Cathelicidin, but not vitamin D, is associated independently with sepsis in pediatric patients with cancer and febrile neutropenia

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Abstract. Sepsis and septic shock are major complications of febrile neutropenia (FN) in pediatric patients with cancer (PPCs). The aim of the present study was to determine the association of vitamin D (VD) and cathelicidin levels with sepsis and septic shock in PPCs with FN. A prospective cohort of PPCs with FN who had previously received cytotoxic chemotherapy was analyzed. At baseline, the plasma levels of VD and cathelicidin were quantified. Patients with sepsis and septic shock were compared with patients with FN without complications. Relative risks (RRs) were calculated with 95% confidence intervals (95% CIs) to determine associations. Multiple logistic regression analysis was performed to adjust the results for the identified confounders. A total of 78 episodes of FN were included; 35 (44.8%) completed their FN treatment without complications, 19 (24.4%) presented with sepsis and 24 (30.8%) progressed to septic shock. The median plasma VD level was 15.2 ng/ml, while the median plasma cathelicidin level was 27.9 ng/ml. Patients with severe VD deficiency (RR, 2.34; 95% CI, 1.17-4.70) and patients with cathelicidin levels >41.5 ng/ml (RR, 2.44; 95% CI, 1.07-5.56) exhibited a higher risk of developing sepsis compared with the control group. Patients with severe VD deficiency had a higher risk of septic shock (RR, 1.96; 95% CI, 1.02-3.79) compared with patients without complications, while cathelicidin levels were not associated with septic shock. After adjusting for confounders, cathelicidin levels >41.5 ng/ml (odds ratio, 5.52; 95% CI, 1.17-26.06) remained as an independent risk factor for progressing to sepsis. In patients who developed septic shock, the multivariate model revealed <700 leukocytes/mm^3 and glucose levels >100 mg/dl as independent risk factors. In conclusion, higher plasma cathelicidin levels were independently associated with progression to sepsis in PPCs with FN.

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Abbreviations: BMI, body mass index; FN, febrile neutropenia; HIMFG, Hospital Infantil de México Federico Gómez; ICU, intensive care unit; IQR, interquartile ranges; MDI, microbiologically documented infection; MLR, multiple logistic regression; OR, odds ratio; RR, relative risks; TTA, time to antibiotic; VD, vitamin D; 95% CI, 95% confidence interval

Key words: cathelicidin, FN, pediatric cancer, sepsis, septic shock, VD

Introduction

Febrile neutropenia (FN) is a common adverse reaction to chemotherapy, which involves an absolute decrease in neutrophils and fever associated with infections that increase the morbidity and mortality of pediatric patients with cancer (PPCs) (1). Consequently, sepsis and septic shock, major complications of FN, are the main causes of intensive care unit (ICU) admission and mortality in patients with malignancies undergoing intensive cytotoxic chemotherapy (2,3). Among pediatric patients with FN, ~20% may experience septic shock (4,5). Previous studies have explored clinical predictors and biomarkers for the early identification of patients at risk of developing severe sepsis or septic shock; however, patient categorization remains a challenge (4,6).

Classically, vitamin D (VD) is involved in calcium and phosphate metabolism, and bone health. Nonetheless, experimental studies have revealed that the VD active form (1,25-dihydroxyvitamin D) exerts immunological activities on both components of the innate and adaptive immune system, as well as endothelial membrane stability (7). In children, VD deficiency has been associated with infection, sepsis and septic shock (8-10). During an infection, Toll-like receptor

activation results in upregulation of both the VD receptor and the VD-activating hydroxylase cytochrome P450 family 27 subfamily B member 1, serving a key role in activating VD signaling, which subsequently induces the production of cathelicidin, a potent antimicrobial peptide effective against a broad spectrum of microorganisms (11,12). Cathelicidin interacts with the microorganism membrane in regions containing anionic components and induces a curvature deformation in the phospholipid bilayer, as well as the translocation of cathelicidin from the outer membrane to the inner surface, resulting in the disruption of membrane homeostasis (12,13). In immunocompromised patients with infection, such as pediatric patients with FN, VD-cathelicidin pathway dysregulation could be involved in the development of complications. Therefore, the aim of the present prospective cohort study was to determine the association of VD and cathelicidin plasma levels with sepsis and septic shock in PPCs with FN.

Materials and methods

Patients. Eligible patients were pediatric patients with any cancer type with FN who previously received cytotoxic chemotherapy between March 2021 and March 2023. A total of 42 patients included in the present study were male and 36 were female. The mean age among participants was 8.68 years (range, 1-17 years). The exclusion criteria were as follow: i) Patients with low-risk FN and complications whose empiric treatment was on an outpatient basis; ii) parents or patients declined to participate; iii) febrile patients without neutropenia; and iv) patients for whom blood samples could not be obtained.

Study design. The present study was a prospective cohort study conducted at the Hemato-Oncology Department, Hospital Infantil de Mexico Federico Gómez (HIMFG; Mexico City, Mexico). The study protocol was approved by the HIMFG Institutional Review Board (approval no. HIM-2021-004; Mexico City, Mexico) and was conducted in accordance with The Declaration of Helsinki. The parents of the patients signed an informed consent form for participation in the present study. In addition, all patients aged >7 years provided written assent. Patients were recruited at the time of hospitalization for FN. Patients were followed up until any of the following scenarios occurred: i) The patient was discharged due to FN resolution; or ii) the patient developed sepsis or septic shock. Data collection included sociodemographic information, cancer diagnosis, and biochemical and hematological parameters at cohort integration.

Definitions. FN was defined as an absolute neutrophil count of <1,000/mm^3 with a single temperature of >38.3˚C or a sustained temperature of ≥38˚C for >1 h (14). Patients with neutrophil counts <100 cells/mm^3 were considered as having profound neutropenia (15). VD deficiency was defined by plasma calcidiol levels <20 ng/ml (50 nmol/l), while patients with levels <5 ng/ml (12.5 nmol/l) were considered to have a severe deficiency (16,17). Hypocalcemia was defined as a blood calcium concentration of <8.0 mg/dl (14). Hypomagnesaemia was defined as a blood magnesium concentration of <1.6 mg/dl (14). Hypokalemia was defined as potassium levels <3.5 mmol/l (14). The body mass index (BMI) was calculated, and patient categorization was performed using the Centers for Disease Control and Prevention BMI percentile calculator for children and adolescents. Cathelicidin levels were categorized using the 50 and 75th percentiles as cut-off points.

Cathelicidin and VD quantification. A blood sample for the determination of VD and cathelicidin levels was taken at the time of cohort integration. The sample was separated by centrifugation at 1,400 x g for 5 min at room temperature to obtain plasma that was aliquoted and stored at -70˚C until analysis. The VD concentration was determined by measuring plasma calcidiol levels using a competitive ELISA kit according to the manufacturer's instructions [25(OH) VD ELISA kit; sensitivity, 1.98 ng/ml; 25(OH) VD Standard; cat. no. ab213966; Abcam]. The cathelicidin plasma concentration was determined using a sandwich ELISA kit according to the manufacturer's instructions [CAMP ELISA Kit (Human); sensitivity, 0.039 ng/ml; Lyophilized CAMP Standard; cat. no. OKCA00619; Aviva Systems Biology Corporation]. Quantification was performed in duplicate, and the arithmetic mean of the measurements was used for statistical analysis.

Statistical analysis. Descriptive statistics were used to analyze the demographic and clinical characteristics of all patients at the baseline. The distribution of quantitative variables was evaluated with the Shapiro-Wilk test. Quantitative variables are presented as the median and interquartile range (IQR), while qualitative variables are presented as the number of patients and percentages. Categorical variables were compared between groups using the χ^2 test, χ^2 for linear trend or Fisher's exact test, as appropriate. Comparisons of quantitative variables were performed using the Mann-Whitney U test. All tests were two-sided, and $P<0.05$ was considered to indicate a statistically significant difference.

Patients with FN were divided into three groups: The first group was made up of patients without complications, the second group consisted of patients who developed sepsis and the third group comprised patients who developed septic shock. Patients with sepsis that progressed to septic shock were included in the septic shock group. For the association analysis, the sepsis and septic shock groups were compared with the group of patients without complications. Associations were determined by calculating relative risks (RRs) and 95% confidence intervals (95% CIs). A backward multiple logistic regression (MLR) analysis was conducted to adjust for confounding variables previously identified between groups. MLR results are presented as the adjusted odds ratio (OR) and 95% CI. All analyses were performed using SPSS version 20 (IBM Corp.).

Results

Patient characteristics. A total of 252 PPCs were identified as potentially eligible to participate in the present study. A total of 53 patients had fever without neutropenia, a blood sample was not obtained in 116 patients and 5 patients decided not to participate. A total of 72 patients experiencing 78 FN episodes were enrolled in the current study.

The median age was 9.0 years (IQR, 7.0 years) and 42 (53.8%) patients were male. Acute lymphoblastic leukemia (ALL; 64.1%) was the most common diagnosis, followed by acute myeloblastic leukemia (AML; 7.7%) and germ cell tumors (5.1%). The median BMI was 16.4 kg/m^2 (IQR, 4.9 kg/m^2), and 44 (56.4%) patients were categorized as normal weight, 23 (29.5%) as underweight and 11 (14.1%) as overweight or obese. The body surface area median was 0.8 m^2 (IQR, 0.57 m^2), and 44 (56.4%) patients had a low socioeconomic status. The median time to antibiotic (TTA) treatment was 58.0 min (IQR, 85.25 min), and 37 (47.4%) FN episodes began antibiotic treatment outside the first h of admission. In terms of hematological parameters, the median values were 900 leukocytes/mm^3 (IQR, 1,260 leukocytes/mm^3), 120 neutrophils/mm^3 (IQR, 210 neutrophils/mm^3), 50 monocytes/mm^3 (IQR, 105 monocytes/mm^3),64,000platelets/mm^3(IQR,151,000platelets/mm^3), 8.1 fl (IQR, 1.2 fl) mean platelet volume and 9.0 g/dl (IQR, 3.7 g/dl) hemoglobin. In terms of biochemical parameters, the median values were 92.0 mg/dl (IQR, 18.0 mg/dl) glucose, 13.9 mg/dl (IQR, 9.0 mg/dl) blood urea nitrogen, 0.48 mg/dl (IQR, 0.31 mg/dl) creatinine, 150.5 U/l (IQR, 37.0 U/l) phosphate alkaline, 51.5 U/l (IQR, 42.0 U/l) alanine transaminase, 28.0 U/l (IQR, 22.0 U/l) aspartate transferase, 231.0 U/l (IQR, 76.5 U/l) lactate dehydrogenase, 2.1 mg/dl (IQR, 0.4 mg/dl) magnesium, 8.2 mg/dl (IQR, 1.6 mg/dl) calcium, 137.0 mmol/l (IQR, 5.0 mmol/l) sodium, 3.9 mEq/l (IQR, 0.9 mEq/l) potassium and 103.0 mEq/l (IQR, 5.5 mEq/l) chloride. Infection was microbiologically documented in 44 (56.4%) patients, and viral (45.4%) and bacterial (43.2%) infections were the most frequent. Baseline sociodemographic, clinical, biochemical and hematological characteristics of the groups are presented in Table I.

VD and cathelicidin levels. The median plasma VD level was 15.2 ng/ml (IQR, 19.4 ng/ml), while the median plasma cathelicidin level was 27.9 ng/ml (IQR, 20.9 ng/ml). VD deficiency was observed in 44 (56.4%) patients, while 7 (9.0%) patients had severe deficiency. Patients with plasma cathelicidin levels >41.5 ng/ml were above the 75th percentile. Plasma levels of VD were not significantly different in patients with ALL (median, 14.86 ng/ml; IQR, 19.1 ng/ml; P=0.49) and AML (median, 21.80 ng/ml; IQR, 28.06 ng/ml; P=0.39) compared with those in patients with solid tumors (median, 14.04 ng/ml; IQR, 20.17 ng/ml). However, the plasma levels of cathelicidin were significantly different in patients with ALL (median, 27.54 ng/ml; IQR, 14.19 ng/ml; P=0.02) and AML (median, 48.20 ng/ml; IQR, 43.14 ng/ml; P=0.006) compared with those in patients with solid tumors (median, 26.73 ng/ml; IQR, 21.72). A total of 35 patients (44.8%) completed their FN treatment without complications, 19 patients presented with sepsis (24.4%) and 24 patients (30.8%) progressed to septic shock. In the study, 2 patients who developed septic shock died (2.5%). The plasma levels of cathelicidin in the patients who died were 16.10 and 16.20 ng/ml.

VD and cathelicidin association with the risk of sepsis and septic shock. In the association analysis, patients with severe VD deficiency (RR, 2.34; 95% CI, 1.17-4.70) and patients with cathelicidin >41.5 ng/ml (RR, 2.44; 95% CI, 1.07-5.56) exhibited a higher risk of developing sepsis compared with patients without complications (Table II). Additionally, patients with creatinine levels >1 mg/dl, hypomagnesemia, AML diagnosis, microbiologically documented infection (MDI), and those whose infection was due to bacteria or fungi also had a higher risk of sepsis (Table II). After adjusting for the aforementioned variables in the MLR, levels of cathelicidin >41.5 ng/ml (OR, 5.52; 95% CI, 1.17-26.06) were independently associated with progression to sepsis (Table III).

Patients with severe VD deficiency (RR, 1.96; 95% CI, 1.02-3.79) had a higher risk of septic shock compared with patients without complications, while plasma levels of cathelicidin were unrelated to septic shock (Table II). In addition, patients with a leukocyte count <700 cells/mm^3, a count of <100 monocytes/mm^3, <50,000 platelets/mm^3, creatinine >1 mg/ml, glucose >100 mg/dl, a temperature >39˚C, MDI, or bacterial or fungal infection also demonstrated a higher risk of septic shock (Table II). While VD and cathelicidin levels were not independently associated with the progression of FN to septic shock in the multivariate analysis, leukocytes <700/mm^3 and glucose levels >100 mg/dl were predictive for septic shock (Table IV).

Discussion

FN is a medical emergency that requires immediate attention; the mortality rate ranges between 0.7 and 10% (18-23). The mortality results in the present study were consistent with this range. This underscores the critical need to understand FN pathophysiology and the factors associated with its complications. Thus, the present study aimed to determine whether VD and cathelicidin plasma levels may be associated with sepsis and septic shock, a major FN complication, in PPCs. To the best of our knowledge, no previous study has explored this hypothesis.

Cathelicidin is a versatile antimicrobial peptide that is expressed in diverse tissues and cell types, including epithelial cells and various immune cells (24). Within the bone marrow, myelocytes and metamyelocytes synthesize cathelicidin, storing the inactive precursor hCAP18 in neutrophil-specific granules for swift release during immune responses (25). In line with this evidence, patients with AML exhibited the highest levels of cathelicidin among all participants in the present study. Furthermore, the observed lower cathelicidin levels in patients with FN, in comparison with patients with other infectious diseases, may be attributed to the depletion of neutrophils, which are an important storage and production site for cathelicidin (24).

Inflammatory stimuli, such as wounds or infections, initiate signaling pathways that activate immune cells, stimulating increased cathelicidin expression, primarily in leukocytes, and its release via neutrophil degranulation (24,26). The present findings indicated that patients with FN and elevated cathelicidin levels upon admission may be at a higher risk of developing sepsis, potentially due to a more severe underlying infection. Notably, patients with significantly higher cathelicidin levels did not develop septic shock, suggesting that cathelicidin may serve as a biomarker of immune responsiveness in immunocompromised individuals. In BALB/c male mice with cyclophosphamide-induced neutropenia and *Pseudomonas aeruginosa*-induced septic shock, a

Table I. Baseline clinical characteristics of pediatric patients with febrile neutropenia (n=78).

Variable	No complication n=35 (%)	Sepsis n=19 (%)	Septic shock n=24 (%)
Male sex, n (%)	19 (54.3)	11 (57.9)	12 (50.0)
Cancer type, n (%)			
Other	10 (28.6)	3 (15.8)	9 (37.5)
Leukemia	25 (71.4)	16 (84.2)	15 (62.5)
Age, ≥12 years, n (%)	8 (22.9)	4 (21.1)	11 (45.8)
Nutritional status, n (%)			
Normal weight	21 (60.0)	8 (42.1)	15 (62.5)
Underweight	10 (28.6)	8 (42.1)	5 (20.8)
Overweight and obesity	4 (11.4)	3 (15.8)	4 (16.7)
Socioeconomic status, n (%)			
Low	22 (62.9)	8 (42.1)	14 (58.3)
Medium	13 (37.1)	11 (57.9)	10 (41.7)
TTA ≥60 min, n (%)	12 (34.3)	11 (57.9)	14 (58.3)
Leukocytes ≤700/mm^3, n (%)	9 (25.7)	6 (31.6)	15 (62.5)[a]
Profound neutropenia, n (%)	15 (42.9)	6 (31.6)	15 (62.5)
Monocytes ≤100/mm^3, n (%)	19 (54.3)	10 (55.6)	22 (91.7)[a]
Platelets ≤50,000/mm^3, n (%)	12 (34.3)	7 (36.8)	15 (62.5)
Creatinine ≥1 mg/dl, n (%)	0 (0.0)	1 (5.3)	7 (29.2)[b]
Hypomagnesemia, n (%)	0 (0.0)	1 (5.3)	0 (0.0)
Hypocalcemia, n (%)	20 (57.1)	13 (68.4)	17 (70.8)
Hypokalemia, n (%)	8 (22.9)	6 (31.6)	9 (37.5)
Glucose ≥100 mg/dl, n (%)	7 (20.0)	7 (36.8)	13 (54.2)[a]
Temperature ≥39.0˚C, n (%)	3 (8.6)	4 (21.1)	6 (25.0)
MDI, n (%)	12 (34.3)	13 (68.4)	19 (79.2)
Microbiological result, n (%)			
No isolation	23 (65.7)	6 (31.6)	5 (20.8)
Fungal	1 (2.9)	2 (10.5)	2 (8.3)
Bacterial	2 (5.7)	3 (15.8)	14 (58.3)
Viral	9 (25.7)	8 (42.1)	3 (12.5)
VD deficiency, n (%)	18 (51.4)	10 (52.6)	16 (66.7)
Severe VD deficiency, n (%)	1 (2.9)	3 (15.8)	3 (12.5)
Cathelicidin ≤28 ng/ml, n (%)	20 (57.2)	6 (31.6)	13 (54.2)
Cathelicidin ≤41.5 ng/ml, n (%)	28 (80.0)	10 (52.6)	22 (91.6)
Median age, years (IQR)	7.0 (7.0)	7.0 (9.0)	11.0 (7.0)[c]
Median BMI, kg/m^2 (IQR)	15.7(3.9)	16.45 (6.84)	17.46 (5.56)[c]
Median BS, m^2 (IQR)	0.76 (0.43)	0.73 (0.71)	1.18 (0.75)[c]
Median leukocytes, mm^3 (IQR)	1000 (900)	900 (2,150)	400 (1,050)[c]
Median neutrophils, mm^3 (IQR)	120 (300)	175.0 (610)	55 (178)
Median monocytes, mm^3 (IQR)	90 (120)	87 (280)	20 (55)[d]
Median hemoglobin, g/dl (IQR)	9.5 (3.6)	8.6 (4.33)	8.8 (2.85)
Median hematocrit, %(IQR)	28.2 (11.1)	28.1 (12.43)	25.45 (8.65)
Median platelets, mm^3 (IQR)	84,000 (187,000)	68,000 (182,250)	28,500 (64,250)[c]
Median MPV, fl (IQR)	8.1 (1.3)	8.0 (1.3)	8.2 (1.43)
Median ALT, U/l(IQR)	51.5 (13)	47.0 (51.75)	48.5 (70.5)
Median AST, U/l (IQR)	28.0 (8.0)	29.5 (27.0)	32.0 (31.5)
Median ALP, U/l (IQR)	150.5 (61.5)	150.5 (53.5)	150.5 (56.8)
Median creatinine, mg/dl (IQR)	0.48 (0.16)	0.42 (0.17)	0.75 (0.93)[c]
Median LDH, U/l (IQR)	231.0 (1.0)	231.0 (84.75)	236.5 (147.0)
Median magnesium, mg/dl (IQR)	2.05 (0.30)	1.85 (0.35)	2.0 (0.58)
Median calcium, mg/dl (IQR)	8.2 (6.5)	8.3 (0.8)	7.85 (1.7)
Median sodium, mEq/l (IQR)	137.0 (2.0)	137.0 (5.25)	134.5 (4.75)

Table I. Continued.

Variable	No complication n=35 (%)	Sepsis n=19 (%)	Septic shock n=24 (%)
Median potassium, mmol/l (IQR)	3.9 (0.5)	3.9 (0.9)	3.9 (1.1)
Median chloride, mEq/l (IQR)	103.0 (2.0)	103.0 (9.0)	102.0 (8.0)
Median glucose, mg/dl (IQR)	89.0 (6.0)	89.0 (29.3)	103.5 (29.8)[e]
Median BUN, mg/dl (IQR)	13.9 (4.42)	10.9 (9.8)	15.85 (19.3)
Median temperature, ˚C (IQR)	38.5 (0.5)	38.7 (0.5)	38.7 (0.6)
Median vitamin D, ng/ml (IQR)	18.5 (19.7)	19.5 (29.1)	11.14 (16.6)
Median cathelicidin, ng/ml (IQR)	23.7 (21.9)	34.4 (36.8)[c]	26.2 (16.7)

TTA, time to antibiotics; MDI, microbiologically documented infection; VD, vitamin D; BMI, body mass index; BS, body surface; MPV, mean platelet volume; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen. [a]χ^2 test, P≤0.01, vs. no complication group. [b]Fisher's exact test, P≤0.001 vs. no complication group. [c]Mann-Whitney U test, P≤0.05 vs. no complication group. [d]Mann-Whitney U test, P≤0.001 vs. no complication group. [e]Mann-Whitney U test, P≤0.01 vs. no complication group.

Table II. Factors associated with the development of sepsis and septic shock in pediatric patients with febrile neutropenia (N=78).

	No complication (n=35)	Sepsis (n=19)		Septic shock (n=24)	
Variable	N (%)	N (%)	RR [95% CI]	N (%)	RR [95% CI]
VD deficiency, yes	18 (51.4)	10 (52.6)	1.03 [0.50, 2.13]	16 (66.7)	1.47 [0.75, 2.88]
Severe VD deficiency, yes	1 (2.9)	3 (15.8)	2.34 [1.17, 4.70][a]	3 (12.5)	1.96 [1.02, 3.79][a]
Cathelicidin, ≤28 ng/ml	20 (57.2)	6 (31.6)	1	13 (54.2)	1
Cathelicidin, 28 to 41.5 ng/ml	8 (22.8)	4 (21.0)	1.44 [0.50, 4.19]	9 (37.5)	1.34 [0.73, 2.49]
Cathelicidin, >41.5 ng/ml	7 (20.0)	9 (47.4)	2.44 [1.07, 5.56][a]	2 (8.3)	0.56 [0.16, 2.06]
Covariates					
Sex, male	19 (54.3)	11 (57.9)	1.05 [0.71, 1.56]	12 (50.0)	1.11 [0.60, 2.05]
Cancer type					
Solid tumor	9 (25.7)	3 (15.8)	1	9 (37.5)	1
Acute lymphoblastic leukemia	25 (71.4)	12 (63.2)	1.30 [0.44, 3.84]	13 (54.2)	0.68 [0.36, 1.30]
Acute myeloblastic leukemia	1 (2.9)	4 (21.0)	3.20 [1.09, 9.36]	2 (8.3)	1.33 [0.53, 3.36]
Age, ≥12 years	8 (22.9)	4 (21.1)	1.04 [0.66, 1.64]	11 (45.8)	1.78 [0.99, 3.21]
Nutritional status					
Normal weight	21 (60.0)	8 (42.1)	1	15 (62.5)	1
Underweight	10 (28.6)	8 (42.1)	1.61 [0.74, 3.53]	5 (20.8)	0.80 [0.36, 1.80]
Overweight and obesity	4 (11.4)	3 (15.8)	1.26 [0.55, 4.40]	4 (16.7)	1.20 [0.54, 2.65]
Socioeconomic status					
Low	22 (62.9)	8 (42.1)	0.58 [0.28, 1.22]	14 (58.3)	0.89 [0.48, 1.66]
Medium	13 (37.1)	11 (57.9)	1	10 (41.7)	1
Time to antibiotics, >60 min	12 (34.3)	11 (57.9)	1.85 [0.89, 3.86]	14 (58.3)	1.78 [0.95, 3.33]
Leucocytes, ≤ 700 mm^3	9 (25.7)	6 (31.6)	0.90 [0.56, 1.44]	15 (62.5)	2.43 [1.28, 4.62][a]
Profound neutropenia, yes	15 (42.9)	6 (31.6)	1.18 [0.80, 1.73]	15 (62.5)	1.61 [0.84, 3.09]
Monocytes, ≤100 mm^3	19 (54.3)	10 (55.6)	0.98 [0.67, 1.45]	22 (91.7)	4.83 [1.27, 18.39][a]
Platelets, ≤50,000 mm^3	12 (34.3)	7 (36.8)	0.96 [0.63, 1.46]	15 (62.5)	1.98 [1.03, 3.78][a]
Creatinine, ≥1 mg/dl	0 (0.0)	1 (5.3)	2.94 [2.02, 4.28][a]	7 (29.2)	3.06 [2.07, 4.52][a]
Hypomagnesemia, ≤1.5 mg/dl	0 (0.0)	1 (5.3)	3.06 [2.07, 4.52][a]	0 (0.0)	UD
Hypocalcemia, ≤8.5 mg/dl	20 (57.1)	13 (68.4)	0.85 [0.58, 1.25]	17 (70.8)	1.44 [0.71, 2.92]
Hypokalemia, ≤3.5 mg/dl	8 (22.9)	6 (31.6)	0.85 [0.51, 1.40]	9 (37.5)	1.48 [0.81, 2.71]

Table II. Continued.

Variable	No complication (n=35) N (%)	Sepsis (n=19) N (%)	Sepsis (n=19) RR [95% CI]	Septic shock (n=24) N (%)	Septic shock (n=24) RR [95% CI]
Glucose, ≥ 100 mg/dl	7 (20.0)	7 (36.8)	0.71 [0.41, 1.25]	13 (54.2)	2.31 [1.27, 4.18][a]
Temperature, ≥39.0˚C	3 (8.6)	4 (21.1)	0.63 [0.26, 1.51]	6 (25.0)	1.85 [1.03, 3.35][a]
MDI, yes	12 (34.3)	13 (68.4)	2.51 [1.12-5.63][a]	19 (79.2)	3.43 [1.48-7.97][a]
Microbiological result					
No isolation	23 (65.7)	6 (31.6)	1	5 (20.8)	1
Fungal	1 (4.2)	2 (25.0)	3.22 [1.10, 9.41][a]	2 (28.6)	3.73 [1.21, 11.53][a]
Bacterial	2 (8.0)	3 (33.3)	2.90 [1.06, 7.96][a]	14 (73.7)	4.90 [2.17, 11.08][a]
Viral	9 (28.1)	8 (57.1)	0.67 [0.41, 1.08]	3 (37.5)	1.40 [0.40, 4.94]

RR, relative risk; 95% CI, 95% confidence interval; VD, vitamin D; TTA, time to antibiotics; MDI, microbiologically documented infection; UD, undetermined. [a]χ^2 test, P<0.05.

Table III. Multiple logistic regression model for the development of sepsis in patients with febrile neutropenia.

Independent risk factor	OR[a]	95% CI Low	95% CI High
Cathelicidin, 28 to 41.5 ng/ml	1.58	0.31	8.10
Cathelicidin, >41.5 ng/ml	5.52	1.17	26.06

OR, odds ratio; 95% CI, 95% confidence interval. [a]Adjusted by creatinine ≥1 mg/dl, hypomagnesemia, severe vitamin D deficiency, cathelicidin 28 to 41.5 ng/ml and cathelicidin >41.5 ng/ml, acute myeloblastic leukemia diagnosis, microbiological result and microbiologically documented infection.

Table IV. Multiple logistic regression model for the development of septic shock in patients with febrile neutropenia.

Independent risk factor	OR[a]	95% CI Low	95% CI Superior
Leucocytes, ≤700 mm^3	29.98	1.95	461.36
Glucose, ≥100 mg/dl	18.01	1.95	166.40
Cathelicidin, 28 to 41.5 ng/ml	6.78	0.62	74.32
Cathelicidin, >41.5 ng/ml	0.17	0.01	2.31

OR, odds ratio; 95% CI, 95% confidence interval. [a]Adjusted by leucocytes ≤700 mm^3, monocytes ≤100 mm^3, platelets ≤50,000 mm^3, creatinine >1 mg/ml, temperature ≥39.0˚C, severe vitamin D deficiency, cathelicidin 28 to 41.5 ng/ml and cathelicidin >41.5 ng/ml, microbiological result, aged ≥12, microbiologically documented infection, glucose ≥100 mg/dl, and time to antibiotics >60 min.

combination of cathelicidin and granulocyte colony-stimulating factor demonstrated the greatest efficacy in protecting against the development of sepsis (27).

In a large multicenter study of infants hospitalized with bronchiolitis, low serum LL-37 levels were independently associated with intensive care use and longer length-of-stay (28). By contrast, in patients with septic shock admitted to the ICU, cathelicidin levels were not associated with in-hospital mortality (29). The present results support the evidence suggesting that cathelicidin levels are not associated with the development of septic shock. Barbeiro *et al* (30) reported that cathelicidin was downregulated during septic shock independently of serum VD levels, while TNF was elevated, indicating a complex regulation of cathelicidin during septic shock. Since multidrug-resistant microorganisms cause serious healthcare-associated infections, even sepsis and death, preclinical studies are evaluating the therapeutic potential of both human and non-human cathelicidins against this type of microorganism (31,32).

In recent years, VD has been recognized as serving a potential role in regulating inflammation and protecting against infection. In the present study, VD deficiency incidence in patients with FN was high, and severe deficiency was associated with the development of sepsis anarciad septic shock, although the association was not independent. Similarly, in pediatric patients with aplastic anemia presenting with FN, a high prevalence of VD deficiency was observed and low levels were associated with an adverse clinical outcome (33). In addition, severe VD deficiency was associated with a higher 30-day mortality in adolescents and adults admitted to hospital with community-acquired pneumonia during winter, while low levels of cathelicidin tended to be associated with increased mortality (34). Adult patients with sepsis admitted to the ICU have been reported to present with lower VD levels compared with healthy controls (35). By contrast, VD was reported to not

be associated with increased mortality in patients with septic shock who were admitted to the ICU, although VD binding protein was associated with in-hospital mortality (29).

Microorganism identification in febrile pediatric patients with neutropenia continues to be a challenge; only between 22 and 34% can be identified (23,36). This difficulty can be explained by the current sensitivity of diagnostic methods, such as microbiological cultures. In the present study, MDI was associated with both sepsis and septic shock, although not independently. One explanation is that patients with a higher degree of infection are identified in the microbiological diagnosis, and thus, are the ones who progress to sepsis and septic shock. In Thai children, MDI has been described as an independent predictor of severe outcomes of FN, such as shock, respiratory failure and death (37). Similarly, in Chilean adults, an association between MDI and increased mortality rates has been observed (38). By contrast, ~50% of pediatric patients with acute leukemia prior to induction have been reported to harbor multidrug-resistant bacteria; this percentage rose to 70% in patients with FN, although this multidrug-resistant bacteria isolation was not associated with negative results in a bivariate analysis (39). Furthermore, in Thai pediatric oncology patients with FN, the isolation of antibiotic-resistant gram-negative bacteria was not associated with septic shock; however, a multivariate analysis was not performed (36).

There is controversy about the benefits of administering antibiotics within the first hour in reducing mortality and ICU admission in patients with FN (40). In this sense, the association of TTA <60 min and secondary complications of FN such as septic shock has been recently analyzed (41,42). Starting antibiotic treatment within 3 h has been reported to be sufficient and reasonable for PPCs with FN (42). In the present study, the TTA revealed a non-significant trend with progression to septic shock, but not with sepsis.

Corticosteroids and L-asparaginase, used in the treatment of pediatric acute leukemia, may cause drug-induced hyperglycemia, which may lead to an increased risk of infections, including cellulitis, bacteremia and fungemia, and an increased incidence of FN (43). In addition, the inflammatory cascade is implicated in the pathogenesis of insulin resistance and hyperglycemia (44). In patients with severe sepsis and septic shock, high levels of resistin, a proinflammatory cytokine derived in part from monocytes, have been associated with raised blood glucose (45). Glucose levels >100 mg/dl on admission for FN were revealed to be independently associated with septic shock. This fact suggests that the increase in glucose may be an early manifestation of the inflammatory stress that the patient is experiencing.

Leukocyte levels <500 cells/mm^3 at admission have been identified as a predictive factor for severe infection complication in pediatric patients with FN in a univariate analysis (46). The present results obtained using a multivariate model revealed that a count of <700 leukocytes/mm^3 was associated with the development of septic shock, but not sepsis.

The main limitation of the present study was the small sample size. Although there were >100 patients that could be recruited, the present study was conducted independently of the treating pediatric oncologists, and for this reason, it was not always possible to obtain the sample at the time of admission for FN. Similarly, the role of other markers, such as procalcitonin and C-reactive protein, could not be evaluated because these measurements were not available for all patients. Notwithstanding, the present study reported that higher plasma cathelicidin levels were independently associated with progression to sepsis in pediatric patients with FN. In addition, severe VD deficiency was associated with sepsis and septic shock, but not independently. Collectively, these findings highlighted the role of the VD-cathelicidin pathway in the development of FN complications.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

PEGG performed investigation, formal analysis, project administration, data curation and writing-original draft. MAPC was involved in conceptualization, funding acquisition, investigation, formal analysis and writing-original draft. KMSJ was involved in Formal analysis, data curation and writing-original draft. LEJV was involved in investigation, data interpretation and writing-original draft. GVR was involved in investigation, data interpretation and writing-original draft. MASR was involved in investigation and writing-original draft. ODCM was involved in conceptualization, funding acquisition, methodology, formal analysis, data interpretation, project administration and writing-original draft. PEGG and ODCM confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was performed in line with the principles of The Declaration of Helsinki. Protocol approval was obtained from the Ethics Committee of Hospital Infantil de México Federico Gómez (03-23-2021/approval no. HIM-2021-004). Written informed consent was obtained from all legal guardians.

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

References

1. Mueller EL, Sabbatini A, Gebremariam A, Mody R, Sung L and Macy ML: Why pediatric patients with cancer visit the emergency department: United States, 2006-2010. Pediatr Blood Cancer 62: 490-495, 2015.
2. Azoulay E, Mokart D, Pène F, Lambert J, Kouatchet A, Mayaux J, Vincent F, Nyunga M, Bruneel F, Laisne LM, *et al*: Outcomes of critically ill patients with hematologic malignancies: Prospective multicenter data from France and Belgium-a groupe de recherche respiratoire en réanimation onco-hématologique study. J Clin Oncol 31: 2810-2818, 2013.
3. Soares M, Bozza FA, Azevedo LC, Silva UV, Corrêa TD, Colombari F, Torelly AP, Varaschin P, Viana WN, Knibel MF, *et al*: Effects of organizational characteristics on outcomes and resource use in patients with cancer admitted to intensive care units. J Clin Oncol 34: 3315-3324, 2016.
4. Avilés-Robles M, Schnur JJ, Dorantes-Acosta E, Márquez-González H, Ocampo-Ramírez LA and Chawla NV: Predictors of septic shock or bacteremia in children experiencing febrile neutropenia post-chemotherapy. J Pediatric Infect Dis Soc 11: 498-503, 2022.
5. Castelán-Martínez OD, Palomo-Colli MA, Barrios-López VE, Silva-Jivaja KM, Juárez-Villegas LE, Castañeda-Hernández G and Sánchez-Rodríguez MA: Efficacy and safety of oral magnesium supplementation in reducing febrile neutropenia episodes in children with solid tumors treated with cisplatin-based chemotherapy: Randomized clinical trial. Cancer Chemother Pharmacol 86: 673-679, 2020.
6. Phillips B: Prospective cohort study of the predictive value of inflammatory biomarkers over clinical variables in children and young people with cancer presenting with fever and neutropenia. F1000Res 10: 1070, 2021.
7. Charoenngam N and Holick MF: Immunologic effects of vitamin D on human health and disease. Nutrients 12: 2097, 2020.
8. Xiao D, Zhang X, Ying J, Zhou Y, Li X, Mu D and Qu Y: Association between vitamin D status and sepsis in children: A meta-analysis of observational studies. Clin Nutr 39: 1735-1741, 2020.
9. de Haan K, Groeneveld ABJ, de Geus HRH, Egal M and Struijs A: Vitamin D deficiency as a risk factor for infection, sepsis and mortality in the critically ill: Systematic review and meta-analysis. Crit Care 18: 660, 2014.
10. Nguyen HB, Eshete B, Lau KHW, Sai A, Villarin M and Baylink D: Serum 1,25-dihydroxyvitamin D: An outcome prognosticator in human sepsis. PLoS One 8: e64348, 2013.
11. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, *et al*: Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311: 1770-1773, 2006.
12. Thennarasu S, Tan A, Penumatchu R, Shelburne CE, Heyl DL and Ramamoorthy A: Antimicrobial and membrane disrupting activities of a peptide derived from the human cathelicidin antimicrobial peptide LL37. Biophys J 98: 248-257, 2010.
13. Henzler Wildman KA, Lee DK and Ramamoorthy A: Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. Biochemistry 42: 6545-6558, 2003.
14. U.S. Department of Health and Human Services: Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0., 2017.
15. Bodey GP, Buckley M, Sathe YS and Freireich EJ: Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. Ann Intern Med 64: 328-340, 1966.
16. Munns C, Zacharin MR, Rodda CP, Batch JA, Morley R, Cranswick NE, Craig ME, Cutfield WS, Hofman PL, Taylor BJ, *et al*: Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: A consensus statement; Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: A consensus statement. Med J Aust 185: 268-272, 2006.
17. Holick MF: Medical progress vitamin D deficiency. N Engl J Med 357: 266-281, 2007.
18. Castelán-Martínez OD, Rodríguez-Islas F, Vargas-Neri JL, Palomo-Colli MA, López-Aguilar E, Clark P, Castañeda-Hernández G and Rivas-Ruiz R: Risk factors for febrile neutropenia in children with solid tumors treated with cisplatin-based chemotherapy. J Pediatr Hematol Oncol 38: 191-196, 2016.
19. Bothra M, Seth R, Kapil A, Dwivedi SN, Bhatnagar S and Xess I: Evaluation of predictors of adverse outcome in febrile neutropenic episodes in pediatric oncology patients. Indian J Pediatr 80: 297-302, 2013.
20. Santolaya ME, Alvarez AM, Avilés CL, Becker A, Mosso C, O'Ryan M, Payá E, Salgado C, Silva P, Topelberg S, *et al*: Admission clinical and laboratory factors associated with death in children with cancer during a febrile neutropenic episode. Pediatr Infect Dis J 26: 794-798, 2007.
21. Erbaş İC, Çakıl Güzin A, Özdem Alataş Ş, Karaoğlu Asrak H, Akans İ, Akyol Ş, Özlü C, Tüfekçi Ö, Yılmaz Ş, Ören H and Belet N: Etiology and factors affecting severe complications and mortality of febrile neutropenia in children with acute leukemia. Turk J Haematol 40: 143-153, 2023.
22. Das A, Trehan A and Bansal D: Risk factors for microbiologically-documented infections, mortality and prolonged hospital stay in children with febrile neutropenia. Indian Pediatr 55: 859-864, 2018.
23. Gupta S, Bonilla M, Gamero M, Fuentes SL, Caniza M and Sung L: Microbiology and mortality of pediatric febrile neutropenia in El Salvador. J Pediatr Hematol Oncol 33: 276-280, 2011.
24. Hancock REW, Haney EF and Gill EE: The immunology of host defence peptides: Beyond antimicrobial activity. Nat Rev Immunol 16: 321-334, 2016.
25. Sørensen O, Arnljots K, Cowland JB, Bainton DF and Borregaard N: The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. Blood 90: 2796-2803, 1997.
26. Park K, Elias PM, Oda Y, Mackenzie D, Mauro T, Holleran WM and Uchida Y: Regulation of cathelicidin antimicrobial peptide expression by an endoplasmic reticulum (ER) stress signaling, vitamin d receptor-independent pathway. J Biol Chem 286: 34121-34130, 2011.
27. Cirioni O, Ghiselli R, Tomasinsig L, Orlando F, Silvestri C, Skerlavaj B, Riva A, Rocchi M, Saba V, Zanetti M, *et al*: Efficacy of LL-37 and granulocyte colony-stimulating factor in a neutropenic murine sepsis due to pseudomonas aeruginosa. Shock 30: 443-448, 2008.
28. Mansbach JM, Hasegawa K, Ajami NJ, Petrosino JF, Piedra PA, Tierney CN, Espinola JA and Camargo CA: Serum LL-37 levels associated with severity of bronchiolitis and viral etiology. Clin Infect Dis 65: 967-975, 2017.
29. Suberviola B, Lavin BA, Jimenez AF, Perez-San Martin S, Garcia-Unzueta M and Santibañez M: Vitamin D binding protein, but not vitamin D or vitamin D-related peptides, is associated with septic shock mortality. Enferm Infecc Microbiol Clin (Engl Ed) 37: 239-243, 2019.
30. Barbeiro DF, Barbeiro HV, Zampieri FG, César Machado MC, Torggler Filho F, Gomes Cunha DM, Goulart AC, Velasco IT, Monteiro da Cruz Neto L, Possolo de Souza H and Pinheiro da Silva F: Cathelicidin LL-37 bloodstream surveillance is down regulated during septic shock. Microbes Infect 15: 342-346, 2013 (In English, Spanish).
31. Nagaoka I, Tamura H and Reich J: Therapeutic potential of cathelicidin peptide LL-37, an antimicrobial agent, in a murine sepsis model. Int J Mol Sci 21: 5973, 2020.
32. Cai S, Qiao X, Feng L, Shi N, Wang H, Yang H, Guo Z, Wang M, Chen Y, Wang Y and Yu H: Python cathelicidin CATHPb1 protects against multidrug-resistant staphylococcal infections by antimicrobial-immunomodulatory duality. J Med Chem 61: 2075-2086, 2018.
33. Gupta V, Kumar V and Singh SK: Low vitamin D levels are associated with an adverse clinical outcome in febrile neutropenia. J Pediatr Hematol Oncol 38: 202-204, 2016.
34. Leow L, Simpson T, Cursons R, Karalus N and Hancox RJ: Vitamin D, innate immunity and outcomes in community acquired pneumonia. Respirology 16: 611-616, 2011.
35. Greulich T, Regner W, Branscheidt M, Herr C, Koczulla AR, Vogelmeier CF and Bals R: Altered blood levels of vitamin D, cathelicidin and parathyroid hormone in patients with sepsis-a pilot study. Anaesth Intensive Care 45: 36-45, 2017.

36. Jungrungrueng T, Anugulruengkitt S, Lauhasurayotin S, Chiengthong K, Poparn H, Sosothikul D and Techavichit P: The pattern of microorganisms and drug susceptibility in pediatric oncologic patients with febrile neutropenia. J Pathog 2021: 6692827, 2021.
37. Suttitossatam I, Satayasai W, Sinlapamongkolkul P, Pusongchai T, Sritipsukho P and Surapolchai P: Predictors of severe adverse outcomes in febrile neutropenia of pediatric oncology patients at a single institute in Thailand. Pediatr Hematol Oncol 37: 561-572, 2020.
38. Rabagliati BR, Fuentes LG, Orellana UE, Oporto CJ, Domínguez MI, Benítez GR, Aedo CI, Ramos GG, Garrido SM and García CP: Etiology of febrile neutropenia episodes among cancer patients from Hospital Clinico Universidad Catolica, Santiago-chile. Rev Chilena Infectol 26: 106-113, 2009 (In Spanish).
39. Jadhav N, Mandal J, Kayal S, Pattnaik J, Madasamy P, Singh J and Dubashi B: Surveillance stool culture and its association with microbiologically documented infection during febrile neutropenia in patients with acute leukemia (AL) undergoing induction chemotherapy. Indian J Hematol Blood Transfus 37: 543-548, 2021.
40. Koenig C, Schneider C, Morgan JE, Ammann RA, Sung L and Phillips B: Association of time to antibiotics and clinical outcomes in patients with fever and neutropenia during chemotherapy for cancer: A systematic review. Support Care Cancer 28: 1369-1383, 2020.
41. De Castro GC, Slatnick LR, Shannon M, Zhao Z, Jackson K, Smith CM, Whitehurst D, Elliott C, Clark CC, Scott HF, *et al*: Impact of time-to-antibiotic delivery in pediatric patients with cancer presenting with febrile neutropenia. JCO Oncol Pract 20: 228-238, 2024.
42. Simon A, Lehrnbecher T, Baltaci Y, Dohna-Schwake C, Groll A, Laws HJ, Potratz J, Hufnagel M and Bochennek K: Time to antibiotics (TTA)-reassessment from the German working group for fever and neutropenia in children and adolescents (DGPI/GPOH). Klin Padiatr 235: 331-341, 2023 (In German).
43. Handattu K, Sharma LK, Vijayasekharan K, Bhat KV, Aroor S and Sudhanshu S: Drug Induced diabetes mellitus in pediatric acute lymphoblastic leukemia: Approach to diagnosis and management. J Pediatr Hematol Oncol 44: 273-279, 2022.
44. Brealey D and Singer M: Hyperglycemia in critical illness: A review. J Diabetes Sci Technol 3: 1250-1260, 2009.
45. Sundén-Cullberg J, Nyström T, Lee ML, Mullins GE, Tokics L, Andersson J, Norrby-Teglund A and Treutiger CJ: Pronounced elevation of resistin correlates with severity of disease in severe sepsis and septic shock. Crit Care Med 35: 1536-1542, 2007.
46. Rondinelli PIP, Ribeiro K de CB and de Camargo B: A proposed score for predicting severe infection complications in children with chemotherapy-induced febrile neutropenia. J Pediatr Hematol Oncol 28: 665-670, 2006.