High parathyroid hormone rather than low vitamin D is associated with reduced event-free survival in childhood cancer

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ABSTRACT:

Background: Vitamin D deficiency is linked to poor cancer outcomes, but the impact of its consequence, elevated parathyroid hormone (PTH) remains understudied. PTH receptor activation influences cancer progression in vitro, yet the effect of elevated PTH on pediatric cancer survival is unexamined.

Methods: This retrospective study examines associations between PTH, 25-OH vitamin D (25OHD), and event-free survival (EFS) and overall survival (OS) in pediatric cancer patients. Laboratory data from 4,349 patients (0-18 years) at a tertiary pediatric cancer unit were analyzed for the highest PTH and lowest 25OHD levels at diagnosis and the following five years. Data on relapse, secondary malignancies, and mortality were stratified by PTH levels above/below the cohort median (47 pg/ml) and 25OHD levels ≤ 30 nmol/L. EFS and OS were analyzed, and hazard ratios (HR) were calculated for the entire cohort and six cancer subgroups.

Results: PTH and 25OHD values were available for 1,286 patients (731 male). Higher PTH associated with inferior EFS in primary malignant brain tumors (HR 1.80 [1.19–2.72]), embryonal (HR 2.20 [1.1–4.43]), and lymphatic malignancies (HR 1.98 [1.05–3.72]). Vitamin D deficiency associated with inferior EFS in embryonal malignancies (HR 2.41 [1.24–4.68]). In a multivariate Cox model, only higher PTH remained significant for inferior EFS.

Conclusions: Elevated PTH may indicate adverse outcomes in certain pediatric cancers.

Impact: This study identifies elevated parathyroid hormone (PTH) as a potential marker for poor outcomes in pediatric cancer patients, emphasizing the need for adequate vitamin D and calcium management.

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INTRODUCTION

Over the past two decades observational, preclinical and clinical studies suggested that vitamin D deficiency may increase the risk for the development of different malignancies while sufficient vitamin D levels may have anti-tumoral effects. [1–4] Proposed mechanisms underlying these anti-cancer effects include anti-proliferative and anti-inflammatory action, induction of apoptosis, stimulation of differentiation, inhibition of invasion and metastasis, and inhibition of angiogenesis, presumably through direct action on the vitamin D receptors. [1,5]

In humans, vitamin D status is mainly determined by exposure of the unprotected skin to sunlight, by (oral) supplementation and, to a small degree, by diet. Vitamin D insufficiency in children and adolescents, commonly defined as 25-OH vitamin D (25OHD) serum levels below 50 nmol/L or deficiency, less clearly defined as 25OHD ranging from 30 nmol/L [6] to 37.5 nmol/l [7] is common especially during winter and spring in the northern hemisphere. [2,8–10]

Despite the common occurrence of vitamin D deficiency, a large body of experimental and observational evidence suggests it poses a risk factor for cancer development, relapse and inferior outcome especially in adults [1,11,12]

However, from an endocrine perspective, active vitamin D (calcitriol) is a steroid hormone which primarily increases intestinal calcium absorption and thus controls the maintenance of a sufficient body calcium pool. Most importantly, this calcium pool is tightly controlled: declining serum calcium levels result in the release of parathyroid hormone (PTH). Via its end-organ receptor PTH1R [13], PTH indirectly stimulates osteoclasts to ensure calcium retrieval from the skeleton through increased bone resorption, in addition to maximizing intestinal calcium absorption by stimulating the hydroxylation of 25-OHD into calcitriol (1,25(OH)2D).[2,14] Therefore, in vitamin D deficiency and in dietary calcium deficiency, PTH elevation and the development of secondary hyperparathyroidism is observed frequently. [15,16]

The two PTH receptors PTH1R and PTH2R are expressed in many tissues including different bone -, kidney- and parathyroid cells as well as gut-, adrenal- and mesenchymal stem cells, T-cells and in the brain. [13]

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Historically, a connection between PTH and cancer has been described when PTH related peptide (PTHrP) was identified in 1988 by three groups as the hormone responsible for humoral hypercalcemia in cancer.[17] PTHrP is encoded by *PTHLH* and exhibits paracrine and endocrine actions via the PTH1R receptor. The hormone was studied extensively in the years following its discovery and was found to be relevant in many different types of cancer. [18,19] Importantly, the role of PTHrP in cancer is not limited to the skeletal effects which result in the development of hypercalcemia, but it was found to modulate initiation, progression and metastasis e.g. in breast cancer [20], in colon cancer [21] and in neuroblastoma tumors *in vitro* [22]

In addition to PTHrP, a potential carcinogenic effect of PTH itself presented when the phase 3 trials for teriparatide (PTH1-34) were prematurely terminated because Fischer rats in the preclinical setting had developed osteosarcoma in a dose-dependent manner when treated with supratherapeutic doses of teriparatide. [23] While therapeutic doses did not lead to the development of osteosarcomas, a boxed warning about this risk was in place for almost 20 years for the use of teriparatide. [24]

Over the last decades, research focused on cancer risk from vitamin D deficiency, with a presumed mechanism of action via reduced vitamin D receptor stimulation. However, an alternative hypothesis, the stimulation of PTH receptors through secondary hyperparathyroidism has not been sufficiently explored to date. Despite the knowledge that low Vitamin D causes secondary hyperparathyroidism and that PTH exerts direct stimulatory actions on many cells and tissues, to the best of our knowledge, PTH has not been directly studied in the context of outcome in childhood malignancies. Hence, the aim of the present study was to investigate a possible association of PTH levels on cancer outcome in a large paediatric cohort.

MATERIALS AND METHODS

Laboratory data of 4349 patients with any childhood malignancy or with a primary benign brain tumor, who were managed between 01/2000 – 7/2021 at a tertiary paediatric cancer unit at the Department of Paediatrics at the University Hospital Essen were obtained. Data were screened for available PTH and 25 OHVD levels in each patient for a five-year period starting from the time of initial diagnosis. Measurement of PTH and 25 OHVD was integrated

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into the routine follow-up of pediatric cancer patients starting in 2012. Subsequently, these measurements were conducted annually as part of a study investigating endocrine and osteological late effects (Acronym: BONE-OK; DRKS#: 00009841)

In each case, the highest plasma PTH and the lowest serum 25OHD during the five-year period were identified and used for further analyses.

To investigate effects of higher vs lower PTH levels in this cohort, the median PTH of the entire cohort was calculated and used as a cutoff to assign cases to either the group with PTH levels at or above the cohort median (PTH+ group) or the group with PTH levels below the cohort median (PTH- group;).

For the analysis on vitamin D the data were grouped according to the presence (25OHD- group) or absence (25OHD+ group) of vitamin D deficiency (serum 25OHD ≤ 30 nmol/L).

From chart review the following information were retrieved: sex, date of birth (DOB), diagnosis (malignancy), date of first diagnosis, age at diagnosis, event-free (EFS) and overall (OS) survival, date of first relapse, secondary malignancy and death. These data were crosschecked and complemented by registry data from the German Childhood Cancer Registry, a nationwide population-based registry on childhood and adolescent cancer (Deutsches Kinderkrebsregister)[25] from the ALL-BFM study center (Akute lymphatische Leukämie – Berlin-Frankfurt-Münster).

Patients were assigned to the following six diagnostic subgroups according to the biological origin of their initial diagnosis: lymphatic malignancy, malignant primary brain tumor, benign primary brain tumor, embryonal malignancy, sarcoma, myeloid malignancy, and the group ‘other’. The group ‘other’ comprises of smaller groups of patients with different underlying malignancies and was not considered for further analysis due to its heterogeneity.

The study was conducted according to the Helsinki principles and was approved by the Research Ethics Committee of the Medical Faculty of the University Duisburg-Essen (# 23-11270-BO).

Statistical analyses

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Statistical analyses and data visualization were performed in R (version 4.3.0; packages survival version 3.5-5, survminer 0.4.9, SubgrPlots 0.1.3, table1 1.4.34, ggplot2, knitr2, https://CRAN.R-project.org/package=survminer. Published online 2021:2021.)[26 - 28].

Values are expressed as the mean ± standard deviation (SD), median and range unless stated otherwise.

Event free survival (EFS) was defined as the time from initial diagnosis to the occurrence of first relapse, secondary malignancy, or death, whichever occurred first. Overall survival (OS) was defined as time from initial diagnosis to death. Of note, nonresponse to treatment was not counted as an event in the analysis. The Kaplan-Meier method was used to estimate overall survival [29]; differences between groups were compared using the two-sided logrank test (Mantel-Cox).[30] To visualize the effect of PTH as a continuous variable on the time-to-event outcome, the r package contsurvplot was applied.[31] The Cox proportional hazard model was used for uni- and multivariate analyses. [32] The timespan from first diagnosis of malignancy to death/event or last contact in case of no event was used as time variable. Sex, age at diagnosis, the presence of vitamin D deficiency and presence of a plasma PTH concentration below or above (= PTH +) the median during the observation period were used as covariables for multivariate analyses. Forest plots for disease-subgroup analyses were modified according to Cuzick [28] to display the difference in the effect of higher PTH and vitamin D deficiency on EFS/OS in subgroups in contrast to the average overall effect in the whole cohort. Differences in the distribution of individual parameters among patient subsets were analyzed using the $\chi 2$ test for categorized variables and the Mann-Whitney U test for continuous variables. For all tests, statistical significance was presumed at $P < 0.05$.

Data Availability Statement

The data generated in this study are available upon request from the corresponding author.

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RESULTS

Cohort characteristics

In total in 1268 cases (731 male) with suitable measurements of plasma PTH (median number of measurements per case: 2, range 1-22) were identified. Out of those, at least one 25OHD measurement was also available in 1252 patients in the five-year period of interest.

Detailed clinical characteristics of the 1268 study participants (mean age 7.30 (± 5.44) years) for PTH analyses are presented in **Table 1**.

The median PTH of the entire group was identified as 47 pg/ml. 634 patients were assigned to the group with PTH levels at or above the median (PTH+ group) and 634 had PTH readings below the median (PTH- group). In table 1 clinical characteristics and highest PTH and lowest 25OHD levels (mean, median, min, max) of the group are provided (**for details see Table 1**).

The PTH+ group was older (8.33 $\pm$ 5.3 years) than the PTH– group (7.34 $\pm$ 5.54 years, p = 0.001), and had more PTH readings (median = 2) than the PTH- group (median = 1,). As expected, 25OHD levels were significantly lower in the PTH+ group compared to the PTH- group. (**for details see Table 1**)

For the analyses of 25OHD, data from 1252 patients (of the 1268) was available. In 638 patients, the lowest 25OHD concentration was $\leq$ 30 nmol/l (25OHD- group) and in 614 the lowest concentration was > 30 nmol (25OHD+ group).

Patients were assigned to the 6 diagnostic cancer groups as following: lymphatic malignancy (n=304), malignant primary brain tumor (n=258), benign primary brain tumor (n=238), embryonal malignancy (n=177), sarcoma (n=168), myeloid malignancy (n=82), and the group 'other' (n=41). A detailed list of all diagnoses and the assignment to the corresponding groups is available in **Supplementary table 1**.

Overall EFS and OS in the entire cohort

As expected, the 5-year EFS/OS differed depending on the underlying malignancy. For detailed information on the 5-year EFS/OS refer to **table 2 and Supplementary Figure 1 A-B**. The highest 5-year-EFS was seen in the lymphatic malignancies (82.1, 77.6 – 86.8% (95% CI)) while the lowest 5-year EFS (56.2, 49.7 – 64.2%) and OS (73.2, 66.9 – 80.2%) was seen in malignant PBT.

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25OHD and EFS/OS

Only in the group of embryonal malignancies was the mean EFS inferior in the 25OHD- group (25OHD ≤ 30 nmol/l) compared to the 25OHD+ group (N = 173, 5-year EFS: 60.3, 47.2 – 76.9% (95%CI) vs 81.7, 73.9 – 90.3%) with an increased hazard ratio (HR) of 2.41 [1.24 – 4.68]. **Figure 1A and Table 2.** Except for the above-mentioned group of embryonal malignancies, vitamin D deficiency during follow-up was not associated with an inferior EFS or OS in the entire cohort or any of the other subgroups in a univariate Cox regression model.

PTH levels ≥ 47 pg/ml (PTH+ group) at any time within the first five years following the cancer diagnosis were associated with significantly inferior EFS in the groups: embryonal malignancies, lymphatic malignancies, and malignant primary brain tumors (see below and **Figure 1B)**. OS was not inferior in any group.

In the group of **embryonal malignancies** (N=177) the mean five-year EFS was lower in PTH+ (66.8%, 57.9 – 56.4%) compared to the PTH- group (83.3%; 74.6 -93.0%). Correspondingly the hazard ratio was increased in the univariate Cox proportional hazard model in the presence of higher PTH (EFS Hazard Ratio (HR) 2.20 [1.1 – 4.43]).

In the group of **lymphatic malignancies** (N=304) the mean five-year EFS was 78.9% (73.0 – 85.3%) in PTH+ compared to 87.7% (81.4. -94.5%) in the PTH- group. The hazard ratio was increased (HR) 1.98 [1.05 – 3.72]).

In the group of **malignant primary brain tumors** (N=258) the mean five-year EFS was 46,9% (37.3 – 60.0%) in PTH+ compared to the PTH- group (64.3%; 55.4. -74.5%). The corresponding hazard ratio was increased (HR) 1.80 [1.19 – 2.72]).

The three corresponding Kaplan Meyer plots for EFS are displayed in **Figure 2 A-C.** Kaplan Meyer plots for OS and EFS for the entire cohort and all subgroups are displayed as **Supplementary Figures 2 A-H and 3 A-H.** In **Figure 3 A-C** survival for the three groups with PTH as a continuous variable are presented.

The HR for Overall survival was not increased in any group.

A multivariate analysis of EFS and OS (dependent variable) including the covariables age at diagnosis, sex, presence/non-presence of PTH ≥ 47 pg/ml and of vitamin D deficiency

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showed a significant association with inferior EFS in the disease groups: embryonal malignancies, lymphatic malignancies and malignant PBT for elevated PTH only. **Figure 4 A-Csuppl.**

DISCUSSION

This study is the first to investigate an effect of higher PTH on event-free and overall survival (EFS/OS) in childhood malignancies, independent of vitamin D. While it is often postulated that vitamin D deficiency increases the risk of cancer development and for inferior outcome[1], studies on effects of PTH are sparse despite its stimulatory effect via PTH1R not only on excessive bone resorption but also other cell types (via PTH1R and PTH2R).

Here, we provide evidence linking higher PTH levels at diagnosis or within the subsequent five-year period to inferior EFS, but unchanged OS, in childhood embryonal and lymphatic malignancies, as well as in childhood malignant primary brain tumors. These findings suggest an increased risk of relapse and second malignancies in paediatric cancer patients with elevated PTH levels within this large paediatric cancer cohort.

Hyperparathyroidism is a common finding in the winter and spring in northern latitudes and even more so in survivors of childhood cancer, as reported by many groups. [9,33–37] . Of note, the actual cutoff to define hyperparathyroidism is heavily debated, in particular in children. [38 39] PTH secretion rises from its baseline to counter decreasing calcium levels in the blood. Since calcium availability depends on the vitamin D status as well as the nutritional intake of calcium, it has been argued that normative values for PTH should be established in calcium- and vitamin D sufficient cohorts only. Indeed, studies have shown that the upper limit of normal for PTH may be 20% [40] or even 25-35% [41] lower than the commonly used cut-off in current use, if investigated in vitamin D sufficient subjects. [42,43] Recent retrospective cross-sectional studies [44,45] also demonstrate an age dependence of serum PTH levels, with lowest values in the youngest age groups. The PTH-25OHD relationship in children differs substantially from adults, in that PTH levels are much lower for the same 25OHD concentrations. [44,45] Studies specifically reporting paediatric PTH reference data for children are discrepant, likely explained by the differing vitamin D status, dietary intakes and fasting duration of the study populations. Reported upper limits of normal range from 35pg/ml [46] to

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60-80 pg/mL. [47] In the absence of an established upper limit for PTH in the age group of interest we therefore decided to use the median PTH (47 pg/ml) of the entire group as a cutoff for this study. As discussed, this is lower than commonly used threshold for adults of 65 pg/ml.

Data on the association between PTH and the outcome of malignancies in humans are limited, especially in children. In one cross sectional study on 295 children with a malignant diagnosis, Jackmann et al. reported no association of PTH with cancer outcome. [16] In adults few studies are available: Cancer-related mortality was studied in 1317 adults (median age 75 years) who participated in the Longitudinal Amsterdam Aging study. In the baseline assessment vitamin D deficiency (25OHD <50nmol/l) was present in about 50% and secondary hyperparathyroidism had developed in almost 5%. No association between PTH and survival was reported. [48]

However, others have investigated the association of PTH and the outcome in patients with breast cancer [49] (no clear association between PTH and outcome in men, but detrimental effects in mice [50]) and in prostate cancer [51]. The latter group reported that baseline PTH levels were negatively associated with OS in patients with prostate cancer in the presence of bone metastases.[52] However, when interpreting these results the suppressing effects of hypercalcemia (due to bone metastases) on PTH and the stimulating effects of hypocalcemia due to anti-resorptive treatment of bone metastases need to be considered and do likely not allow an evaluation of PTH on survival as an independent marker.

Kang et al. studied serum PTH in 115 patients with multiple myeloma (a malignancy arising in the bone marrow from abnormal plasma cells) and found inferior progression-free survival in the group with high PTH levels compared to the group with normal PTH levels. In addition, serum PTH at diagnosis was associated with risk factors and clinical outcome. [53]

Inferior EFS was detected in three groups of childhood malignancies that may seem unrelated at first glance. However, in all three groups tumors arise from tissues or cell types with known expression of PTH1R or PTH2R. For the group of **lymphatic malignancies** it is well established that PTH-signaling is not only involved in the fate of the mesenchymal stem cells of the bone marrow niche [54] but also directly influences T-cells.[55] In addition, accumulating evidence shows the bone marrow niche is critical to the maintenance and

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retention of hematopoietic stem cells (HSC), including leukemia stem cells (LSC). [56–60] In fact, LSC-induced microenvironmental reprogramming of the bone marrow niche contributes significantly to leukemogenesis.[61] Activation of the PTH receptor on osteoblasts also increases stem cell number [62–64] and pharmacologic use of PTH increases the number of HSCs mobilized into the peripheral blood for stem cell harvests in mice. [65,66] Moreover, Ballen et al. showed that PTH was effective in mobilization of HSCs from bone marrow in patients who previously had 1 or 2 unsuccessful stem cell mobilization attempts. [67] Interestingly, we did not observe inferior EFS/OS in the smaller group of children with myeloid malignancies; an observation that warrants further investigation.

Beyond lymphatic malignancies inferior EFS was observed in children with **malignant primary brain tumors**. As reviewed by Dettori et al [13] there is almost ubiquitous expression of PTH receptors in the central nervous system, opening the possibility for direct actions of PTH on a cellular level.

Furthermore, for the group of to the group of **embryonal malignancies** it was shown that neuroblastoma cell lines not only respond to PTH in culture [61] but that PTHrP differentially regulates neuroblastoma cell via action on PTH1R [22]. Cell lines of Retinoblastoma, the entitiy representing the largest proportion in the group of embryonal malignancies, respond to PTH as well. Phosphorylation of rb is PTH-dependent and E2F3, which is a candidate for tumor progression in retinoblastoma [68] belongs to the group of E2F which is targeted and stimulated by PTH [69].

Together, these experimental data and the observations of our study suggest that higher and elevated PTH levels could alter the fate of tumor cells and the release of leukemic stem cells from the bone marrow, thereby affecting EFS. It is possible that to some degree the previously described deleterious effects of a vitamin D deficiency on cancer outcome may be mediated by elevation of PTH itself.

Strength and Limitations of the study

The present study is by far the largest study of its kind in children, investigating a potentially relevant association. Even in common vitamin D deficiency, it is well known that bone

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pathology only starts once PTH rises. Similar effects on cancer development may potentially exist that warrant further research on this topic.

The main limitations of this study are caused by the retrospective design: While complete and structured clinical data on EFS and OS were obtained, the laboratory assessments of PTH and 25OHD were not part of routine laboratory evaluation until 2012 and therefore did not follow a protocol but were prompted by clinical standards. Thus, the timing and frequency of the PTH and 25OHD assessments differed between the patients and PTH was measured more frequently in patients who were found to have developed hyperparathyroidism than in patients without hyperparathyroidism.

Given the hypothesis that elevated PTH levels may impact EFS and OS in cancer patients, the analysis was focused on the highest PTH level recorded for each patient during the 5-year period from diagnosis. To align with this analysis and investigate the hypothesis that low 25 OHVD is detrimental to EFS/OS, the lowest recorded 25 OHVD was also analyzed.

The reported association of high PTH with lower EFS in some cancers was noted in the absence of a temporal relationship. One possible explanation is that patients diagnosed with elevated PTH at any point during follow-up may have experienced similar unfavorable calcium homeostasis earlier, which went undetected due to the lack of laboratory assessment. Fully addressing this matter would require a prospective study design

In summary, this study highlights the potential role of elevated parathyroid hormone (PTH) as a prognostic marker for poor outcomes in pediatric cancer patients. The findings suggest that higher PTH levels are associated with inferior event-free survival (EFS) in certain cancer subtypes, including malignant primary brain tumors, embryonal and lymphatic malignancies. These results underscore the importance of monitoring PTH levels in pediatric cancer patients and ensuring adequate vitamin D and calcium intake to potentially mitigate adverse outcomes. This research contributes to the understanding of biochemical markers in cancer prognosis and supports the need for integrated metabolic management in pediatric oncology.

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TABLES

Table 1: Clinical characteristics of the group of patients with parathyroid hormone (PTH) levels below the group-median (47 pg/ml) (**PTH- group,** first column), the patients with PTH levels ≥ the median (**PTH+ group,** middle column) and the entire group (last column). PTH measurements were obtained at any time during the 5-years following the initial diagnosis. The number of PTH measurements during follow up, age, sex and diagnostic groups, the highest PTH, the corresponding 25-hydroxy vitamin D serum level (25OHD), the lowest 25OHD, and the distribution as well as p-values for the PTH+ and PTH - group are provided.

	PTH < Median (PTH-; N=634)	PTH ≥ Median (PTH+; N=634)	*p-Value*	Entire Cohort (N=1268)
Number of PTH Measurements				
Mean (SD)	2.18 (2.02)	4.16 (3.16)	< 0.001	3.17 (2.83)
Median [Min, Max]	1.00 [1.00, 14.0]	4.00 [1.00, 22.0]		2.00 [1.00, 22.0]
Age at Diagnosis (years)				
Mean (SD)	7.34 (5.54)	8.33 (5.30)	0.0011	7.84 (5.44)
Median [Min, Max]	5.94 [0, 18.3]	8.04 [0.0438, 17.9]		7.04 [0, 18.3]
Follow-up Time (years)				
Mean (SD)	5.14 (3.92)	5.67 (3.57)	0.012	5.41 (3.76)
Median [Min, Max]	4.51 [0.05, 17.30]	5.27 [0.09, 18.18]		4.93 [0.05, 18.18]
Sex				
Male	376 (59.3%)	355 (56.0%)	0.256	731 (57.6%)
Female	258 (40.7%)	279 (44.0%)		537 (42.4%)
Diagnostic Groups				
Lymphatic L/L	110 (17.4%)	194 (30.6%)	< 0.001	304 (24.0%)
MalignantPBT	151 (23.8%)	107 (16.9%)		258 (20.3%)
BenignPBT	133 (21.0%)	105 (16.6%)		238 (18.8%)
Embryonal	99 (15.6%)	78 (12.3%)		177 (14.0%)
Sarcoma	93 (14.7%)	75 (11.8%)		168 (13.2%)
Myeloid	28 (4.4%)	54 (8.5%)		82 (6.5%)
Other	20 (3.2%)	21 (3.3%)		41 (3.2%)
Highest PTH (pg/ml)				
Mean (SD)	32.6 (9.01)	81.6 (55.8)	< 0.001	57.1 (46.9)
Median [Min, Max]	33.0 [6.40, 46.9]	68.5 [47.0, 1030]		**47.0** [6.40, 1030]
Corresponding 25OHD (nmol/l)				
Mean (SD)	47.6 (23.9)	41.3 (25.7)	< 0.001	44.5 (25.0)
Median [Min, Max]	44.8 [10.0, 146]	36.3 [10.0, 248]		41.0 [10.0, 248
Diagnosis to Highest PTH				
Mean (SD)	1.60 (1.52)	1.75 (1.39)	0.78	1.67 (1.46)
Median [Min, Max]	1.01 [0.00, 5.00]	1.37 [0.00, 4.99]		1.25 [0.00, 5.00]
Highest PTH to Event				
Mean (SD)	-0.32 (1.87)	-0.13 (2.14)	0.374	-0.22 (2.02)
Median [Min, Max]	-0.27 [-4.68, 7.32]	-0.22 [-4.81, 9.69]		-0.26 [-4.81, 9.69]
Lowest 25OHD (nmol/L)				
Mean (SD)	41.1 (22.7)	29.3 (20.2)	< 0.001	35.2 (22.3)
Median [Min, Max]	35.8 [9.25, 146]	24.5 [8.25, 230]		29.5 [8.25, 230]
Diagnosis to Lowest 25OH				
Mean (SD)	2.01 (2.54)	2.40 (2.58)	0.008	2.20 (2.56)
Median [Min, Max]	0.75 [0.00, 12.72]	1.30 [0.00, 14.93]		1.01 [0.00, 14.93]
5y-Overall Survival				
Percent	88.7	87.9	0.284	88.3
% CI	85.9 – 91.6	85.1 – 90.7		86.3 – 90.3

(Abbreviations: L/L = Leukemia or Lymphoma; PBT = primary brain tumor)

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600 **Table 2: Five-year overall survival (OS) and event free survival (EFS)** for the group of patients with parathyroid
601 hormone (PTH) levels below the median (47 pg/ml) of the group (**PTH- group,** first column), the patients with
602 PTH levels ≥ the median (**PTH+ group,** second column), the group with deficient (25OHD ≤ 30 nmol/l) vitamin D
603 levels (**25OHD– group**, third column), the group with sufficient vitamin D levels (**25OHD+ group**, fourth
604 column), and the entire group (last column). PTH and 25OHD measurements were obtained at any time during
605 the 5-years following the initial diagnosis.

606

	PTH < Median (PTH-)	PTH ≥ Median (PTH+)	25OHD ≤ 30 (25OHD -)	25OHD > 30 (25OHD +)	Entire Cohort
Entire Cohort	N = 634	N = 634	N = 638	N = 614	N = 1268
Deaths	N = 58	N = 78	N = 54	N = 78	N = 136
5y-Overall Survival	88.7	87.9	88.1	88.8	88.3
Percent / 95% CI	85.9 – 91.6	85.1 – 90.7	85.4 – 90.9	85.9- 91.9	86.3 – 90.3
Events	N = 159	N = 188	N = 160	N = 179	N = 347
5y-Event-free Survival	71.0	68.7	70.9	68.7	69.7
Percent / 95% CI	67.1 – 75.0	64.9 – 72.7	67.3 – 74.8	64.7 – 73.1	67.0 – 72.6
Benign PBT	N = 133	N= 105	N = 126	N = 111	N = 238
Deaths	N = 4	N =3	N = 4	N =3	N = 7
5y-Overall Survival	97.8	97.5	99.1	95.7	97.6
Percent / 95% CI	94.7 - 100	94.1 - 100	97.4 - 1	91.1 - 100	95.3 – 100
Events	N = 53	N =33	N = 45	N = 40	N = 86
5y-Event-free Survival	57.4	67.7	68.2	55.3	62.3
Percent / 95% CI	49.4 – 67.4	59.0 – 77.7	60.4 – 77.0	46.0 – 66.6	56.1 – 69.1
Embryonal	N = 99	N= 78	N = 53	N = 120	N = 177
Deaths	N = 4	N = 6	N = 3	N = 7	N = 10
5y-Overall Survival	96.3	93.9	91.0	96.8	95.0
Percent / 95% CI	92.3 - 100	88.1 - 100	82.8 - 100	93.4 - 100	91.3 – 98.8
Events	N = 12	N =23	N = 17	N = 18	N =35
5y-Event-free Survival	83.3	66.8	60.3	81.7	75.1
Percent / 95% CI	74.6 – 93.0	56.4 – 79.2	47.2 – 76.9	73.9 – 90.3	68.0 – 82.9
Lymphatic L/L	N = 110	N= 194	N = 195	N = 104	N = 304
Deaths	N = 4	N = 12	N = 5	N = 10	N = 16
5y-Overall Survival	95.5	93.1	94.2	94.4	94.0
Percent / 95% CI	91.3 – 100	89.3 – 97.0	90.7 – 97.8	89.6 – 99.4	91.1- 96.9
Events	N = 13	N = 40	N = 15	N = 35	N = 53
5y-Event-free Survival	87.7	78.9	82.0	84.5	82.1
Percent / 95% CI	81.4 – 94.5	73.0 – 85.3	76.5 – 87.9	77.2 – 92.4	77.6 – 86.8
Malignant PBT	N = 151	N= 107	N = 107	N = 149	N = 258
Deaths	N = 24	N = 32	N = 21	N = 33	N = 56
5y-Overall Survival	74.8	71.0	68.4	78.9	73.2
Percent / 95% CI	66.2 – 84.5	61.6 – 81.7	59.2 – 78.9	70.4 – 88.-5	66.9 – 80.2
Events	N = 40	N =51	N = 46	N = 43	N = 91
5y-Event-free Survival	64.3	46.9	58.3	55.1	56.2
Percent / 95% CI	55.4 -74-5	37.3 – 58.9	49.0 – 69.4	45.6 – 66.6	49.7 – 64.2
Myeloid Malignancy	N = 28	N= 54	N = 58	N = 23	N = 82
Deaths	N = 4	N = 8	N = 3	N = 9	N = 12
5y-Overall Survival	85.3	86.3	85.1	87.0	85.9
Percent / 95% CI	73.0 – 99.7	77.3 – 96.3	76.1 – 95.2	74.2 – 100	78.6 – 94.0
Events	N = 8	N = 14	N = 7	N = 14	N = 22
5y-Event-free Survival	70.6	74.6	74.1	69.6	73.2

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Percent / 95% CI	55.3 – 90.0	63.5 – 87-6	63.3 – 86.8	53.1 – 91.2	64.0 – 83.7
Sarcoma	N = 93	N= 75	N = 77	N = 88	N = 168
Deaths	N = 17	N = 12	N = 15	N = 13	N = 29
5y-Overall Survival	76.4	76.6	78.5	75.3	76.5
Percent / 95% CI	67.1 – 86.9	65-.7 – 89.4	68.6 – 89.8	65.0 – 87.3	69.2 – 84.6
Events	N = 30	N = 22	N = 26	N = 24	N = 52
5y-Event-free Survival	61.9	64.9	64.4	62.0	63.1
Percent / 95% CI	51.6 – 74.1	54 .0 – 78.1	53.7 – 77.1	51.2 – 75.0	55.4 – 71.9
Other	N = 20	N= 21	N = 22	N = 19	N = 41
Deaths	N = 1	N = 5	N = 3	N = 3	N = 6
5y-Overall Survival	94.1	78.5	89.6	82.2	85.9
Percent / 95% CI	83.6 – 100	61.8 – 99.8	77.0 – 100	65.8 – 100	75.0 – 98.2
Events	N = 3	N = 5	N = 4	N = 4	N = 8
5y-Event-free Survival	81.4	80.4	83.2	77.8	80.7
Percent / 95% CI	64.5 - 100	64.9 – 99.6	67.4 - 100	60.8 – 99.6	68.7 – 94.7

(Abbreviations: L/L = Leukemia or Lymphoma; PBT = primary brain tumor)

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612 **FIGURE LEGENDS**

613 **Figure 1 A-B: Event-free survival in the entire group and in six diagnostic groups**

614 Hazard ratios (HR) and 95% confidence interval (CI) for the presence of a Vitamin D
615 deficiency (25 OH vitamin D level at or below 30 nmol/l) at any time during the five years
616 following the initial diagnosis is shown in 1A, and for the presence of parathyroid hormone
617 (PTH) levels at or above 47 pg/ml (= median of the group) is shown in 1B. A univariate
618 analysis was performed.

619 **Figure 2 A-C: Kaplan Meyer plots showing event free survival in subgroups**

620 The Kaplan Meyer plots show the event-free survival in the groups of embryonal
621 malignancies (2A) lymphatic malignancies (2B) and malignant primary brain tumor (PBT) (2C)
622 for patients with PTH $\geq$ the median (47 pg/ml) of the entire group (orange line) and patients
623 with PTH levels below the median (grey line). For the analyses the highest PTH at diagnosis
624 or during the following 5-year period was used. P values from paired log rank tests are
625 provided.

626 **Figure 3 A-C: Event-free survival in subgroups with PTH as a continuous variable**

627 Visualizing of the highest measured PTH on time-to-event (as event free survival) in the
628 subgroups of embryonal malignancies (3A) lymphatic malignancies (3B) and malignant
629 primary brain tumor (PBT) (3C). For the analyses the highest PTH at diagnosis or during the
630 following 5-year period was used.

631

632 **Figure 4 A-C: Multivariate Cox proportional hazard model for event-free survival**

633 In figure 4 the variables 'Vitamin D deficiency' (defined by a 25 OH vitamin D level $\leq$ 30
634 nmol/l), 'higher PTH' (defined by a PTH level $\geq$ 47 pg/ml), Sex and Age at diagnosis were
635 investigated for event free survival (EFS) in the groups: embryonal malignancies (4A)
636 lymphatic malignancies (4B) and malignant primary brain tumor (PBT) (4C).

637

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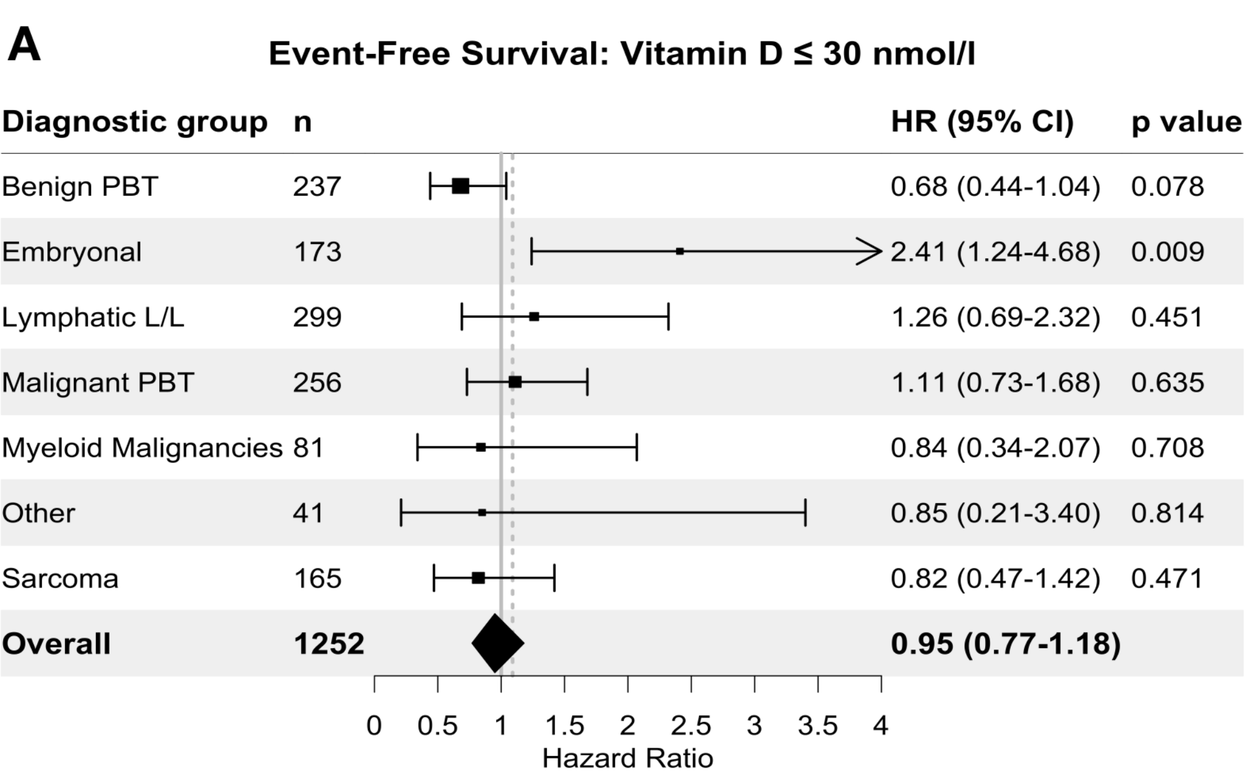

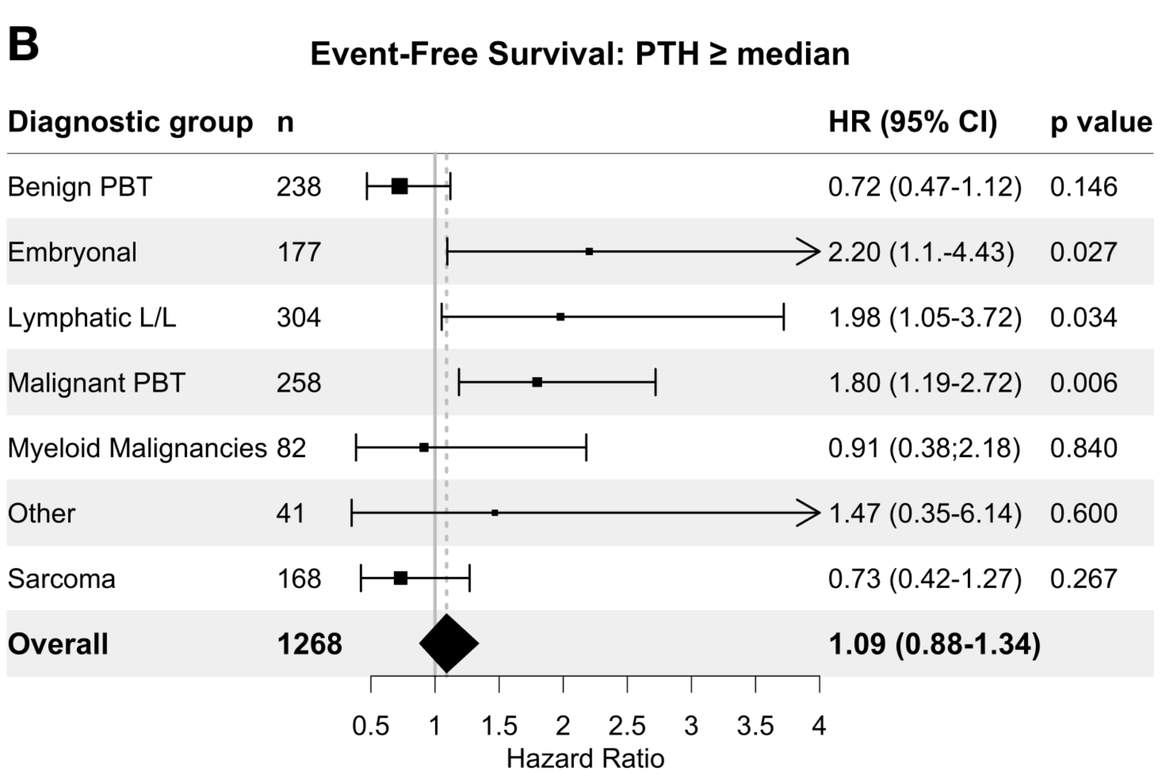

Figure 1.

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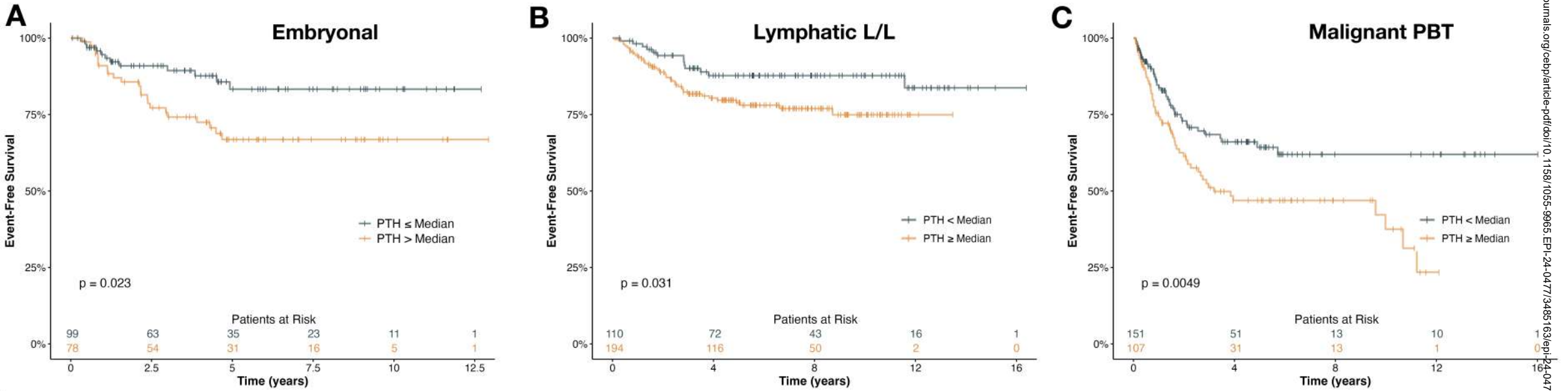

Figure 2.

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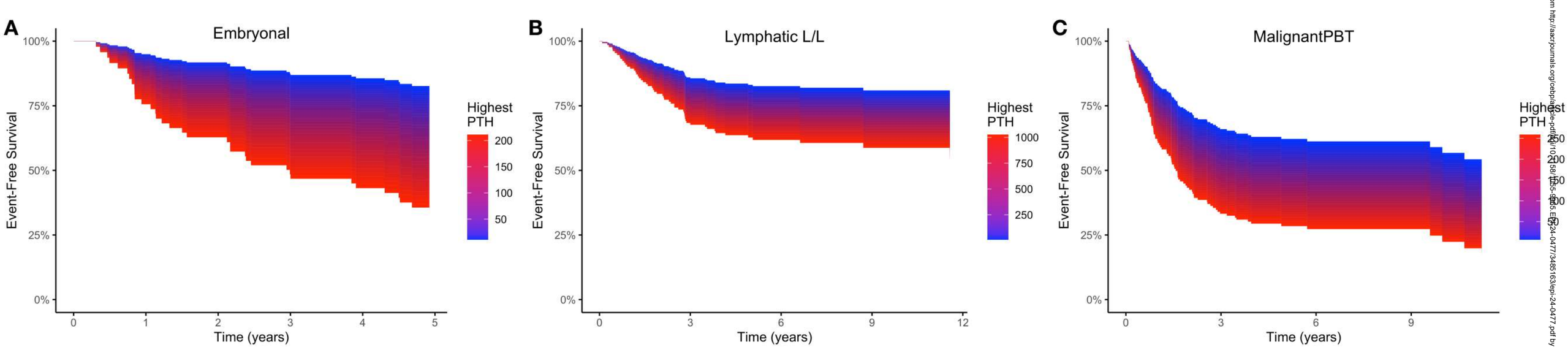

Figure 3.

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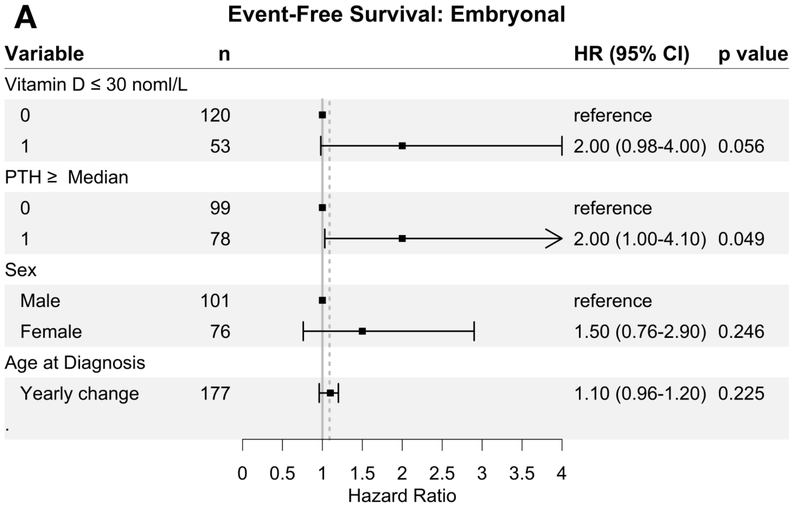

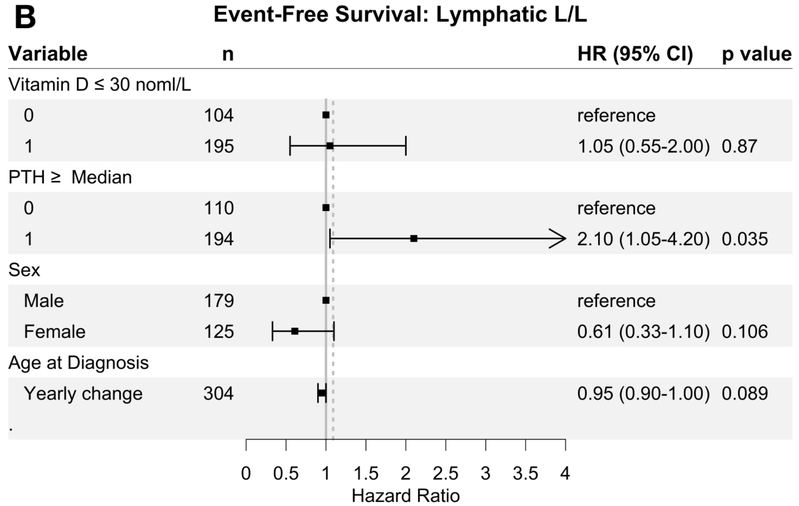

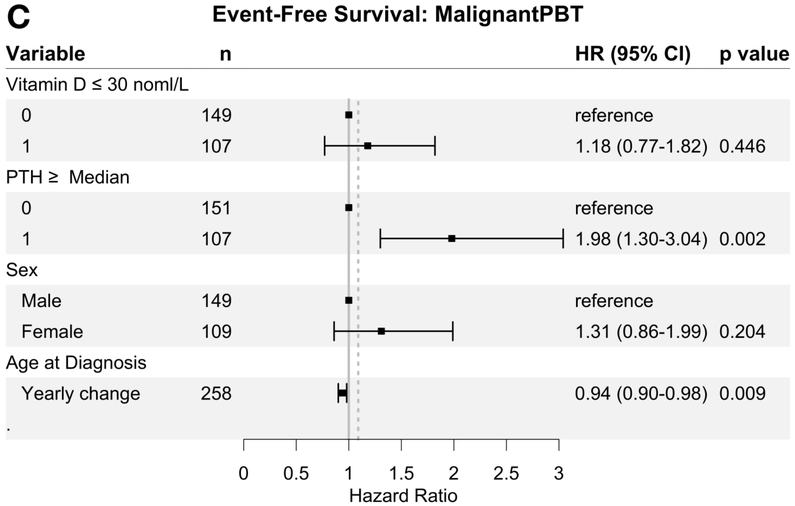

Figure 4.

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