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Original Research

High prevalence of severe hypovitaminosis D in patients with advanced gastric cancer treated with first-line chemotherapy with or without anti-EGFR–directed monoclonal antibody (EXPAND trial) showing no prognostic impact

Radka Obermannova [a,b,f], Dalibor Valik [b,d,f], Dirk Hasenclever [c], Lenka Zdrazilova-Dubska [b,d,f], Ulrich Hacker [e], Regina Demlova [b,f], Iveta Selingerova [b,d], Florian Lordick [e,*]

[a] *Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute and Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[b] *Regional Center of Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic*
[c] *Institute for Medical Informatics, Statistics and Epidemiology, Medical Faculty of University Leipzig, Germany*
[d] *Department of Laboratory Medicine, Masaryk Memorial Cancer Institute, Brno, Czech Republic*
[e] *1st Medical Department (Hematology, Cell Therapy, Medical Oncology and Hemostaseology), University Cancer Center Leipzig (UCCL), University Leipzig Medical Center, Germany*
[f] *Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

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Abstract ***Purpose:*** The goal of our analysis was to study pretherapeutic circulating 25-OHD plasma levels in patients with previously untreated advanced gastric cancer treated in the randomised controlled phase III Erbitux (cetuximab) in combination with Xeloda (capecitabine) and cisplatin in advanced esophago-gastric cancer (EXPAND) trial (NCT00678535) and to explore whether low 25-OHD plasma levels are associated with worse prognosis and may compromise the clinical efficacy of cetuximab.
Methods: Six hundred thirty patients with available pretherapeutic 25-OHD plasma levels and treated with chemotherapy based on capecitabine and cisplatin, or chemotherapy and cetuximab, were included. The Cox proportional hazard regression model was used to analyse the association between low 25-OHD and survival in both treatment arms.

* *Corresponding author*: 1st Medical Department (Hematology, Cell Therapy, Medical Oncology and Hemostaseology), University Cancer Center Leipzig (UCCL), University Leipzig Medical Center, Liebigstr. 22, D – 04103, Leipzig, Germany. Fax: +493419712569.
E-mail address: florian.lordick@medizin.uni-leipzig.de (F. Lordick).

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Results: Majority of study patients were found to have severe vitamin D deficiency. No prognostic impact of 25-OHD plasma levels could be found in our patient cohort, and there was no indication of an interference of 25-OHD plasma levels and the efficacy of treatment with the anti–epidermal growth factor receptor monoclonal antibody cetuximab.
Conclusions: Although majority of patients with advanced gastric cancer show hypovitaminosis D deficiency, there is no proof for a negative impact on survival or reduced treatment response. A prospective study is needed to investigate the potential benefit of vitamin D supplementation in this patient cohort during first-line chemotherapy.

1. Introduction

Prognosis of patients with advanced gastric cancer is very poor. Although palliative chemotherapy prolongs overall survival (OS) and improves symptom control [1,2], the role of targeted therapy in gastric cancer is still modest. Except for trastuzumab, other biologically targeted agents including the epidermal growth factor receptor (EGFR)–targeted monoclonal antibodies cetuximab and panitumumab failed to improve survival outcomes [3–5]. The reasons for treatment failure in the respective studies are complex and not completely understood, encompassing weaknesses in the respective study designs, inadequate patient selection, tumour heterogeneity and various host–disease interactions [4–6].

Several environmental factors have been studied in gastric cancer, and a strong causal association was demonstrated for infection of the gastric mucosa with *Helicobacter pylori* [7]. Vitamin D (25-OHD) has been implicated in epidemiology of several common malignancies including gastrointestinal tumours [8–11]. A number of studies reported severe deficiency of vitamin D in breast and patients with colorectal cancer. Therapeutic potential of vitamin D and related compounds in cancer has been postulated [12–15] based on its pleiotropic biological properties such as modulation of cell proliferation and differentiation [16,17]. Promising data were recently published in patients with metastatic colorectal cancer. Patients with higher postdiagnostic levels of 25-OHD had better OS and better disease outcome [18,19]. An association between higher levels of vitamin D and better survival outcomes was also observed in diffuse large B-cell lymphoma treated with the anti-CD20 monoclonal antibody rituximab [20]. It was hypothesised that vitamin D deficiency impairs antibody-dependent cell-mediated cytotoxicity (ADCC) and substitution of vitamin D plasma levels may improve rituximab-mediated cellular cytotoxicity. The same concept was described in a cetuximab-treated colorectal cancer [21] ex-vivo model and thus may also be applied in other malignancies where monoclonal antibodies are used in treatment and where ADCC is assumed to be a mode of activity, as it has been postulated for cetuximab [22]. Vitamin D is a prohormone most noted for the regulation of calcium and phosphate levels in circulation, and thus of bone metabolism. Inflammatory and immune cells not only convert inactive vitamin D metabolites into calcitriol, the active form of vitamin D, but also express the nuclear receptor of vitamin D that modulates differentiation, activation and proliferation of these cells [23].

The Erbitux (cetuximab) in combination with Xeloda (capecitabine) and cisplatin in advanced esophagogastric cancer (EXPAND) trial was a large, open-label, randomised, controlled, phase III trial comparing capecitabine and cisplatin with and without the EGFR-directed monoclonal antibody cetuximab in patients with advanced gastric and oesophagogastric junction cancer [4]. The primary end-point of the EXPAND study was progression-free survival, with OS being observed as a secondary end-point. Survival outcomes were similar between treatment groups: progression-free survival was 4.4 versus 5.6 months and OS was 9.4 versus 10.7 months with cetuximab combination and control treatment, respectively. Overall response rates were 29% with cetuximab and 30% with control. Because of the similar safety profiles in both arms, the negative results of this trial cannot be explained by toxicity.

To the best of our knowledge, no data on vitamin D levels and its potential influence on prognosis of patients with advanced gastric cancer are available. The goal of our retrospective analysis was to study circulating 25-OHD plasma levels in patients treated in the EXPAND trial and to explore whether low 25-OHD plasma levels are associated with worse prognosis and may impede on cetuximab clinical efficacy.

2. Methods

2.1. Patients

The EXPAND trial (clinical registration number: EudraCT, number 2007-004219-75) included adults aged 18 years or older with histologically confirmed locally advanced unresectable (M0) or metastatic (M1)

adenocarcinoma of the stomach or oesophagogastric junction. The study design, patient characteristics and trial results are available in the original publication [4]. Treatment consisted of 3-week cycles of twice-daily capecitabine 1000 mg/m^2 (on days 1–14) and intravenous cisplatin 80 mg/m^2 (on day 1), with or without weekly cetuximab (400 mg/m^2 initial infusion on day 1 followed by 250 mg/m^2 per week thereafter). 25-OHD plasma levels were measured in all EXPAND patients with available baseline plasma samples. The analysis data set for the study presented here consists of 630 EXPAND study patients with valid follow-up for whom baseline vitamin D pretherapeutic levels were measured.

2.2. Blood samples

Analysis of 25-OHD plasma levels was performed at Masaryk Memorial Cancer Institute (MMCI) in Brno, Czech Republic, using Abbott Architect chemiluminescent immunoassay (Abbott Laboratories, Illinois, USA). The plasma specimens for analysis were stored at −80 °C at the biobank of University Cancer Center Leipzig and shipped to MMCI in dry ice. To reduce analytical variabilities, the whole sample set was analysed over the shortest possible period of time (from 9.11. to 10.11.2016) using one reagent lot and one calibration function. The measurement was performed in an ISO15189-accredited clinical laboratory.

2.3. Statistical analyses

The distribution of 25-OHD levels was illustrated using histogram of untransformed values. Cutoff values to characterise patients with insufficient and deficient vitamin D levels were taken from the literature [24]. OS was estimated using the Kaplan–Meier method. Comparison of survival curves was performed using the log-rank test. The Cox proportional hazard regression model was used to analyse the association between low 25-OHD and survival (OS) in both treatment arms.

2.4. Role of the sponsor and funding source

Analyses presented here were sponsored by the participating institutions and by the Czech Ministry of Health grants no. 17–29389A. The EXPAND trial was financially supported by Merck Serono GmbH, an affiliate of Merck KGaA, Darmstadt, Germany. Merck KGaA, Darmstadt, Germany, reviewed the manuscript for medical accuracy only before journal submission. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

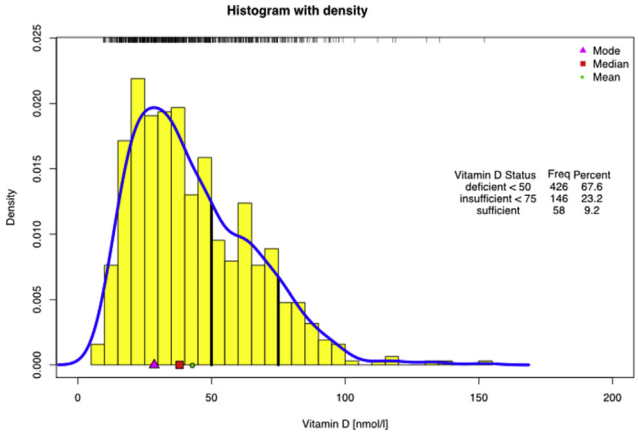

Fig. 1. Histogram of 25-OHD baseline plasma levels in 630 patients with advanced gastric cancer treated within the EXPAND trial.

3. Results

3.1. Distribution of 25-OHD plasma levels

The distribution of pretherapeutic vitamin D plasma levels (baseline) are shown in Fig. 1. Applying accepted criteria of 25-OHD insufficiency (plasma levels < 75 nmol/l) and deficiency (<50 nmol/l) [24], the EXPAND patient cohort was found to be heavily 25-OHD deficient already at baseline, before the start of any treatment. Median vitamin D plasma level was 38.2 nmol/l (interquartile range (IQR) 25.8; 58.9), and mean was 43.5 nmol/l. Some slight differences in vitamin D plasma levels were seen between specific subgroups (Table 1).

Table 1
Vitamin D plasma levels (nmol/l) according to different patient characteristics within the study population.

Variable	Mean	SD	p-value
Age <65	43.31	22.65	0.787
Age 65+	43.84	21.83	
Female	40.11	22.68	0.0317
Male	44.60	22.22	
ECOG PS 0	45.31	23.14	0.0207
ECOG PS 1	41.17	21.27	
Asian	39.35	18.37	0.00534
Non-Asian	44.65	23.32	
BMI under 18.5	34.23	19.26	0.00802
BMI 18.5 to 25	44.16	22.59	
BMI 25 to 30	44.36	22.76	
BMI over 30	45.32	21.67	
N mets less than 2	41.49	20.77	0.00927
N mets 2 or more	46.40	24.38	

BMI, body mass index (kg/m^2); ECOG PS, Eastern Cooperative Oncology Group performance status; N mets, number of metastases; SD, standard deviation.

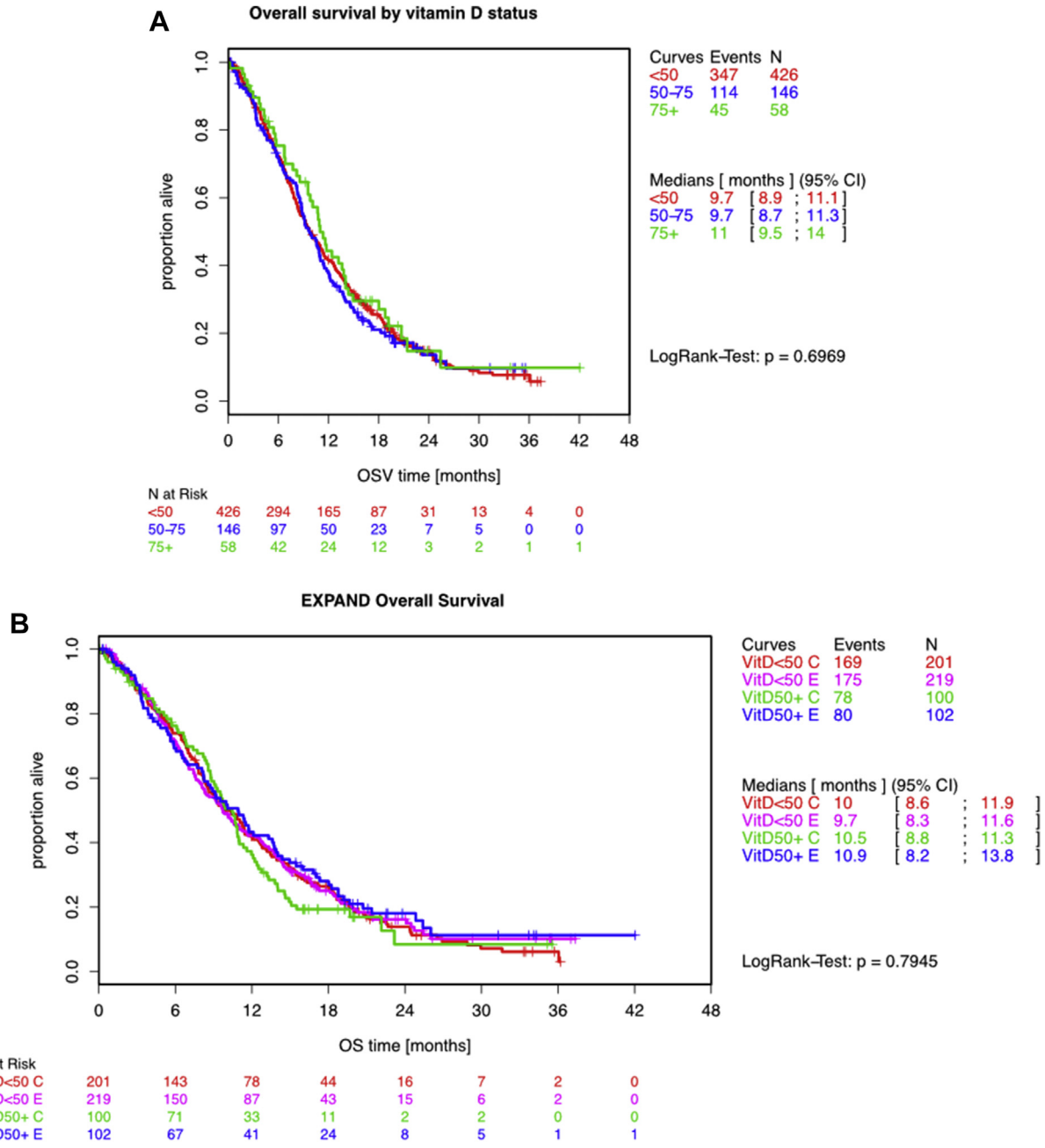

Fig. 2. A) OS by vitamin D status deficient <50 nmol/l, insufficient 50–75 nmol/l and 75 + nmol/l. (B) OS by vitamin D status deficient <50 nmol/l and 50 + nmol/l and by treatment arm. C, control arm; E, experimental arm; OS, overall survival; CI, confidence interval.

3.2. Vitamin D deficiency and OS

Fig. 2A shows the Kaplan–Meier curves for OS by vitamin D status as defined in the literature. There is no evidence of a prognostic role for vitamin D status on OS (Log rank test p = 0.70). This also holds true when vitamin D is entered linearly into a Cox regression model. Adding the treatment arm and an interaction term into the Cox model, there is no suggestion of a prognostic impact of vitamin D in either treatment arm, and thus, no suggestion that vitamin D deficiency affects the treatment arms differently. Fig. 2B shows the Kaplan–Meier curves for OS by vitamin D status and the by treatment arm. There is no evidence of a prognostic role for vitamin D in either of the two arms, for any treatment interaction or for any predictive role (log-rank test p = 0.79).

3.3. 25-OHD seasonality and deficiency

The date of 25-OHD evaluation was analysed in the context of seasonality in the particular region. As expected, we observed the highest levels of 25-OHD in

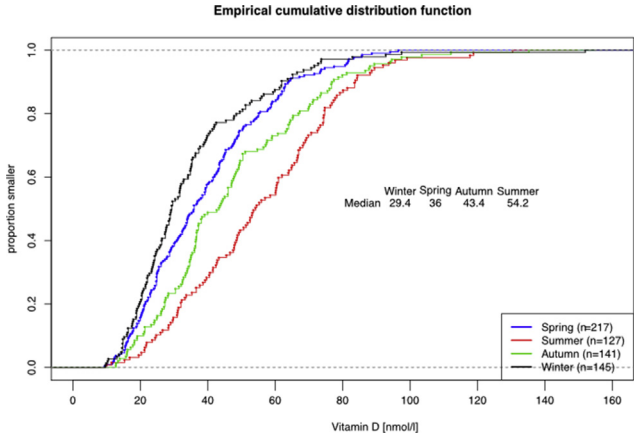

Fig. 3. Empirical cumulative distribution function of Vitamin D by seasons. Spring: April to June (northern hemisphere, NH), October to December (southern hemisphere, SH); summer: July to September (NH), January to March (SH); autumn: October to December (NH), April to June (SH); winter: January to March (NH), July to September (SH).

samples drawn during the summer season and the lowest values in winter cases (Fig. 3).

4. Discussion

The primary goal of our study was to investigate a possible association of pretherapeutic plasma 25-OHD levels with survival in a global population of patients with advanced gastric cancer and to determine if low plasma 25-OHD levels interfere with treatment outcomes in patients treated with chemotherapy alone or with chemotherapy and the anti-EGFR monoclonal antibody cetuximab. We found that a majority of patients with advanced gastric cancer were highly 25-OHD deficient and did not observe statistically significant differences in OS in patients who were 25-OHD deficient and those who had less severe 25-OHD deficiency or normal plasma levels.

These findings are in contrast to patients with colorectal cancer where the subgroup with higher post-diagnostic vitamin D plasma levels had longer OS than patients with lower levels [18,25,26]. Fuchs *et al.* [27] recently published data from adjuvant colorectal cancer CALGB 89803 trial; they examined the influence of the predicted 25-OHD scores on cancer recurrence and mortality (disease-free survival) and found that patients in the highest quintile of 25-OHD had a better OS than patients in the lowest. These results confirmed previous observations from the Cancer and Leukemia Group B (CALGB) 80405 study performed by Ng *et al.* [18]. In a recently published study from our group, we could also find prognostic differences between patients with colorectal cancer with high and with low 25-OHD plasma levels [19]. The differences between our current gastric cancer study and data from colorectal cancer might be explained by specific differences in tumour biology between gastric and colorectal cancer but also by the usually more severely altered nutritional status of patients with advanced gastric cancer [28].

The chronobiological pattern of seasonal variations of 25-OHD levels described in many observational studies was also found in our analysis. Higher 25-OHD plasma levels were observed during summer/autumn season, and conversely, the lowest 25-OHD plasma levels were measured in the winter/early spring season. This is generally consistent with previous observations [29] showing substantial fluctuations in plasma levels of 25-OHD throughout the year. As the EXPAND trial recruited patients from three continents and from the Northern as well as the Southern hemispheres, the phenomenon of season-dependent 25-OHD plasma levels is of worldwide significance. These variations may thus further substantiate clinical considerations towards oral supplementation of vitamin D in the winter season. In addition, this finding brings more confidence in the accuracy of our assay to underline the robustness of our measurements.

The second goal of our analysis was to examine if there is a prognostic difference in patients treated with cetuximab. We assumed that low 25-OHD plasma levels are associated with an adverse prognosis in these patients because of interactions of vitamin D with the immune system. ADCC significantly contributes to the antitumour effects of monoclonal antibodies, including cetuximab [22]. This association has been observed in malignant lymphoma treated with rituximab [20], and ex vivo data are available in colorectal cancer treated with cetuximab [20,21]. However, in patients with advanced gastric cancer, we observed no significant differences in the cetuximab treatment arm. This is probably due to the lacking efficacy of cetuximab in this disease. However, as we observed severe deficiency of vitamin D in the majority of patients, it may be important to study whether early vitamin D supplementation in patients with advanced gastric cancer improves their prognosis. Of note, some targeted drugs such as pertuzumab and TDM-1 have shown efficacy in other diseases but failed in studies for metastatic gastric cancer [30,31]. The reasons for these failures are certainly complex and not entirely understood, but profound hypovitaminosis D might be one of the concomitant factors. A prospective study is needed to investigate the potential benefit of vitamin D supplementation in this patient cohort during first-line chemotherapy. Whether or not Vitamin D supplementation can be of any benefit for a gastric cancer population, like it has been shown for patients with colorectal cancer [32], needs to be demonstrated.

One might question the precision and accuracy of the vitamin D measurement after plasma storage for 3–5 years and transportation to another laboratory. However, 25-hydroxyvitamin D3 measured in biological

fluids such as plasma is a quite stable species [33,34], provided the conditions of storage are constant which was the case during this study.

This study has some limitations: first, it is an unplanned retrospective analysis. However, the analysis has been carried out within the framework of one of the biggest prospective randomised controlled studies ever carried out in previously untreated advanced gastric cancer. Sampling of clinical data and biomaterial were defined per protocol and closely monitored. Another criticism could be that we analysed only pretherapeutic baseline samples, while it could also be interesting and of prognostic information how plasma 25-OHD levels developed in the course of treatment. However, we feel that with the severe deficiency observed in the majority of patients and the lack of any prognostic impact, further analysis might be expensive but futile and most probably without any clinical impact. Finally, we are lacking information on the lifestyle of the patients of our cohort, for example, if they followed more indoor or more outdoor activities, and we also did not analyse racial and gender differences in 25-OHD plasma levels.

In summary, although seasonal differences in the 25-OHD plasma levels of patients with advanced gastric cancer were found, the majority of them have a severe vitamin D hypovitaminosis. No prognostic impact of 25-OHD plasma levels could be found in our patient cohort, and there was no indication of an interference of 25-OHD plasma levels and the efficacy of treatment with the anti-EGFR monoclonal antibody cetuximab.

Conflict of interest statement

R.O. has had consulting/advisory roles for Amgen, Roche, Servier and Bayer; served on speakers' bureaus for Amgen, Roche and Eli Lilly and Company and received research funding from Merck. F.L. had consulting/advisory roles for Amgen, Astellas, Biontech, BMS, Eli Lilly, Elsevier and Merck Sharp Dohme; received honoraria for lectures, article writing or reviewing from Amgen, Astra Zeneca, BMS, Eli Lilly, Elsevier, Excerpta Medica, Imedex, Infomedica, Iomedico, AG, Medscape, MedUpdate GmbH, Merck Sharp Dohme, Merck Serono, Oncovis Gmbh and Springer Nature Group and received research support from BMS. D.V., D.H., L.Z.-D., U.H., R.D. and I.S. declare no conflict of interest.

Contributorship statement

All authors of this manuscript participated in the study design, manuscript preparation and writing and interpretation of results and agree with publication of the manuscript in the form presented.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.05.011.

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