assay
PCT	Procalcitonin	ng/ml	immunoturbidimetry
FBC	Full blood count		photometry
Chol	Cholesterol	mg/dl	photometry
Tri	Triglycerides	mg/dl	photometry
HDL	High-density lipoproteins	mg/dl	photometry
LDL	Low-density lipoproteins	mg/dl	photometry
VLDL	Very-low-density lipoproteins	mg/dl	photometry
PT	Prothrombin time	%	coagulometry
aPTT	Act. partial thromboplastin time	s	coagulometry
Fib	Fibrinogen	mg/dl	coagulometry

Table 13 Laboratory parameters measured in the K119 study profile

K120 study profile

Abbreviation	Name	Unit	Method
Na^+	Sodium	mmol/l	indirect potentiometry
K^+	Potassium	mmol/l	indirect potentiometry
Cl^-	Chloride	mmol/l	indirect potentiometry
PO_4^-	Phosphate	mg/dl	indirect potentiometry
Mg^{2+}	Magnesium	mmol/l	indirect potentiometry
Ca^{2+} total	Total calcium	mmol/l	photometry
Ca^{2+} ion	Ionised calcium	mmol/l	potentiometry
Crea	Creatinine	mg/dl	photometry
Urea	Urea	mg/dl	photometry
ALAT	Alanine-aminotransferase	U/l	photometry
ASAT	Aspartate-Aminotransferase	U/l	photometry
GGT	Gamma-glutamyl transferase	U/l	photometry
AP	Alkaline phosphatase	U/l	photometry
Bili total	Total bilirubin	mg/dl	photometry
TP	Total protein	g/dl	photometry
Alb	Albumin	g/dl	photometry
CRP	C-reactive protein	mg/l	immunoturbidimetry
NT-pro-BNP	N-terminal brain natriuretic peptide	pg/ml	electrochemiluminescence assay
FBC	Full blood count		photometry
Chol	Cholesterol	mg/dl	photometry
Tri	Triglycerides	mg/dl	photometry
HDL	High-density lipoproteins	mg/dl	photometry
LDL	Low-density lipoproteins	mg/dl	photometry
VLDL	Very-low-density lipoproteins	mg/dl	photometry
PT	Prothrombin time	%	coagulometry
aPTT	Act. partial thromboplastin time	s	coagulometry
Fib	Fibrinogen	mg/dl	coagulometry

Table 14 Laboratory parameters measured in the K120 profile

2.5.2 Vitamin D analysis techniques

Chemiluminescence assay: The IDS-iSYS 25OHD^S assay (IDS Immunodiagnostic Systems, Tyne & Wear, United Kingdom) was used as a widely available, relatively quick, simple and financially favourable measurement method for vitamin D. This assay based on the IDS-iSYS Multi-Discipline Automated System was intended and licensed for the "quantitative determination of 25(OH)D and other hydroxylated metabolites in human serum" according to its description.

The technical basis for this assay was chemiluminescence technology. Summarised briefly, to perform tests with this assay, 10µl of samples were pre-treated to denature VDBP. These pre-treated samples were then neutralised in assay buffer and antibodies specific for 25(OH)D labelled with acridinium were added. Samples were then incubated. Afterwards, magnetic particles linked to 25(OH)D were added and the samples were incubated once more. The previously added magnetic particles were then attracted by a magnet. Next, samples were washed and further trigger reagents were added. Finally, the light emitted by the acridinium label was measured; the light emitted was inversely proportional to the concentration of 25(OH)D in the original sample.

This assay's reportable range was 7 - 125 ng/ml. Values below 7 ng/ml were reported as "<7 ng/ml" by the laboratory. The limit of blank (LoB), limit of detection (LoD) and limit of quantitiation (LoQ) for this assay were 0.6 ng/ml, 2.4 ng/ml and 7.0 ng/ml, respectively.

Rates of cross reactivity with vitamin D metabolites were stated as follows: 25(OH)D_3 102%, cholecalciferol 0%, ergocalciferol 1%, 3-epi-25(OH)D_3 1%. Pearson's correlation coefficient for the correlation with LC-MS/MS as the gold standard was reported to be 0.93 for this assay.

LC-MS/MS kit: ClinMass® (Recipe GmbH, Munich, Germany) complete kit and additional components for on-line analysis of 25(OH)D_2 and 25(OH)D_3 in serum (order numbers MS7000, MS7031, MS7035, MS7014, MS7015 and MS7082) were used for mass spectrometric analyses.

With this kit, the 3-epi-25(OH)D was claimed to be separated reliably from the analyte peak. An additional analytical column with a run time of four minutes was used in order to obtain baseline separation. For analysis, 50 µl of sample (either calibration, control or patient sample) were mixed with 150µl of IS/P solution (internal standard and precipitation agent), incubated

at 4°C and then centrifuged. 10 to 50 µl of the resulting solution were injected into the LC-MS/MS couple for analysis.

2.6 Ethical implications

Approval of this study by the institutional review board of the Medical University of Graz (IRB00002556) was sought and granted before its conduction (decision 27-161 ex 14/15). Applications for extension of the ethics approval were filed and approved before expiration of the decision in accordance with the ethics committee's requirements.

All patients were informed about the study and its interventions both verbally by a physician and by a written patient information document approved by the ethics committee. All patients willing to participate in the study provided written informed consent after sufficient time for consideration.

2.7 Trial registration

This study was registered in the German Clinical Trials Registry (DRKS, Deutsches Register Klinischer Studien, www.drks.de) under the identifier DRKS00009216. It was thus also listed in the World Health Organisation's International Clinical Trials Registry Platform (ICTRP, http://apps.who.int/trialsearch).

2.8 Data collection and management

Data were collected on paper-based case report forms (CRF) during the conduction of the study. These data were then collected in a spreadsheet (.XLSX format) using common commercially available software (Microsoft Excel® 2019, Microsoft Corp., Redmond, USA).

To ascertain data validity, laboratory measurements were additionally retrieved from the central laboratory documentation system by an employee of the Clinical Institute of Medical and Chemical Laboratory Diagnostics. The predefined study codes were used to identify relevant laboratory data sets. These were then associated with corresponding data sets in the study spreadsheet using admission numbers as identifiers. No personal data were documented in the data file to ensure anonymity.

After completion of data collection, this spreadsheet was imported into an IBM SPSS Statistics 2018 file (.SAV format) using the built-in import tool. All variables were adjusted according to their respective data type; data fields were manually checked for plausibility following data

type conversion. 25(OH)D values below the lower detection limit of ECLIA were imputed as 3.5 ng/ml for all analyses according to convention.

2.9 Statistical Analysis

Statistical analyses (except for Passing-Bablok regression analysis) were conducted using IBM SPSS Statistics 25 (IBM Corp., Armonk, USA, 2018) provided via Citrix® (https://citrix.medunigraz.at) by the Medical University of Graz.

Graphs were generated using Excel® 2019 (Microsoft Corp., Redond, USA, 2019) with Analyse-it® package "Method Validation Edition" (Analyse-it Software Ltd., Leeds, United Kingdom). Passing-Bablok regression analysis was also conducted using this package.

p values below 0.05 were generally considered significant.

2.9.1 General patient characteristics

Baseline demographic data (e.g. age, gender) and indicators of physical status (e.g. American Society of Anesthesiologists (ASA) physical status classification) were described as median values ± interquartile range (IQR).

2.9.2 Vitamin D measurements

25(OH)D and 1,25$(OH)_2$D levels measured by the techniques under investigation were presented as median values ± interquartile range at all time points. Overall changes were evaluated for statistical significance using Friedman test as a non-parametric test for multiple related samples. Changes from baseline vitamin D status at different time points were assessed using Wilcoxon rank-sum test as a non-parametric test for two related samples.

2.9.3 Laboratory measurements and fluid status parameters

Fluid input (including priming fluid for cardio-pulmonary bypass) and fluid output were routinely assessed at every study timepoint by the responsible anaesthesiologist or ICU staff, respectively. Fluid balance was calculated as the difference between fluid input and fluid output.

Relevant routine laboratory results, i.e. measures of serum ions implicated in the vitamin D axis such as Ca^{2+} and measures of inflammation such as leucocytes and C-reactive protein (CRP)) were presented as median values ± interquartile ranges (IQR). Changes over time were

evaluated for statistical significance using Friedman test as a non-parametric test for multiple related samples.

2.9.4 Vitamin D, fluid status and inflammation

Changes in vitamin D levels over time, both absolute and relative, were assessed for linear correlation with fluid balance and markers of inflammation (CRP and leukocytes) using Pearson's correlation coefficient.

2.9.5 Measurement methods

Mean difference and Pearson's correlation coefficient were calculated for crude comparison of the investigated of vitamin D measurement methods (ECLIA immunoassay and LC-MS/MS).

Due to the known limitations of this approach, analyses as proposed by Bland and Altman [171] as well as regression analysis using the procedure postulated by Passing and Bablok [172] were additionally conducted.

Agreement in the categorial diagnosis of vitamin D deficiency between measurement methods was assessed using Cohen's kappa coefficient (κ). The generally agreed upon cut-off value for the overall population of 25(OH)<20 ng/ml [108] as well as 25(OH)D<12 ng/ml as a potential cut-off value in critical illness derived from a previous study [160] were used to define vitamin D deficiency for this analysis.

2.9.6 Diagnostic use and utility

Diagnostic utility of vitamin D assessment in the perioperative phase and during critical illness by both LC-MS/MS and ECLIA was assessed by comparison of measurements at timepoints t2 to t5 with baseline LC-MS/MS measurements as the gold standard.

True positive rate (TPR, *sensitivity*), true negative rate (TNR, *specificity*), positive predictive value (PPV) and negative predictive values were calculated according to derivation formulas listed in **Table 15**.

Again, two definitions of vitamin D deficiency as described in section 2.9.5 were used and evaluated.

Measure	Abbreviation	Derivation
Sensitivity	TPR	$TPR = \frac{n_{true\ positive}}{n_{true\ positive} + n_{false\ negative}}$
Specificity	TNR	$TNR = \frac{n_{true\ negative}}{n_{true\ negative} + n_{false\ positive}}$
Positive Predictive Value	PPV	$PPV = \frac{n_{true\ positive}}{n_{true\ positive} + n_{false\ positive}}$
Negative Predictive Value	NPV	$NPV = \frac{n_{true\ negative}}{n_{true\ negative} + n_{false\ negative}}$

Table 15 Derivation formulas for measures of diagnostic utility

3 RESULTS – FINDINGS

3.1 Patient groups

Patients were screened for inclusion into the study according to inclusion and exclusion criteria listed in chapter 2.3. Initially, 70 patients provided written informed consent for participation in the study.

Of these, a total of four patients withdrew their consent for participation; two before study-related procedures were undertaken (one due to participation in another study that precluded the inclusion into other studies, one for unknown reasons), one withdrew consent for participation in another trial after perioperative measurements were performed. The "Perioperative Phase" patient group thus included 66 patients.

Availability of study resources – both personnel and measurement capacities – allowed for further sampling in measurements during intensive care treatment in 26 of the above patients; these patients made up the "Critical Care Phase" patient group.

A study flow chart is presented as **Figure 9**. Analyses conducted in the whole "Perioperative Phase" patient group are reported in chapter 3.2, results from further measurements performed solely in the "Critical Care Phase" are presented in chapter 3.3.

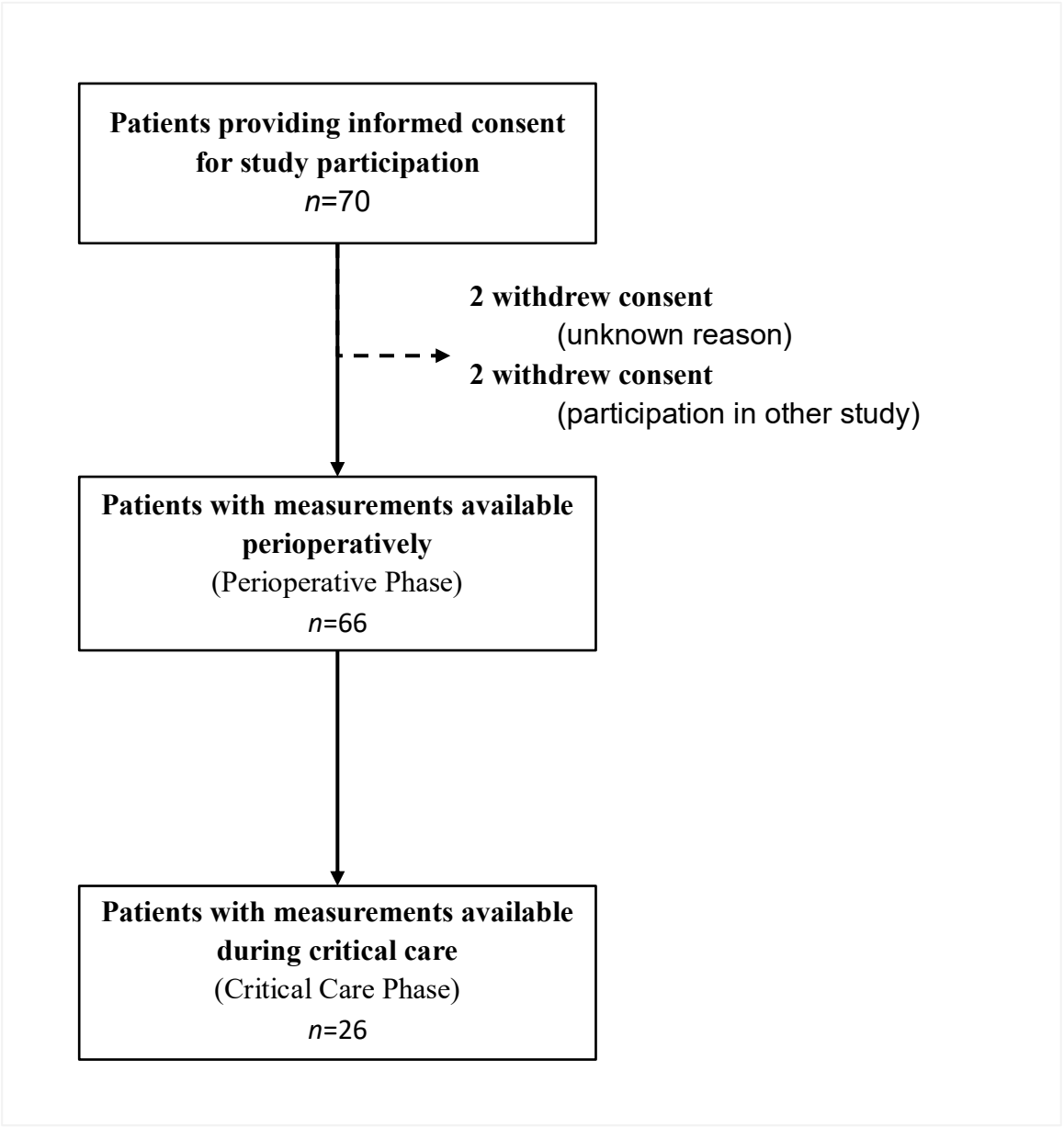

Figure 9 Study flow chart

3.2 Perioperative phase

3.2.1 Patient group

Perioperative samples were collected from 66 patients; characteristics of the "perioperative phase" patient group are listed in **Table 18**. Analyses presented in section 3.2 were conducted in these 66 patients and the respective blood samples collected from them.

Variable	Value
***n* of patients**	66
Age [years] (median, IQR)	70 (64-76)
Sex (n, %)	
male	42 (64%)
female	24 (36%)
Ethnicity (n, %)	
Caucasian	66 (100%)
BMI (median, IQR)	28 (25-31)
ASA score (median, IQR)	4 (3-4)
Pre-existing conditions and treatments (n, %)	
chronic kidney disease	19 (29%)
chronic hepatic disease	1 (1%)
osteoporosis	7 (11%)
vitamin D treatment	8 (12%)
Baseline laboratory parameters (median, IQR)	
Creatinine [mg/dl]	0.92 (0.78 – 1.09)
25(OH)D [ng/ml]	19.4 (13.6 – 24.6)
Ca^{2+} total [mmol/l]	2.27 (2.22 – 2.33)
Ca^{2+} ionised [mmol/l]	1.17 (1.14 – 1.20)
PTH [pg/ml]	54.9 (32.1 – 74.9)
Type of surgery (n, %)	
Coronary Artery Bypass Grafting (CABG)	28 (42%)
Aortic Valve Replacement (AVR)	13 (20%)
CABG + Valve Replacement	12 (19%)
CABG + Aortic Root Replacement	2 (3%)
Aortic Root Replacement	3 (5%)
AVR + ASD repair	1 (1%)
AVR + Aortic Root Replacement	6 (9%)
Multi Valve Replacement	1 (1%)

Table 16 Baseline characteristics of the overall perioperative patient group.

These patients remained in the ICU for a median of 3 (2-5) days and were admitted to the hospital for a median of 15 (13-20) days. One patient died during intensive care treatment; both ICU mortality and in-hospital mortality were therefore 1.5%.

3.2.2 Vitamin D trends

25-hydroxy-vitamin D trends

Median 25(OH)D levels measured by ECLIA changed significantly during perioperative fluid loading (p=0.028). Median 25(OH)D levels were 19.4 ng/ml (13.6-24.6 ng/ml) at baseline (t1); they dropped to 16.8 ng/ml (10.0-21.7 ng/ml, p=0.006 for comparison with t1) until the end of cardiopulmonary bypass (t2); they were 17.6. ng/ml (10.7-23.7 ng/ml, p=0.002 for comparison with t1) upon admission to the ICU (t3). (**Figure 10**)

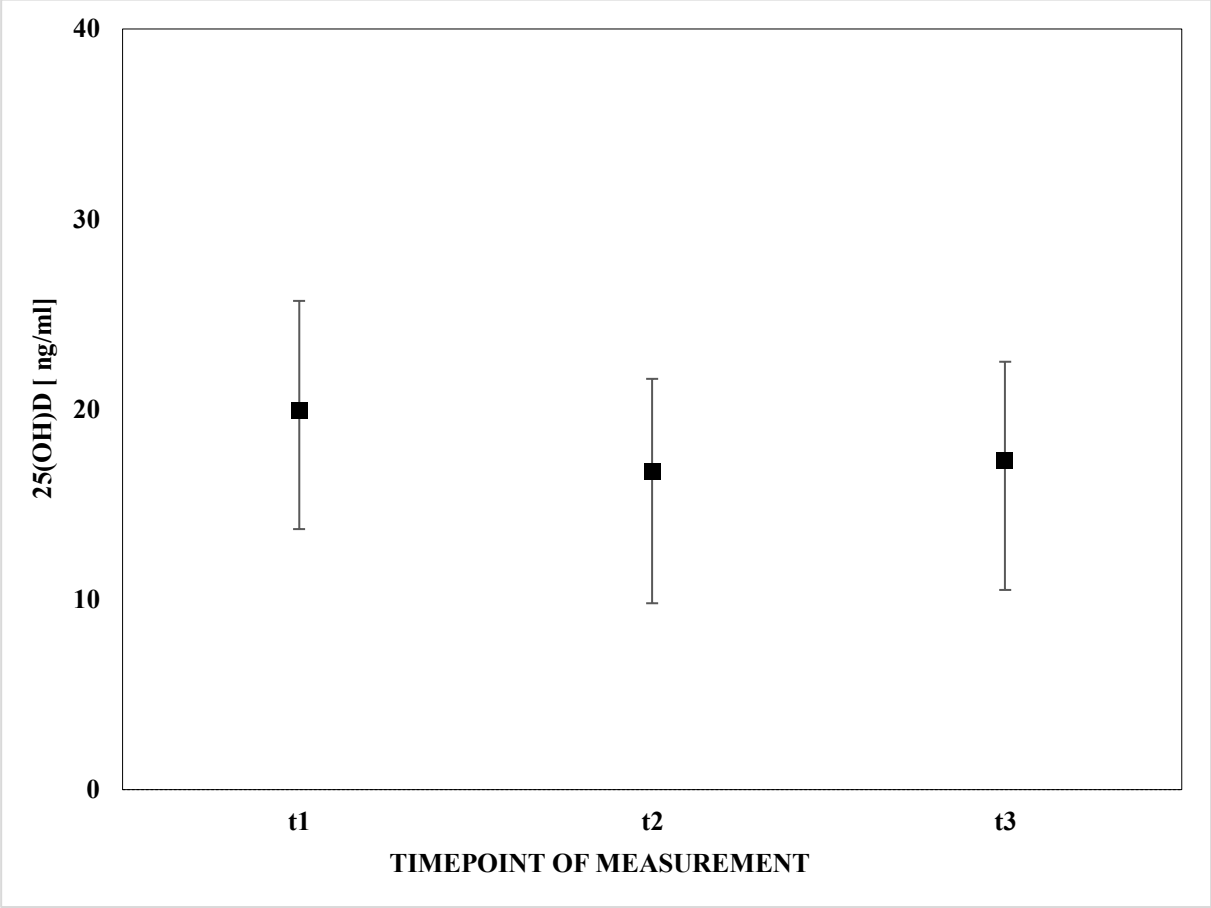

Figure 10 Median and interquartile ranges of 25(OH)D measured by ECLIA during the perioperative phase.

1,25-dihydroxy-vitamin D trends

Median $1,25(OH)_2D$ levels measured by ECLIA were also significantly altered with perioperative fluid loading ($p<0.001$). While mean $1,25(OH)_2D$ was 75 pmol/l (44-98 pmol/l) preoperatively (t1), median levels were only 40 pmol/l (25-59 pmol/l, $p<0.001$ for comparison with t1) after cardiopulmonary bypass (t2) and 40 pmol/l (23-58 pmol/l, $p<0.001$ for comparison with t1) at admission to the intensive care unit (t3) (**Figure 11**).

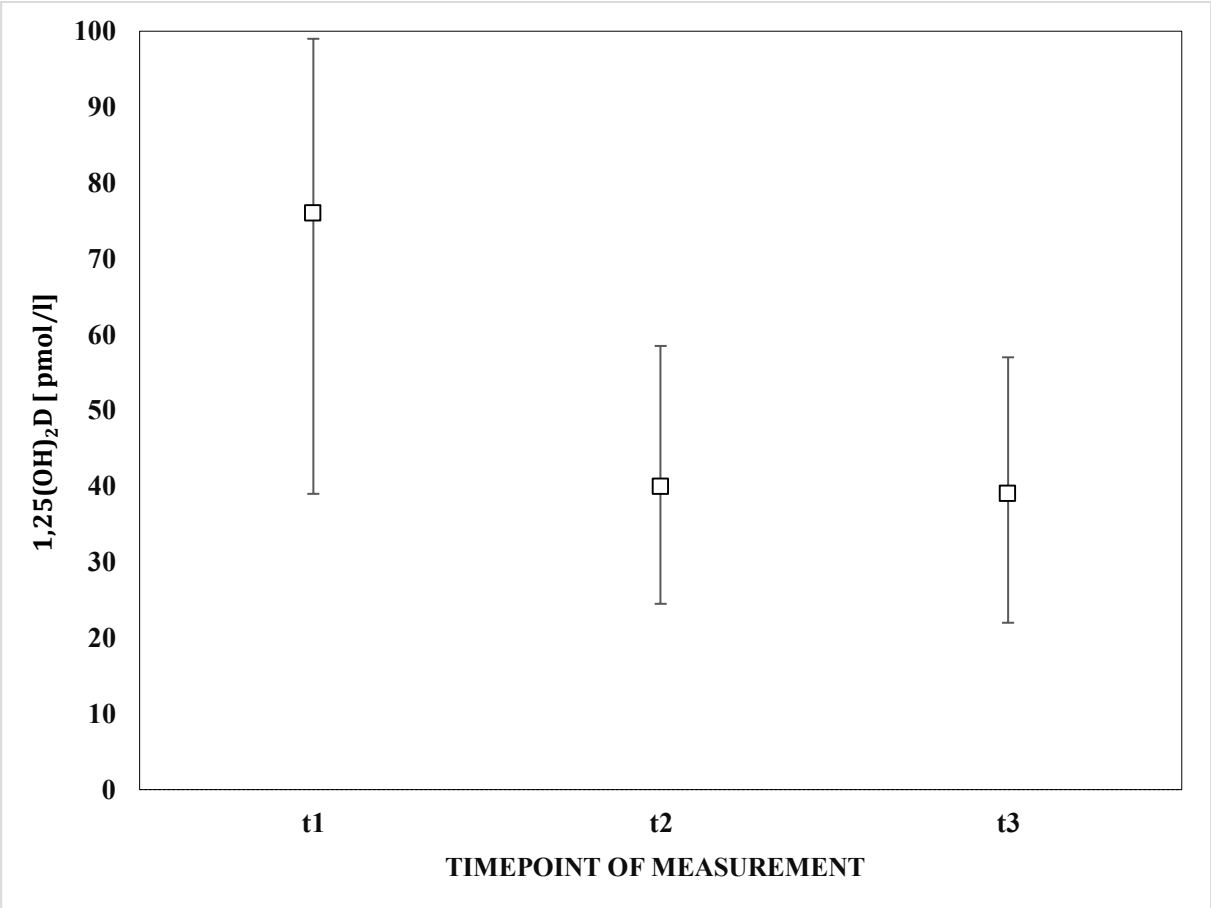

Figure 11 Median and interquartile ranges of $1,25(OH)_2D$ measured by ECLIA during the perioperative phase.

3.2.3 Influence of fluid loading

Fluid loading led to positive fluid balances all throughout the perioperative phase, markers of plasma cell and protein contents therefore all dropped significantly. Details are presented in **Table 17**.

	t1	t2	t3	*p*
Fluid input [ml]	-	2975 (2800 – 3683)	1622 (1088 – 2614)	-
Fluid balance [ml]	-	2800 (2388 – 3683)	928 (20 – 1344)	-
Haematocrit [%]	36 (31-39)	27 (25 – 30)	32 (29 – 34)	<0.001
Albumin [g/dl]	3.6 (3.4 – 3.8)	2.5 (2.3 – 2.7)	2.9 (2.6 – 3.1)	<0.001
Total protein [g/dl]	6.2 (5.8 – 6.6)	4.3 (3.8 – 4.7)	4.8 (4.3 – 5.1)	<0.001

Table 17 Markers and measurements of fluid status at investigation time points in the perioperative phase.

No definitive correlation between fluid input and fluid balance and changes in 25(OH)D levels could be found. Pearson's correlation coefficient was -0.131 (p=0.19) for absolute change in 25(OH)D levels and fluid input, -0.023 (p=0.82) for relative change in 25(OH)D levels and fluid input, -0.205 (p=0.036) for absolute change in 25(OH)D levels and fluid balance and -0.153 (p=0.12) for relative change in 25(OH)D levels and fluid balance, respectively.

3.3 Critical care phase

3.3.1 Patient group

Further samples in the critical care phase were collected from 26 patients out of the aforementioned perioperative patient group; their characteristics are listed in **Table 18**.

Variable	Value
***n* of patients**	26
Age [years] (median, IQR)	67 (60-76)
Sex (n, %)	
male	19 (73%)
female	7 (27%)
Ethnicity (n, %)	
Caucasian	26 (100%)
BMI (median, IQR)	26 (24-28)
ASA score (median, IQR)	4 (3-4)
Pre-existing conditions and treatments (n, %)	
chronic kidney disease	8 (31%)
chronic hepatic disease	0 (0%)
osteoporosis	1 (4%)
vitamin D treatment	2 (8%)
Baseline laboratory parameters (median, IQR)	
Creatinine [mg/dl]	0.91 (0.75 – 1.05)
25(OH)D [ng/ml]	21.7 (13.8 - 26.0)
Ca^{2+} total [mmol/l]	2.29 (2.20 - 2.34)
Ca^{2+} ionised [mmol/l]	1.18 (1.15 - 1.20)
PTH [pg/ml]	48.2 (30.6 - 60.1)
Type of surgery (n, %)	
Coronary Artery Bypass Grafting (CABG)	12 (46%)
Aortic Valve Replacement (AVR)	4 (15%)
CABG + AVR	5 (19%)
AVR + Atrial septal defect repair	1 (4%)
Aortic Aneurysm Repair + AVR	4 (15%)

Table 18 Baseline characteristics in patients with samples collected in the critical care phase. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); outcome data removed.

These patients remained in the ICU for a median of 3 (2-5) days and were admitted to the hospital for a median of 14 (13-15) days. All patients were alive at discharge from the hospital; both ICU mortality and in-hospital mortality were therefore 0%.

3.3.2 Vitamin D trends

25-hydroxy-vitamin D trends

Median 25(OH)D levels measured by ECLIA were 21.7 ng/ml (13.8-26.0 ng/ml) at baseline (t1). Changes over time were not statistically significant overall (p=0.29).

Measured with this assay, median levels of 25(OH)D fell to 18.4 ng/ml (14.2-23.1 ng/ml, p=0.15 for comparison with t1) during surgery on cardiopulmonary bypass (t2); they were 18.9 ng/ml (10.3-23.7 ng/ml, p=0.09 for comparison with t1) upon admission to the ICU (t3); were at 21.2 ng/ml (11.6-26.3 ng/ml p=0.29 for comparison with t1) in the morning of the first postoperative day (t4) and were 7.6 ng/ml (13.8-22.0 ng/ml, p=0.16 for comparison with t1) in the morning of the second postoperative day (t5) (**Figure 12**).

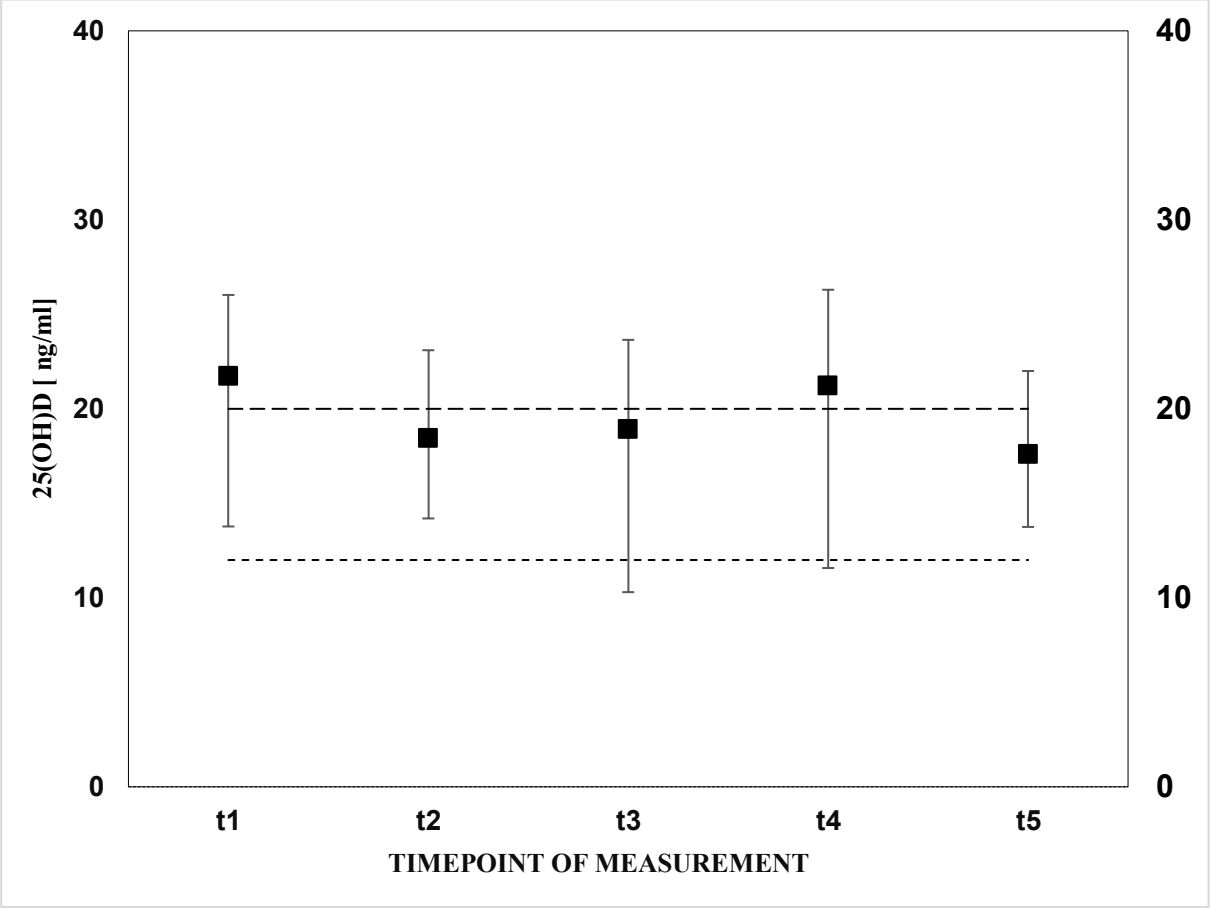

Figure 12 Median and interquartile ranges of 25(OH)D measured by ECLIA in the critical care phase patient group. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); focused presentation of one single trend in changed layout.

Median 25(OH)D levels measured by LC-MS/MS were 19.8 ng/ml (12.1-25.0 ng/ml) at baseline (t1). Changes over time were statistically significant overall ($p<0.001$).

By measurement with this technique, median 25(OH)D levels dropped to 12.5 ng/ml (7.5-16.3 ng/ml, $p=0.001$ for comparison with t1) over the course of surgery on CPB (t2); they remained roughly the same at 12.1 ng/ml (8.8-15.1 ng/ml, $p<0.001$ for comparison with t1) until ICU admission (t3); then changed little up to the morning of the first postoperative day (t4), where they were 12.9 ng/ml (7.2-18.1 ng/ml, $p=0.004$ for comparison with t1) and rose again slowly until the morning of the second postoperative day (t5), where they were 15.1 ng/ml (8.5-17.6 ng/ml, $p<0.001$ for comparison with t1) (**Figure 13**).

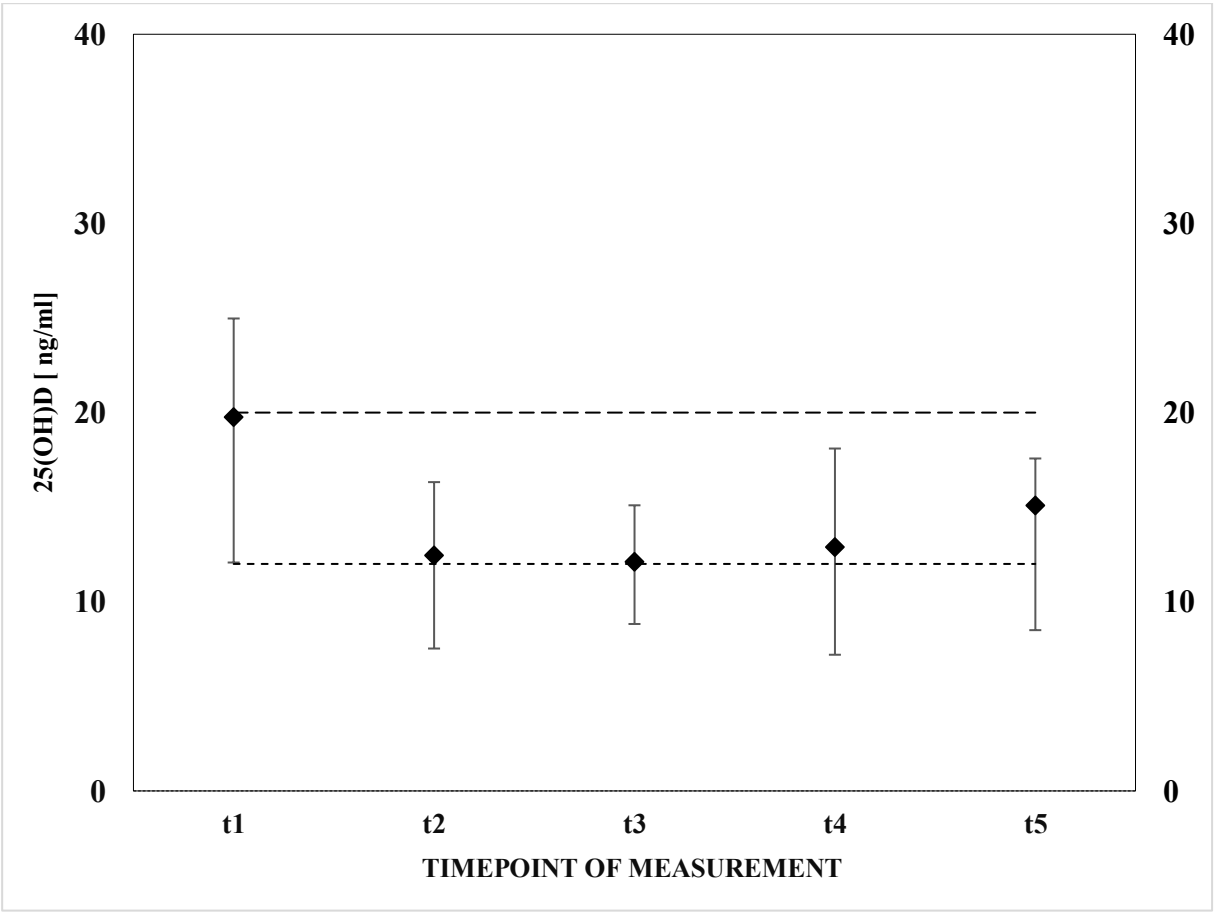

Figure 13 Median and interquartile ranges of 25(OH)D measured by LC-MS/ in the critical care phase patient group. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); focused presentation of one single trend in changed layout.

1,25-dihydroxy-vitamin D trends

Median baseline (t1) levels were 76 pmol/l (36-89 pmol/l). Changes over time were statistically significant overall ($p<0.001$).

Following fluid loading and inflammation due to initiation of CPB and conduction of surgery, median $1,25(OH)_2D$ levels dropped significantly to 34 pmol/l (21-51 pmol/l) at t2 ($p<0.001$ for comparison with t1). $1,25(OH)_2D$ levels hardly changed from that time point on. Upon postoperative admission to ICU (t3), median levels were 35 pmol/l (19-54 pmol/l, $p<0.001$ for comparison with t1). There was no discernible recovery of $1,25(OH)_2D$ levels during treatment and stay in ICU; median levels were 34 pmol/l (23-64 pmol/l, $p=0.002$ for comparison with t1) on postoperative day 1 (t4) and 29 pmol/l (18-64 pmol/l, $p<0.001$ for comparison with t1) on postoperative day 2 (t5) (**Figure 14**).

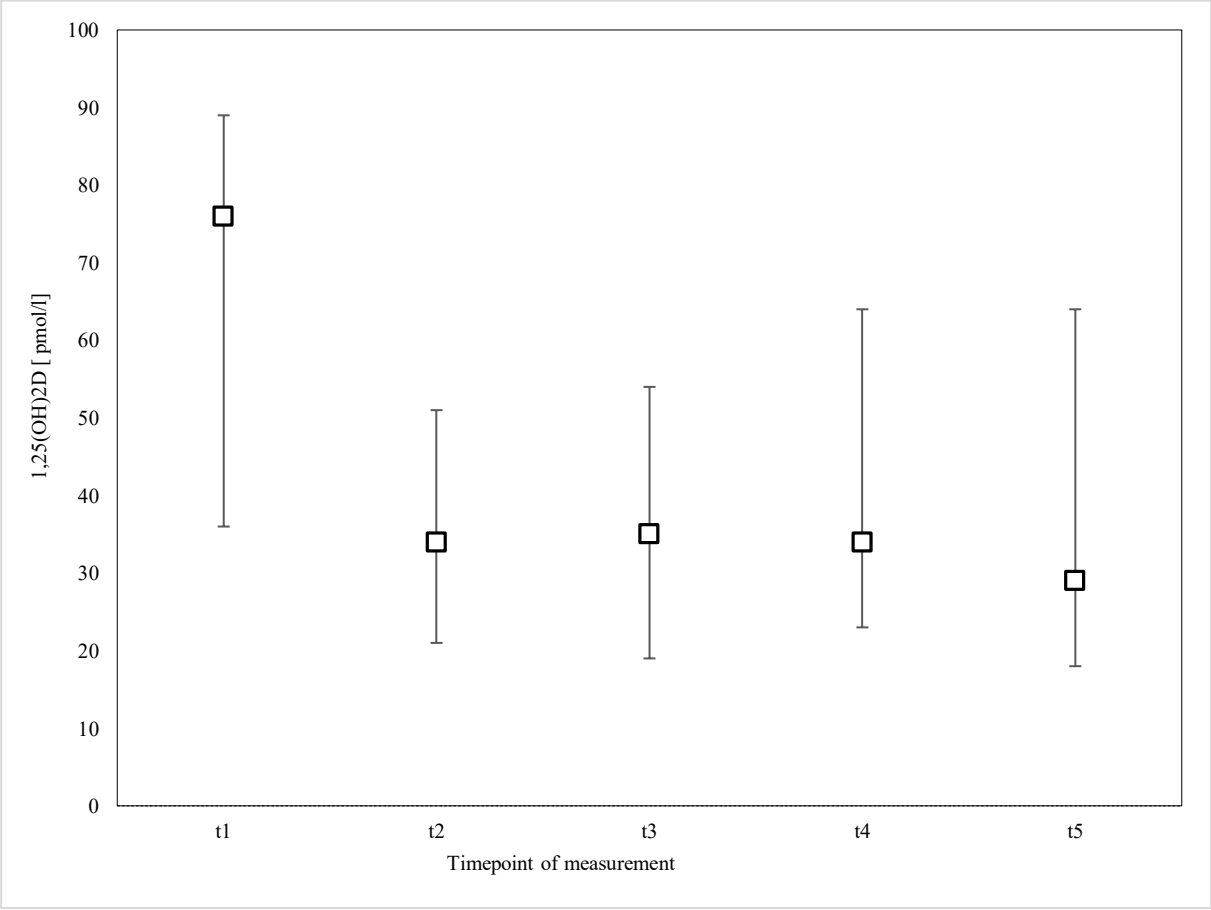

Figure 14 Median and interquartile ranges of $1,25(OH)_2D$ measured by ECLIA in the critical care phase patient group. Created using data published in [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); figure previously unpublished.

3.3.3 Influence of fluid loading and inflammation

Fluid loading during the operative period was pronounced due to the use of cardio-pulmonary bypass in patients undergoing major surgery; a median of 3.8 (3.1-4.2) litres of fluid were applied from the beginning of the operation until the end of cardiopulmonary bypass, an additional 1.7 (1.1-2.4) litres of fluid were added until admission to the intensive care unit. More than 4 litres of fluid were given on each of the following two observation days (**Table 19**).

As was to be expected in major cardiac surgery, median fluid balance was highly positive intraoperatively (t2) and immediately postoperatively (t3), but less so at the first and second postoperative day during treatment in the intensive care unit. Haemodilution was apparent in other laboratory measurements as well; haematocrit, albumin levels and total protein levels dropped significantly especially intraoperatively (**Table 19**).

Markers of inflammation – both C-reactive protein and leukocyte count - rose slowly from the pre-operative baseline, where no inflammation was to be assumed, until the end of the observation time frame (**Table 19**).

	t1	**t2**	**t3**	**t4**	**t5**	***p***
Fluid input [ml]	-	3815 (3100 – 4170)	1665 (1104-2358)	4834 (4020 – 5786)	4155 (3003 – 45688)	-
Fluid balance [ml]	-	3700 (3100 - 4111)	920 (-535 – 1378)	1895 (1265 – 3038)	124 (-201 – 825)	-
Haematocrit [%]	35.5 (31.0-38.4)	26.2 (25.1-28.7)	31.0 (26.0-34.4)	29.5 (25.6-32.9)	27.8 (24.8-30.4)	0.002
Leukocytes [G/l]	4.8 (4.2-5.8)	7.2 (6.0-10.4)	9.7 (6.4-12.1)	10.3 (8.2-12.2)	11.7 (9.7-12.7)	<0.001
CRP [mg/l]	1.3 (0.7-2.3)	1.1 (0.6-2.0)	2.0 (0.7-4.6)	63.4	146.0	<0.001

				(50.4-77.6)	(92.4-203.5)	
Albumin [g/dl]	3.5 (3.3-3.7)	2.4 (2.2-2.7)	2.8 (2.4-3.0)	3.0 (2.8-3.3)	2.9 (2.6-3.1)	<0.001
Total protein [g/dl]	6.0 (5.7-6.3)	4.3 (3.7-4.6)	4.7 (4.2-5.0)	5.1 (4.6-5.5)	5.2 (4.9-5.7)	<0.001

Table 19 Markers and measurements of fluid status and inflammation at investigation time points. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); focused presentation of parameters relevant to this section in changed layout.

Although fluid balance and trends in vitamin D measured by the gold standard LC-MS/MS during the whole observation period were found to be correlated, the correlation was weak: Pearson's coefficient was -0.445 ($p<0.001$) for absolute changes in 25(OH)D between time points and the respective fluid balances and -0.316 ($p=0.016$) for relative changes in 25(OH)D between time points and the respective fluid balances.

No correlation between markers of inflammation and 25(OH)D levels measured by LC-MS/MS during the observation period could be discerned: Pearson's coefficient was -0.037 ($p=0.740$) for 25(OH)D and leukocyte count at the respective timepoints and -0.152 ($p=0.169$) for 25(OH)D and CRP at the respective timepoints.

3.3.4 Effects on the vitamin D axis and measures of bone metabolism

Despite the marked reduction in both 25(OH)D and $1,25(OH)_2D$ levels described above, no real compensatory reaction was discernible; median levels of parathyroid hormone actually dropped significantly from median baseline (t1) levels of 48 pg/ml (31-60 pg/ml) to a low of 29 pg/ml (21-44 pg/ml) on t2 until they essentially recovered at t4 ($p=0.033$).

This might be attributed to dilution during surgery on CPB, since median albumin levels and median levels of total serum calcium demonstrated a similar behaviour, while median ionized calcium levels varied little perioperatively.

Similarly, neither median alkaline phosphatase levels, median osteocalcin levels nor median beta cross laps levels indicated increased bone turnover, as would be expected in vitamin D

deficiency states. These findings are obviously limited by the relatively short observation time frame for slower processes such as bone metabolism (**Table 20**).

	t1	t2	t3	t4	t5	*p*
PTH [pg/ml]	48.2 (30.6-60.1)	29.1 (21.2-44.0)	39.8 (23.3-55.7)	44.6 (28.0-70.2)	51.1 (33.8-73.5)	0.033
Ca^{2+}, total [mmol/l]	2.29 (2.20-2.34)	2.11 (2.06-2.20)	2.20 (2.08-2.25)	2.12 (2.03-2.23)	2.08 (2.00-2.14)	<0.001
Ca^{2+}, ionized [mmol/l]	1.18 (1.15-1.20)	1.19 (1.17-1.23)	1.18 (1.16-1.27)	1.11 (1.06-1.15)	1.10 (1.05-1.14)	<0.001
Albumin [g/dl]	3.5 (3.3-3.7)	2.4 (2.2-2.7)	2.8 (2.4-3.0)	3.0 (2.8-3.3)	2.9 (2.6-3.1)	<0.001
AP [U/l]	49 (39-58)	38 (32-42)	37 (30-45)	39 (30-45)	44 (37-48)	<0.001
OC [ng/ml]	20.5 (12.0-23.3)	12.8 (9.4-14.0)	12.8 (8.0-17.1)	10.2 (7.0-15.3)	8.4 (6.5-15.7)	<0.001
ß-CTx [ng/ml]	0.33 (0.24-0.48)	0.19 (0.15-0.28)	0.26 (0.17-0.34)	0.32 (0.17-0.50)	0.43 (0.20-0.53)	0.008

Table 20 Measurements associated with the vitamin D axis and bone metabolism at investigation time points. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); focused presentation of parameters relevant to this section in changed layout.

3.3.5 Agreement between LC-MS/MS and ECLIA

Noticeable differences between 25(OH)D levels measured by ECLIA and LC-MS/MS could be identified. Numerical agreement between the two methods under investigation was acceptable at best when assessed by linear correlation; Pearson's r was 0.73 ($p<0.001$) [1]. Intercept and

slope calculated by Passing-Bablok regression were 1.040 (95% CI -0.569 – 3.356) and 1.271 (95% CI 1.096 – 1.429), respectively (**Figure 15**).

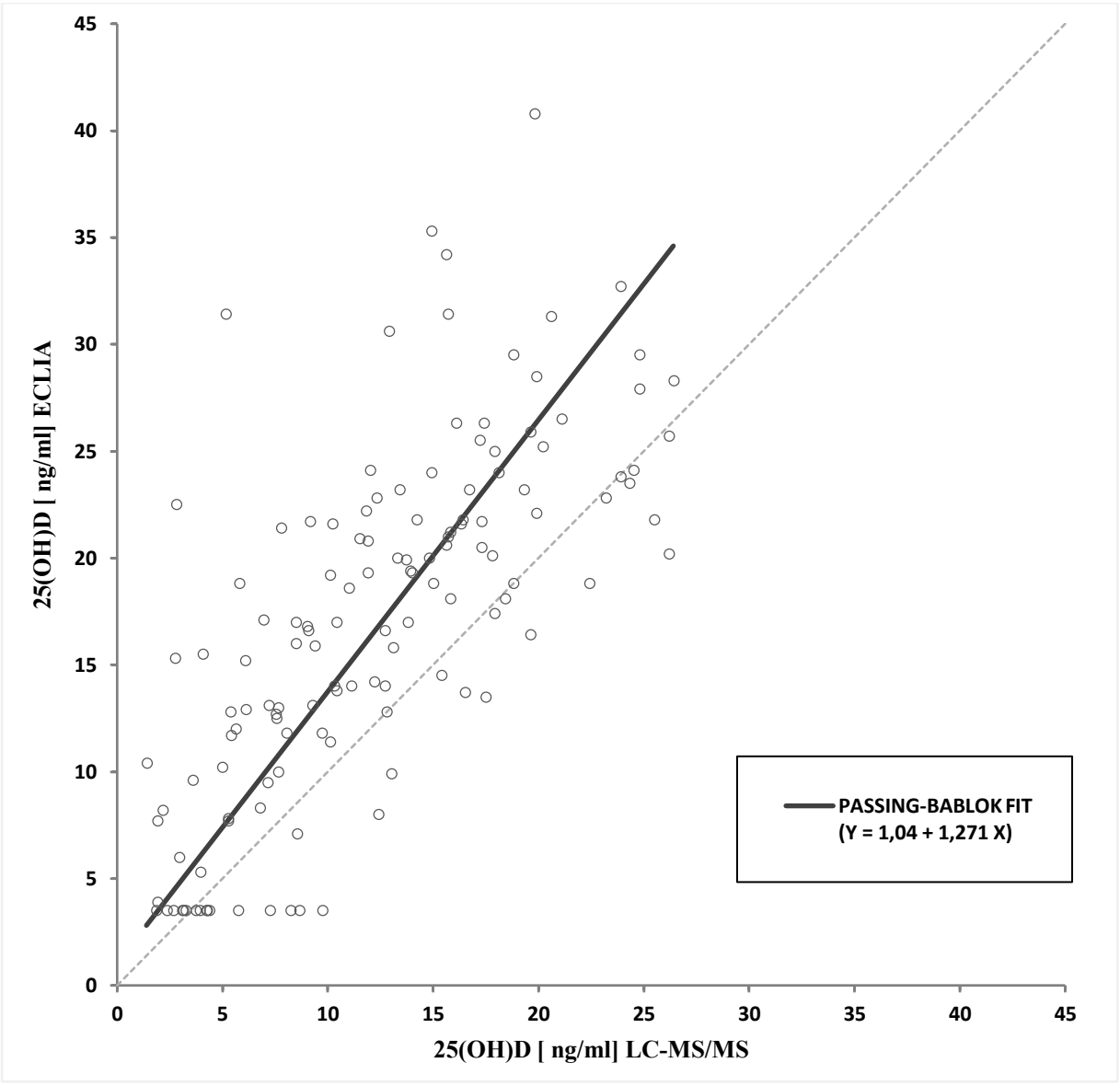

Figure 15 Regression analysis and Passing-Bablok fit for 25(OH)D measured by LC-MS/MS and ECLIA. Created using data published in [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); figure previously unpublished.

Mean difference between ECLIA and LC-MS/MS was 4.8 ng/ml (±5.7). Bland-Altman analysis comparing the two methods of measurement is provided in **Figure 16**.

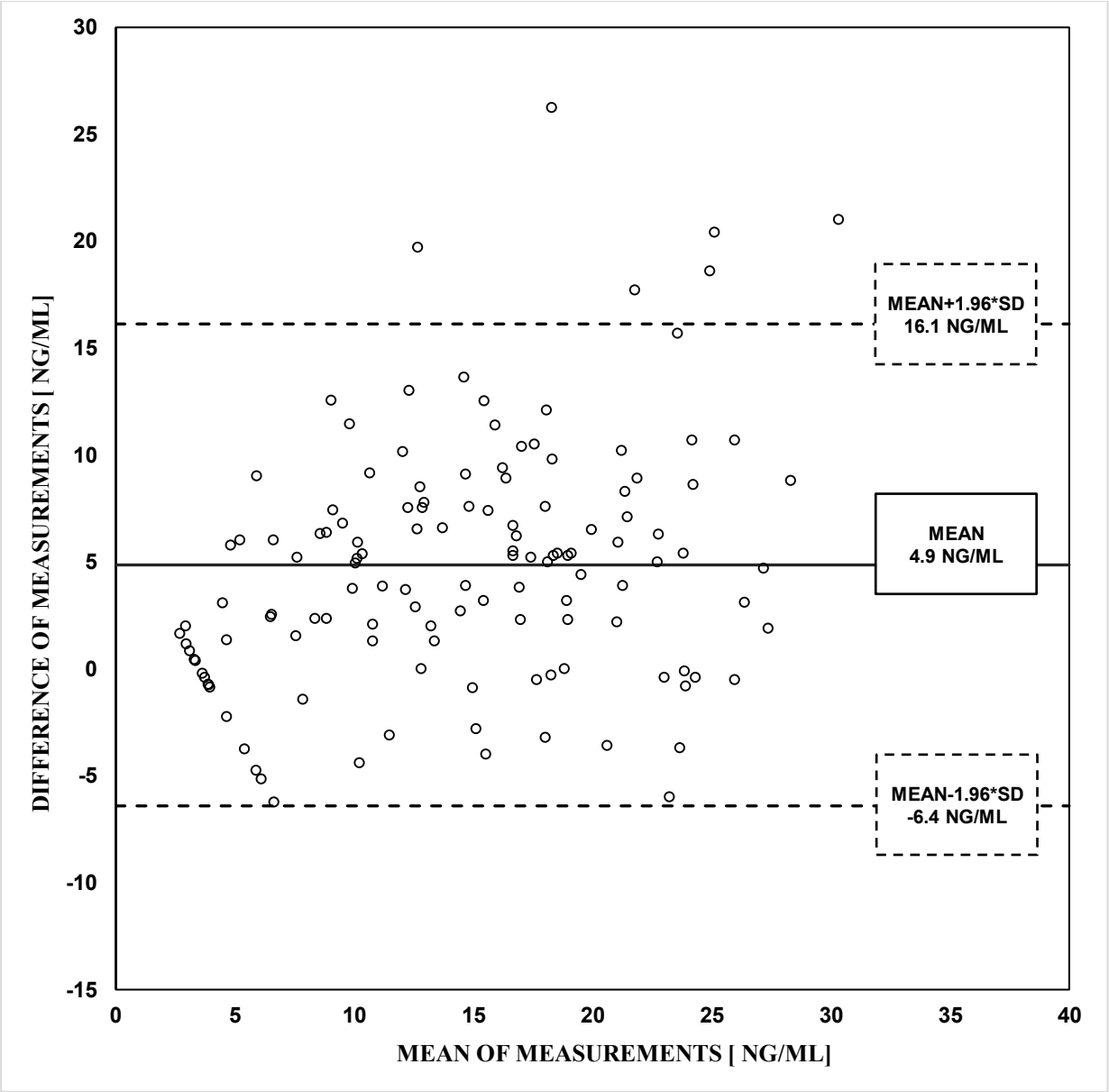

Figure 16 Bland-Altman-plot comparing 25(OH)D measurement by ECLIA and LC-MS/MS in the critical care group. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); changed layout.

Limited agreement between ECLIA and LC-MS/MS was found with regards to the categorical diagnosis of vitamin D deficiency [1].

When vitamin D deficiency was defined as 25(OH)D<20 ng/ml, the two methods agreed in only 91 (70%) samples; Cohen's κ was 0.291 (p<0.001). Using a cut-off value 25(OH)D<12 ng/ml, the two methods agreed in 95 (73%) samples; Cohen's κ was 0.469 (p<0.001).

3.3.6 Reliability of LC-MS/MS and vitamin D epimers

Epimers of 25(OH)D were not found in any of the measured samples. The biologically inactive C3-epimer did therefore not contribute to the vitamin D levels assessed by LC-MS/MS [1].

3.3.7 Diagnostic use and utility

Diagnostic characteristics of both LC-MS/MS and ECLIA were assessed in samples obtained intra- and postoperatively, i.e. those from time points t2 – t5 (n=104). The chosen gold standard for comparison was vitamin D deficiency at baseline, i.e. a 25(OH)D level below 20 ng/ml measured by LC-MS/MS at t1.

The use of 20 ng/ml as a cut-off following fluid loading would lead to low specificity with both ECLIA and LC-MS/MS (Specificity 63% and 13%, respectively) and to concomitant high rates of over-diagnosis (indicated by PPVs of 76% and 64%, respectively). In contrast, use of a cut-off value of 12/ ng/ml would result in low sensitivity, but higher specificity for ECLIA and LC-MS/MS alike (98% and 85%, respectively) (**Table 21**) [1].

	Sensitivity	**Specificity**	**PPV**	**NPV**
ECLIA				
25(OH)D<20 ng/ml	73%	63%	76%	60%
25(OH)D<12 ng/ml	38%	98%	96%	49%
LC-MS/MS				
25(OH)D<20 ng/ml	98%	13%	64%	83%
25(OH)D<12 ng/ml	72%	85%	89%	65%

Table 21 Diagnostic characteristics of ECLIA and LC-MS/MS used with different cut-off values for the diagnosis of vitamin D deficiency. Derived from [1] under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); changed layout.

4 DISCUSSION

The present dissertation and the underlying research project shed light on vitamin D and its metabolites in critically ill patients, their "normal" levels in intensive care, changes to be expected throughout critical illness and its treatment and how to best assess these values both in scientific and clinical practice. There has been a clear need for additional insights into these issues, as the previously existing body of evidence has been insufficient to guide clinicians and researchers in their decision making.

4.1 Answers to research questions

RQ1 What vitamin D metabolite should be used to assess vitamin D status in critically ill patients?

The most commonly used metabolite to assess vitamin D status is 25-hydroxy-vitamin D. Its use seems prudent, since it represents the storage form of vitamin D that makes up for most of the vitamin D in the human body and has a half-life of a few weeks. More or less clear associations between 25(OH)D levels and several health-related endpoints have been drawn in the past, mostly in non-critically ill patients. However, it can be hypothesised that unwanted effects of what is effectively a hormone deficiency would mostly stem from low levels of the effective metabolite 1,25-dihydroxy-vitamin D. That would especially be the case if levels of 25(OH)D and $1,25(OH)_2D$ were to diverge significantly in critically ill patients.

This study indeed demonstrates that 25(OH)D and $1,25(OH)_2D$ follow different patterns with regards to their changes over time in the perioperative phase and during intensive care. While levels of both metabolites are reduced by fluid loading and inflammation (discussed in answer to RQ2), 25(OH)D levels recover relatively predictably after around two days after the initial insult, while $1,25(OH)_2D$ levels remain depressed, as does its major regulator, parathyroid hormone: there appears to be no immediate compensatory counter-regulation of parathyroid hormone or immediate effect on bone metabolism as assessed by alkaline phosphatase, beta cross laps and osteocalcin.

With that in mind it is to be recommended to use 25(OH)D as a marker of vitamin D status in critically ill patients. It represents the metabolite best evaluated for this purpose in the general

population and its association with outcomes has repeatedly been demonstrated in critical care cohorts. Further research is needed in the future to draw definitive conclusions whether there are similar – or even more pronounced – associations for $1,25(OH)_2D$ levels and outcomes as well.

RQ2 What vitamin D levels are to be expected in intensive care patients and how do they change over the course of critical illness and its treatment?

Vitamin D deficiency may be common in patients planned to undergo major cardiac surgery on cardiopulmonary bypass already preoperatively. In this investigation, median serum 25(OH)D levels have been found to be 19.8 ng/ml (12.1-25.0 ng/ml) at baseline before surgery. This finding lends itself to further research into possible ways of best possible preparation for major surgery possibly leading to outcome improvements for this select patient group in the future.

More importantly though, median vitamin D levels on ICU admission – that is, following fluid loading due to cardiopulmonary bypass and inflammation due to a major surgical procedure – have been found to be significantly lower than at baseline, at 12.1 ng/ml (8.8-15.1 ng/ml) in this study. This is in line with findings from previous studies that report vitamin D deficiency in critically ill patients to be even more frequent than in the overall population [136,151].

Comparison of measurements performed before and after surgery imply that these findings however may be overestimated. Fluid loading and inflammation have been found to lead to an immediate, but variable reduction of 25(OH)D levels by approximately 40% in this study. If vitamin D status would regularly be assessed upon admission to intensive care units, prevalence of deficiency may thus be overestimated, at least after major surgery. After the first phase of immediate fluid loading and inflammation, 25(OH)D levels increase again even without addition of exogenous vitamin D. However, they do not seem to fully return to baseline within the observation period of 48 hours.

The active metabolite of vitamin D, $1,25(OH)_2D$, follows a different pattern of change over the course of the perioperative phase and intensive care treatment. While $1,25(OH)_2D$ levels are immediately reduced by fluid loading and inflammation as well, they do not seem to recover at all during the observed phase of early critical illness. This is in line with previous studies exploring this topic [173].

RQ3 Can possible changes in vitamin D status be predicted and can premorbid vitamin D levels be inferred from measurements performed later on?

It would be desirable for health care providers to be able to infer pre-morbid vitamin D status from measurements performed during critical illness to work around the effects of haemodilution and inflammation described above. Several measurements to assess both fluid status and inflammatory status are routinely performed in intensive care medicine to guide decision making and treatments:

Volume of fluid applied perioperatively and during resuscitation in critical illness and fluid output are closely monitored. Fluid balance – the difference of fluid input and output – is a parameter routinely used by clinicians to guide treatment with regards to volume status. Markers of inflammation – especially leukocyte count and C-reactive protein – are laboratory markers usually measured daily in intensive care units to assess inflammatory status and to monitor the effectivity of anti-infective treatments.

Findings from this study suggest that inference of pre-morbid vitamin D status from downstream measurements corrected for fluid balance and markers of inflammation is not viable in critically ill patients. 25(OH)D levels correlated too poorly with fluid balance, white blood count and CRP to allow for re-calculation of vitamin D levels before fluid loading and inflammation.

RQ4 What cut-off values should be used to diagnose vitamin D deficiency in critically ill patients?

Cut-off values for 25(OH)D to define vitamin D deficiency in the general population are derived from studies examining multiple health-related endpoints that are not necessarily as relevant in critically ill patients. 25(OH)D is used as a marker for vitamin D status since it represents the storage form of vitamin D. This presumed stability is not evident in critical illness, when large fluid shifts and systemic inflammation occur, as demonstrated in this study.

Using the now generally agreed upon cut-off value of 25(OH)D<20 ng/ml for diagnosis of vitamin D deficiency in intensive care would lead to significant overdiagnosis. Specificity for this definition has been found to be as low as 13% in this study if LC-MS/MS is used as the

measurement technique. Such a high false-positive rate can neither be accepted in research environments nor in everyday clinical practice.

A stricter definition with a cut-off value of 25(OH)D<12 ng/ml has physiological rationale, as levels as low as this are invariably associated with disease states such as rickets in children; patients with 25(OH)D levels this low have also been found to benefit from correction of vitamin D deficiency during critical care in the VITdAL-ICU trial [160]. Results of the present investigation support the use of such a narrower definition of vitamin D deficiency; specificity for has been found to be 85% or 98% if LC-MS/MS or ECLIA are used, respectively.

Such a strict definition obviously is associated with decreased sensitivity of the tests applied. Indeed, true-positive rates of only 72% or 28% have been found for LC-MS/MS and ECLIA with this cut-off value in this study. It seems reasonable to trade sensitivity for specificity in these circumstances, though. Every intervention that has yet to prove benefit for patients should always be applied to those who might benefit most initially. This is also the case in treatment of vitamin D deficiency in critically ill patients [166].

RQ5 Which measurement methods should be employed to assess vitamin D status in intensive care patients?

With increasing availability of liquid chromatography – mass spectrometry for both scientific and clinical purposes, this method has become the accepted gold standard in the assessment of vitamin D status in the overall population. The use of LC/MS-MS is still relatively slow and expensive, though, and it might delay testing if samples have to be transported to external laboratories or if a waiting period has to be passed to maximise the number of samples to be measured during one run of LC-MS/MS.

Modern immunoassays for vitamin D measurement are readily available and comparably cheap, therefore their use would notably aid clinical and scientific practice. Their validity has been questioned in critically ill patients and other select patient populations though. On the other hand, many LC-MS/MS test kits may also detect the biologically inactive C3-epimer and would overestimate vitamin D status, if the epimer was present in sizeable amounts.

This study has also assessed the occurrence of 3-epi-25(OH)D in critically ill patients and has compared the gold standard of LC-MS/MS and ECLIA, a popular electrochemiluminescence assay, to test its validity in intensive care medicine. Contrary to what previous experience might have suggested, no C3-epimers have been found in the measured samples. This is one more argument for LC-MS/MS as the gold standard of vitamin D measurement and extends this role into the field of critical care.

The validity of the electrochemiluminescence assay, on the contrary, has been found to be lacking when compared to LC-MS/MS in the setting of critical illness. Agreement between the methods both for measured values and for the categorical diagnosis of vitamin D deficiency was limited in all conducted comparative analyses. Sole use of these assays for assessment of vitamin D status can therefore not be recommended; if available, LC-MS/MS should preferably be employed to measure vitamin D levels in critically ill patients.

Immunoassays can still be employed for screening purposes in this patient population when used together with stricter cut-off values as described in the above section. This study demonstrates that ECLIA has 98% specificity and thus 96% positive predictive value for pre-morbid vitamin D deficiency assessed by the gold standard when used with a cut-off value of 25(OH)D<12 ng/ml even after fluid loading and during inflammation.

4.2 Implications for clinical practice

Although vitamin D deficiency in critically ill patients has received considerable scientific interest in the last years, no definitive recommendations for everyday clinical practice in the setting of perioperative medicine and critical care have been issued as of yet.

Guidelines aimed at "patients at risk for vitamin D deficiency" [108] suggest adults between 19 and 70 years of age require at least 600 IU of vitamin D per day to maximize bone health and muscle function. Adults aged 70 years and above are said to require at least 800 IU of vitamin D per day to achieve the same goal. It is emphasised that at least 1500 to 2000 IU per day are needed to raise blood levels of 25(OH)D above 30 ng/ml consistently in all adults.

The current European Society for Clinical Nutrition and Metabolism (ESPEN) guideline on clinical nutrition in the intensive care unit [157] states that "in critically ill patients with measured low plasma levels (25-hydroxy-vitamin D<12.5 ng/ml, or 50nmol/l) vitamin D_3 can be supplemented" and that "in critically ill patients with measured low plasma levels (25-hydroxy-vitamin D<12.5 ng/ml, or 50nmol/l) a high dose of vitamin D_3 (500,000 IU) as a single dose can be administered within a week after admission". These recommendations are categorised as a good practice point and grade 0 recommendation, respectively.

The latter recommendation is primarily based on findings of the VITdAL-ICU trial [160]. While the proposed dose of vitamin D to be used for treatment of vitamin D deficiency in intensive care units is very similar to the regimen used in this study, the 25(OH)D cut-off value is considerably lower than the one used in the trial (25(OH)D<20 ng/ml). However, a statistically significant benefit in mortality has only been found in a subgroup of severely deficient patients (25(OH)D<12 ng/ml), leading to this recommendation.

Findings from the current study reinforce current ESPEN recommendations regarding cut-off values for supplementation and treatment by providing additional evidence on usual changes in vitamin D levels during critical illness. Levels of circulating 25(OH)D have been shown to decrease during inflammation and fluid loading; it is therefore prudent to use lower values than in the general population to avoid overdiagnosis. A cut-off of 12 ng/ml was associated with high specificity for pre-existing vitamin D-deficiency in this study.

Furthermore, this study adds knowledge on how to measure vitamin D in critically ill patients that should be considered in everyday clinical practice and should be addressed in future

iteration of relevant guidelines. Readily available, comparably fast and cheap assays such as the ECLIA used in this study make expeditious assessment of vitamin D status possible. They are, however, notably inaccurate as demonstrated in this study. Their availability and speed still make them a worthwhile option for diagnostics, especially when swift decision making is imperative, as in critical illness. For more precise measurements, the use of LC-MS/MS should certainly be preferred. The superiority of this measurement technique has been reinforced in this study by the finding that its results do not seem to be distorted by the biologically inactive C3-epimer in critically ill patients.

In addition to the "how" to diagnose vitamin D deficiency in intensive care medicine, this study has also shed light on the question as to "when" to measure. This is important, since critical illness can usually not be anticipated apart from major elective surgical procedures requiring intensive care postoperatively. While in this select patient group, it would be possible to measure vitamin D levels during preoperative evaluation, as done in this study, this approach is not applicable to the majority of patients who are admitted to intensive care units due to acute illness and injury or emergency surgical procedures.

Findings of this study suggest not to measure vitamin D levels directly at ICU admission, when systemic inflammation may be at its peak, both due to underlying pathology and due to surgical interventions, and when requirements for fluid resuscitation may be the highest. It seems more prudent to postpone blood collection and measurement for 24 to 48 hours following an impactful event to be able to assess vitamin D status relatively reliably.

4.3 Implications for future research

The aforementioned paucity of high-quality evidence to support recommendations regarding vitamin D supplementation or even high-dose therapy in critically ill patients makes further research in the area inevitable. With most current knowledge derived from retrospective studies and a single randomised controlled trial [160] there is an urgent need for further interventional studies. At the time of writing, two randomised controlled trials have been designed to investigate vitamin D therapy in critically ill patients:

The European VITDALIZE study (ClinicalTrials.gov identifier NCT03188796) is a follow-up trial to the previous VITDAL-ICU trial expanding the Austrian network to Belgium, Germany and the UK with a substantially larger sample size (planned 2400). It aims to assess the impact of high-dose cholecalciferol treatment on 28-day all-cause mortality (primary endpoint) and morbidity (hospital length of stay, hypercalcemia and hospital readmissions) in critically ill patients. The trial is conducted at academic and non-academic hospitals in several European countries and steered from the study centre at the Medical University of Graz, Austria.

In this study, adult patients who have been admitted to ICU for 72 hours or less upon screening and who are expected to require further critical care for at least 48 hours are included, if they are found to be vitamin D deficient. The cut-off for vitamin D deficiency chosen for this trial is 25(OH)D≤12 ng/ml. Participating units may use whatever measurement method is available in their respective institutions; if assays with low sensitivity are used, patients may also be included if they fulfil the aforementioned inclusion criteria and have undetectable levels of 25(OH)D.

Similarly, the American VIOLET (Vitamin D to Improve Outcomes by Leveraging Early Treatment) trial (ClinicalTrials.gov identifier NCT03096314) has aimed to “assess the efficacy and safety of early administration of cholecalciferol in reducing mortality and morbidity” in vitamin D deficient patients. This study has been confined to patients at “high risk for ARDS and mortality”, though. Patient recruitment has been conducted in emergency departments, hospital wards, operating rooms and intensive care units in hospitals participating in the PETAL (Prevention & Early Treatment of Acute Lung Injury) Network.

Measurements of 25(OH)D levels for screening purposes have been conducted either by hospital laboratories using whatever measurement method is availably or a point-of-care device

(FastPack IP, Qualigen Inc). The cut-off value for patients to be considered vitamin D deficient and therefore to be randomised and included into the study has been 25(OH)D<20 ng/ml. Study medication has been applied enterally, i.e. either orally or via a nasogastric or orogastric tube. Half of randomised patients have received high-dose cholecalciferol (540,000 units), the other half receive a placebo.

At the time of writing, the VITDALIZE study is recruiting patients in centres in Austria, and Belgium and has included several hundreds of patients. As a pragmatic trial, its end-date cannot yet be foreseen. On the contrary, the VIOLET trial has been terminated following its first interim analysis on October 6th, 2018, barely a year after start of patient inclusion. At this timepoint, 1360 patient had been included into the study, still short of fifty percent of the originally estimated 3000 patients to be recruited. The trial's results have finally been published on December 26th, 2019 [165].

While high-dose enteral supplementation of cholecalciferol has again been proven to be effective in correcting vitamin D deficiency in critically ill patients, as demonstrated by the fact that mean levels of 25(OH)D at day 3 were 46.9±23.2 ng/ml in the intervention group and only 11.4±5.6 ng/ml in the placebo group, a patient-oriented benefit in outcomes has not been demonstrated; 90-day all-cause, all-location mortality as the primary endpoint of interest in this study has been found to be 23.5% in the vitamin D group and 20.6% in the placebo group (p=0.26).

Moreover, mortality rates have been reported to even be higher in the vitamin D group than in the placebo group in the subgroups of patients with sepsis or infection, facility residence, pneumonia, infection and pre-randomisation acute respiratory distress syndrome. Notably, none of all deaths in this trial have been were causally linked to vitamin D treatment; all pre-specified adverse events related to the application of vitamin D, i.e. hypercalcemia, kidney stones, fall-related fractures, have not differed between the treatment group and the placebo group.

This apparent futility of promising interventions in randomised-controlled trials is a common occurrence in interventional studies in intensive care medicine. A study from 2008 has found that out of 72 multicentre randomised controlled trials carried out in adult patients in intensive care units, 55 (76%) fail to demonstrate any statistically significant impact on mortality as the

primary outcome [174]. In trials on ARDS – a key inclusion criterion for the VIOLET study – the rate of positive interventional studies might even be as low as 9% [175].

A notable difference between these two large RCTs on vitamin D in critically ill patients is obviously the cut-off values for vitamin D deficiency and therefore inclusion of patients: while the terminated VIOLET trial uses 25(OH)D<20 ng/ml as its definition, the still ongoing VITDALIZE study has chosen 25(OH)D<12 ng/ml as its criterion. Findings from this study certainly support the lower cut-off value as the more suitable definition for vitamin D deficiency in critically ill patients.

This approach allows for the inclusion of patients who are more likely to be vitamin D deficient by usual definitions before ICU admission and associated interventions and thus makes it more likely that the intervention aids those who might benefit most from it. This has been proposed as a key necessity for vitamin D trials to succeed in intensive care medicine [166].

4.4 Strengths and limitations

This study project is a pilot study in a relatively unexplored area of intensive care medicine and is thus subject to several limitations.

Its pilot character has allowed for little pre-emptive calculations, especially concerning sample size, so several estimations have served as the basis for the study protocol. These estimates could not be reached in their entirety within the constraints of study time and funding; the final sample size is therefore lower than what was considered desirable and achievable during planning of the study. Nevertheless, this investigation is still based on the largest cohort of patients included in a study on this very topic and therefore gives the most comprehensive insight into changes of vitamin D status after major cardiac surgery and in critical illness.

This study has aimed to describe usual changes in vitamin D status during fluid loading and critical illness in adult patients in general, yet the included patient cohort encompassed patients undergoing major cardiac surgery requiring cardio-pulmonary bypass only. While this may somewhat reduce generalisability of results, the decision to solely include this patient group was deemed to be without any alternative to make patient education and informed consent ahead of fluid possible loading and guarantee intensive care unit admission following the surgical procedure. Of note, the low mortality rate observed in this patient cohort is also not be representative of critically ill patients in general.

A major strength of this study is the parallel use of the measurement technique that is considered the gold standard in vitamin D assessment (LC-MS/MS) and a more affordable and thus more readily available test assay (ECLIA). This not only allows for comparison of the two measurement methods in critically ill patients, but makes immediate translation of this study's findings into clinical practice possible. The sole use of the gold standard would preclude the application of these results in many institutions, since LC-MS/MS is still not readily available in most hospitals outside of research environments.

A major limitation is that findings in elective major cardiac surgery patients may not be transferable to medical or neurological ICU patients with different baseline criteria and different treatment approaches.

4.5 Conclusion

Diagnosis of vitamin D deficiency in states of fluid loading and inflammation, such as the perioperative period and critical illness, can be complicated by haemodilution. Inference of baseline values from downstream measurements is not reliably possible.

Stricter definitions, such as serum 25(OH)D levels lower than 12 ng/ml may therefore be used to diagnose vitamin D deficiency with a substantially lower false-positive rate compared to the previously used cut-off of 20 ng/ml.

Measurements by commonly available chemiluminescence assays may deviate noticeably from the mass spectrometry gold standard; however, they are acceptably reliable in the acute setting when vitamin D deficiency has to be diagnosed rapidly.

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6 APPENDIX

6.1 **Initial ethics committee approval**

Ethikkommission

Medizinische Universität Graz

Auenbruggerplatz 2, A-8036 Graz
ethikkommission@medunigraz.at
Tel.: +43 / 316 / 385-13928, Fax: -14348

VOTUM
gültig bis 27.02.2016

EK-Nummer:	27-161 ex 14/15
Studientitel:	VITdAL-ICU DIAGNOSIS: prospective evaluation of diagnosis of vitamin D deficiency in critical care
Prüfer:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein Medizinische Universität Graz
Sponsor:	Klin. Abt. f. Endokrinologie u. Stoffwechsel, Univ.-Klin. f. Innere Medizin
Ansprechpartner:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein, 8036 Graz, Auenbruggerplatz 2
CRO:	-
Antragsteller:	Univ.-Klin. f. Anästhesiologie u. Intensivmedizin
Ansprechpartner:	Dr. Paul Zajic, 8036 Graz, Auenbruggerplatz 29

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 08.01.2015 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 26.12.2014, begutachtet im 'expedited Review' am 08.01.2015		
✓	Antragsformular ECS	26.12.2014
	Originalprotokoll Study Protocol VITdAL ICU DIAGNOSIS v10 1.0	22.12.2014
	Informed Consent Form Consent Form VITdAL-ICU DIAGNOSIS v10 1.0	24.12.2014
Dokumente eingegangen am 12.02.2015 (in der nächsten Begutachtung mitbegutachtet)		
✓	Antragsformular ECS unterschrieben	15.01.2015
	Originalprotokoll 1.2	12.02.2015
✓	Informed Consent Form 1.1	12.02.2015
✓	Sonstiges: Sponsorerklärung undatiert	
✓	Sonstiges: Stellungnahme zur Bearbeitungsmitteilung undatiert	
Dokumente eingegangen am 26.02.2015, begutachtet im 'expedited Review' am 27.02.2015		
✓	Originalprotokoll 1.3	25.02.2015

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Es handelt sich um eine Studie im Rahmen einer Dissertation.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
UID: ATU 575 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 948 400 04, Raiffeisen Landesbank Steiermark BLZ 38000 Konto-Nr. 49510.

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

Dieses Votum gilt für ein Jahr ab dem Datum der Ausstellung. Bei längerer Studiendauer ist rechtzeitig vor Ablauf der Gültigkeit des Votums ein Zwischenbericht vorzulegen (Berichtsformular), um eine etwaige Verlängerung zu erlangen.

Graz, 27. Februar 2015

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.DDr.Hans-Peter Kapfhammer
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
UID: ATU 575 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 948 400 04, Raiffeisen Landesbank Steiermark BLZ 38000 Konto-Nr. 49510.

6.2 **Ethics committee approval of amended protocol**

Ethikkommission

Medizinische Universität Graz

Auenbruggerplatz 2, A-8036 Graz
ethikkommission@medunigraz.at
Tel.: +43 / 316 / 385-13928, Fax: -14348

FOLGEVOTUM

gültig bis 27.02.2016

EK-Nummer:	27-161 ex 14/15
Studientitel:	VITdAL-ICU DIAGNOSIS: prospective evaluation of diagnosis of vitamin D deficiency in critical care
Prüfer:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein Medizinische Universität Graz
Sponsor:	Univ.Klinik für Innere Medizin
Ansprechpartner:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein, 8036 Graz, Auenbruggerplatz 15
CRO:	-
Antragsteller:	Univ.-Klin. f. Anästhesiologie u. Intensivmedizin
Ansprechpartner:	Dr. Paul Zajic, 8036 Graz, Auenbruggerplatz 29

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 08.01.2015 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 26.12.2014, begutachtet im 'expedited Review' am 08.01.2015	
✓ Antragsformular ECS	26.12.2014
Originalprotokoll Study Protocol VITdAL ICU DIAGNOSIS v10 1.0	22.12.2014
Informed Consent Form Consent Form VITdAL-ICU DIAGNOSIS v10 1.0	24.12.2014
Dokumente eingegangen am 12.02.2015 (in der nächsten Begutachtung mitbegutachtet)	
✓ Antragsformular ECS unterschrieben	15.01.2015
Originalprotokoll 1.2	12.02.2015
✓ Informed Consent Form 1.1	12.02.2015
✓ Sonstiges: Sponsorerklärung undatiert	
✓ Sonstiges: Stellungnahme zur Bearbeitungsmitteilung undatiert	
Dokumente eingegangen am 26.02.2015, begutachtet im 'expedited Review' am 27.02.2015	
✓ Originalprotokoll 1.3	25.02.2015
Dokumente eingegangen am 13.04.2015, begutachtet im 'expedited Review' am 22.04.2015	
✓ Originalprotokoll 1.4	12.04.2015
✓ Informed Consent Form 1.2	13.04.2015
✓ Sonstiges: Stellungnahme zum Amendment Protokoll undatiert	

Datum Erstvotum: 27.02.2015

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Es handelt sich um eine Studie im Rahmen einer Dissertation.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

Graz, 22. April 2015

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.DDr.Hans-Peter Kapfhammer
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at

6.3 **Extended ethics committee approval**

Ethikkommission

Medizinische Universität Graz

Auenbruggerplatz 2, A-8036 Graz
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Tel.: +43 / 316 / 385-13928, Fax: -14348

FOLGEVOTUM
gültig bis 27.02.2017

EK-Nummer:	27-161 ex 14/15
Studientitel:	VITdAL-ICU DIAGNOSIS: prospective evaluation of diagnosis of vitamin D deficiency in critical care
Prüfer:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein Medizinische Universität Graz
Sponsor:	Univ.Klinik für Innere Medizin
Ansprechpartner:	Ass.-Prof. Priv.-Doz. Dr. Karin Amrein, 8036 Graz, Auenbruggerplatz 15
CRO:	-
Antragsteller:	Univ.-Klin. f. Anästhesiologie u. Intensivmedizin
Ansprechpartner:	Dr. Paul Zajic, 8036 Graz, Auenbruggerplatz 29

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 08.01.2015 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 26.12.2014, begutachtet im 'expedited Review' am 08.01.2015		
✓	Antragsformular ECS	26.12.2014
	Originalprotokoll Study Protocol VITdAL ICU DIAGNOSIS v10 1.0	22.12.2014
	Informed Consent Form Consent Form VITdAL-ICU DIAGNOSIS v10 1.0	24.12.2014
Dokumente eingegangen am 12.02.2015 (in der nächsten Begutachtung mitbegutachtet)		
✓	Antragsformular ECS unterschrieben	15.01.2015
	Originalprotokoll 1.2	12.02.2015
✓	Informed Consent Form 1.1	12.02.2015
✓	Sonstiges: Sponsorerklärung undatiert	
✓	Sonstiges: Stellungnahme zur Bearbeitungsmitteilung undatiert	
Dokumente eingegangen am 26.02.2015, begutachtet im 'expedited Review' am 27.02.2015		
✓	Originalprotokoll 1.3	25.02.2015
Dokumente eingegangen am 13.04.2015, begutachtet im 'expedited Review' am 22.04.2015		
✓	Originalprotokoll 1.4	12.04.2015
✓	Informed Consent Form 1.2	13.04.2015
✓	Sonstiges: Stellungnahme zum Amendment Protokoll undatiert	
Dokumente eingegangen am 09.02.2016, begutachtet im 'expedited Review' am 15.02.2016		
✓	Zwischenbericht	09.02.2016

Datum Erstvotum: 27.02.2015

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung

nach AMG bzw. MPG handelt.

Es handelt sich um eine Studie im Rahmen einer Dissertation.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

Graz, 15. Februar 2016

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

SIGNATURE REMOVED IN THIS DISSERTATION

Univ.Prof.Dr.Hermann Toplak
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

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6.4 **Patient information and consent form**

PATIENTINNENINFORMATION UND EINWILLIGUNGSERKLÄRUNG ZUR TEILNAHME AN DER BEOBACHTUNGSSTUDIE

VITDAL-ICU DIAGNOSIS

Prospektive Evaluierung der Diagnose des Vitamin D-Mangels in der Intensivmedizin

Sehr geehrte Teilnehmerin, sehr geehrter Teilnehmer!

Wir laden Sie ein an der oben genannten Beobachtungsstudie teilzunehmen. Die Aufklärung darüber erfolgt in einem ausführlichen Gespräch.

Ihre Teilnahme an dieser Studie erfolgt freiwillig. Sie können jederzeit ohne Angabe von Gründen aus der Studie ausscheiden. Die Ablehnung der Teilnahme oder ein vorzeitiges Ausscheiden aus dieser Studie hat keine nachteiligen Folgen für Ihre medizinische Betreuung.

Beobachtungsstudien sind Studien, bei denen in der Regel nur Daten aufgezeichnet und ausgewertet werden, die im Rahmen der normalen Patientenversorgung anfallen. In manchen Fällen kann es auch sein, dass zusätzliche, nicht belastende Untersuchungen oder Befragungen vorgenommen werden. In keinem Fall wird die für Sie vorgesehene Behandlung durch Ihre Studienteilnahme verändert. Beobachtungsstudien sind notwendig, um zusätzliche Erkenntnisse über bereits bewährte medizinische Verfahren zu gewinnen.

Zu dieser Beobachtungsstudie, sowie zur Patienteninformation und Einwilligungserklärung wurde von der zuständigen Ethikkommission eine befürwortende Stellungnahme abgegeben.

WAS IST DER ZWECK DIESER STUDIE?

Der Zweck dieser Beobachtungsstudie ist es, den Verlauf der Blutkonzentration von Vitamin D über die Dauer großer Operationen und der Behandlung auf Intensivstationen hinweg nachvollziehen zu können. Vitamin D ist ein für viele körpereigene Prozesse wichtiger Nährstoff, der vermutlich starken Schwankungen während der intensivmedizinischen Betreuung unterliegt. Sie werden um die Teilnahme an dieser Studie gebeten, da für Sie eine Behandlung an der Intensivstation im Anschluss an Ihre Operation vorgesehen ist.

WIE LÄUFT DIE BEOBACHTUNGSSTUDIE AB?

Diese Studie wird an unserem Klinikum durchgeführt, es werden insgesamt ungefähr 100 Personen daran teilnehmen. Ihre Teilnahme wird sich über die Dauer Ihrer Behandlung an unserer Klinik erstrecken.

Folgende Maßnahmen werden ausschließlich aus Studiengründen durchgeführt:

Blutproben werden zu verschiedenen Zeitpunkten Ihres Krankenhausaufenthalts abgenommen und analysiert. Die erste Analyse erfolgt bereits im Rahmen der präoperativen Durchuntersuchung. Mehrheitlich wird versucht, aus diesen Blutproben auch sämtliche anderen Laboruntersuchungen, die im Rahmen Ihrer medizinischen Betreuung notwendig werden, durchzuführen. Sollte dies nicht möglich sein, werden insgesamt bis zu 100ml Blut – das entspricht etwa der Menge von 20 Teelöffeln – abgenommen. Diese Menge ist für Sie ungefährlich.

Worin liegt der Nutzen einer Teilnahme an der Beobachtungsstudie?

Es ist nicht zu erwarten, dass Sie aus Ihrer Teilnahme an dieser Studie unmittelbaren gesundheitlichen Nutzen ziehen werden. Nach Abschluss der Studie kann der Sie behandelnde Arzt bzw. die Sie behandelnde Ärztin jedoch etwaige Mangelzustände ausgleichen oder vorbeugende Maßnahmen treffen. Auch ein Screening auf Laktoseintoleranz kann Ihnen aus dem für die Studie abgenommenen Blut angeboten werden. Eine finanzielle Vergütung Ihrer Teilnahme erfolgt nicht.

Gibt es Risiken, Beschwerden und Begleiterscheinungen?

Nein.

In welcher Weise werden die im Rahmen dieser Beobachtungsstudie gesammelten Daten verwendet?

Sofern gesetzlich nicht etwas anderes vorgesehen ist, haben nur die Studienärzte und deren Mitarbeiter Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden („personenbezogene" Daten). Weiters können ggf. Beauftragte von in- und ausländischen Gesundheitsbehörden, der zuständigen Ethikkommission und Personen, die vom Studienleiter der Studie mit der Kontrolle der Datenqualität beauftragt wurden, Einsicht in diese Daten nehmen, um die Richtigkeit der Aufzeichnungen zu überprüfen. Diese Personen sind zur Verschwiegenheit verpflichtet.

Die Weitergabe der Daten erfolgt ausschließlich zu statistischen Zwecken und Sie werden ausnahmslos nicht namentlich genannt. Auch in etwaigen wissenschaftlichen Veröffentlichungen der Daten dieser Studie werden Sie nicht namentlich genannt.

Die Bestimmungen des Datenschutzgesetzes in der geltenden Fassung werden eingehalten.

Möglichkeit zur Diskussion weiterer Fragen

Für weitere Fragen im Zusammenhang mit dieser Studie stehen Ihnen Ihr Studienarzt und seine Mitarbeiter gern zur Verfügung.

Assoz. Prof. Priv.-Doz. Dr. Karin Amrein, MSc

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EINWILLIGUNGSERKLÄRUNG

Name des Patienten in Druckbuchstaben .....

Geb.Datum: ..... Code: .....

Ich habe dieses Informationsblatt gelesen und verstanden. Alle meine Fragen wurden beantwortet und ich habe zurzeit keine weiteren Fragen mehr.

Mit meiner persönlich datierten Unterschrift gebe ich hiermit freiwillig mein Einverständnis, dass meine Daten gespeichert und ohne direkten Personenbezug für wissenschaftliche Zwecke verwendet werden dürfen. Mir ist bekannt, dass zur Überprüfung der Richtigkeit der Datenaufzeichnung Beauftragte der zuständigen Behörden und der Ethikkommission, sowie mit der Kontrolle der Datenqualität beauftragte Personen Einblick in meine personenbezogenen Krankheitsdaten nehmen dürfen.

Ich weiß, dass ich diese Zustimmungen jederzeit und ohne Angabe von Gründen widerrufen kann.

Eine Kopie dieser Patienteninformation und Einwilligungserklärung habe ich erhalten. Das Original verbleibt beim Studienarzt.

.....

(Datum und Unterschrift des Patienten)

.....

(Datum, Name und Unterschrift des verantwortlichen Arztes)

(Der Patient erhält eine unterschriebene Kopie der Patienteninformation und Einwilligungserklärung, das Original verbleibt im Studienordner des Studienarztes.)

6.5 **Case Report Form (CRF)**

VITDAL-ICU DIAGNOSIS **CASE REPORT FORM** **V1.0, BLATT 1**

Blatt 1 und 2 des CRF sind nach Aufklärung und Einschluss des/der Patient/in im Rahmen der präoperativen Untersuchung anzulegen.

PATIENTENETIKETT	**BASISDEMOGRAPHISCHE DATEN**
	Größe: ..... cm Gewicht: ..... kg Populations-gruppe: ◻ kaukasisch ◻ arabisch ◻ asiatisch ◻ schwarzafrikanisch ◻ ..... Lebens-situation: ◻ unabhängig ◻ häusliche Pflege ◻ institutionelle Pflege

PRÄOPERATIVE PHASE **PDU-DATUM:**

Operations-Indikation: _____

Geplante Operation: _____

ASA-Score: ◻ 1 ◻ 2 ◻ 3 ◻ 4 ◻ 5

Osteoporose: ◻ nein ◻ ja

Niereninsuffizienz: ◻ nein ◻ ja

Leberschaden: ◻ nein ◻ ja

Epilepsie: ◻ nein ◻ ja

Vitamin D-Substitution: ◻ nein ◻ ja ..... IE/d

Blatt 1 ist im Anschluss im Ordner „VITDAL-ICU DIAGNOSIS Investigator Site File PDU" abzulegen, Blatt 2 der Krankenakte beizulegen.

GESAMTVERLAUF wird durch Studienteam ausgefüllt!

ZEITLICHER VERLAUF

KH-Aufnahme: _____

ICU-Aufnahme: _____

ICU-Entlassung: _____

KH-Entlassung: _____

MEDIZINISCHER VERLAUF

Entlassungs-zustand: ◻ lebend ◻ verstorben

Entlassungs-zielort: ◻ Eigenheim ◻ Pflegeheim ◻ Krankenanstalt

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VITDAL-ICU DIAGNOSIS **CASE REPORT FORM** **V1.0, BLATT 2**

PATIENTENETIKETT

INTRAOPERATIVE PHASE

OP-DATUM:

ANÄSTHESIEFREIGABE, OPERATIONSBEGINN **ABNAHME-ZEITPUNKT:**

Flüssigkeitseinfuhr: ..... ml Flüssigkeitsausfuhr: ..... ml

Katecholamingabe: ◻ nein ◻ ja Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

ABGEHEN VON HERZ-LUNGEN-MASCHINE **ABNAHME-ZEITPUNKT:**

Flüssigkeitseinfuhr: ..... ml Flüssigkeitsausfuhr: ..... ml

Katecholamingabe: ◻ nein ◻ ja Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

OPERATIONSENDE, TRANSFER AN INTENSIVSTATION **ABNAHME-ZEITPUNKT:**

Flüssigkeitseinfuhr: ..... ml Flüssigkeitsausfuhr: ..... ml

Katecholamingabe: ◻ nein ◻ ja Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

Blatt 2 ist nach dem Transfer an die Intensivstation im Ordner „VITDAL-ICU DIAGNOSIS Investigator Site File ICU" abzulegen.

POSTOPERATIVE PHASE

POSTOPERATIVER TAG 1 **ABNAHME-ZEITPUNKT:**

Flüssigkeit: Einfuhr: ..... ml Ausfuhr: ..... ml Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Atmungsform: ◻ spontan ◻ assistiert ◻ kontrolliert Katecholamingabe: ◻ nein ◻ ja

Inflammation: ◻ nein ◻ Pneumonie ◻ Harnwegsinfekt ◻ SIRS ◻ Sepsis ◻ .....

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

POSTOPERATIVER TAG 2 **ABNAHME-ZEITPUNKT:**

Flüssigkeit: Einfuhr: ..... ml Ausfuhr: ..... ml Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Atmungsform: ◻ spontan ◻ assistiert ◻ kontrolliert Katecholamingabe: ◻ nein ◻ ja

Inflammation: ◻ nein ◻ Pneumonie ◻ Harnwegsinfekt ◻ SIRS ◻ Sepsis ◻ .....

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

POSTOPERATIVER TAG 3 **ABNAHME-ZEITPUNKT:**

Flüssigkeit: Einfuhr: ..... ml Ausfuhr: ..... ml Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Atmungsform: ◻ spontan ◻ assistiert ◻ kontrolliert Katecholamingabe: ◻ nein ◻ ja

Inflammation: ◻ nein ◻ Pneumonie ◻ Harnwegsinfekt ◻ SIRS ◻ Sepsis ◻ .....

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

POSTOPERATIVER TAG 4 **ABNAHME-ZEITPUNKT:**

Flüssigkeit: Einfuhr: ..... ml Ausfuhr: ..... ml Vitamin D-Gabe: ◻ nein ◻ ja ..... IE

Atmungsform: ◻ spontan ◻ assistiert ◻ kontrolliert Katecholamingabe: ◻ nein ◻ ja

Inflammation: ◻ nein ◻ Pneumonie ◻ Harnwegsinfekt ◻ SIRS ◻ Sepsis ◻ .....

Blutproduktgabe: ◻ nein ◻ ..... EK ◻ ..... TK ◻ ..... FFP ◻ ..... IE PCC ◻ ..... g Albumin

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POSTOPERATIVER TAG 5	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG 6	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG 7	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

POSTOPERATIVER TAG	ABNAHME-ZEITPUNKT:	

Flüssigkeit: Einfuhr: ...... ml Ausfuhr: ...... ml Vitamin D-Gabe: □ nein □ ja .... IE
Atmungsform: □ spontan □ assistiert □ kontrolliert Katecholamingabe: □ nein □ ja
Inflammation: □ nein □ Pneumonie □ Harnwegsinfekt □ SIRS □ Sepsis □ ....
Blutproduktgabe: □ nein □ .... EK □ .... TK □ .... FFP □ .... IE PCC □ .... g Albumin

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6.6 **Screen-print: Publication derived from this dissertation**

original article

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Vitamin D assessment in perioperative medicine and critical care

A prospective observational pilot study

Paul Zajic · Stefan Heschl · Michael Schörghuber · Petra Srekl-Filzmaier · Tatjana Stojakovic · Viktoria Weixler · Sieglinde Zelzer · Karin Amrein

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Summary

Background There is controversy about the impact of acute illness on vitamin D levels. This study was carried out to assess the influence of perioperative fluid loading on 25-hydroxy-vitamin D [25(OH)D] levels. The study evaluated the clinical utility of a commonly available chemiluminescence assay (ECLIA, IDS-iSYS) and liquid chromatography/mass spectrometry (LC-MS/MS) in the diagnosis of vitamin D deficiency in this setting.

Methods In this prospective observational pilot study in adult patients undergoing cardiovascular surgery on cardiopulmonary bypass (CPB), blood samples drawn at preoperative baseline (t1), after weaning from CPB (t2), on intensive care unit (ICU) admission (t3) and on the first (t4) and second (t5) postoperative days were analyzed.

Results A total of 26 patients (130 samples) were included in this study. Fluid loading by CPB led to a median reduction of 25(OH)D by –22.6% (range –54.5% to –19.5%) between t1 and t2. Cohen's kappa (κ) for method agreement for vitamin D deficiency (tested cut-off values 20 ng/ml and 12 ng/ml), was $\kappa = 0.291$ ($p < 0.001$) and $\kappa = 0.469$ ($p < 0.001$), respectively. The mean difference between measurements by ECLIA and LC-MS/MS was 4.8 ng/ml (±5.7), Pearson's r for correlation was 0.73 ($p < 0.001$). The biologically inactive C3-epimer did not contribute to 25(OH)D levels assessed by LC-MS/MS.

Author contributions PZ and KA designed the study, SH, PSF, MS, VW and PZ performed patient screening, information and inclusion, SZ performed laboratory measurements, PZ and KA performed the statistical analyses, PZ wrote the manuscript, PZ, SH, MS, PSF, TS, VW and KA critically revised the manuscript for important intellectual content. All authors approved of the manuscript ahead of submission.

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