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ORIGINAL PAPER

The human cathelicidin hCAP-18 in serum of children with haemato-oncological diseases

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Summary

The human cathelicidin hCAP-18 (pro-LL-37) is the pro-protein of the antimicrobial peptide LL-37. hCAP-18 can be produced by many different cell types; bone marrow neutrophil precursors are the main source of hCAP-18 in the circulation. Neutrophil count is used as a marker for myelopoiesis but does not always reflect neutrophil production in the bone marrow, and thus additional markers are needed. In this study, we established the reference interval of serum hCAP-18 level in healthy children and compared serum hCAP-18 levels between different diagnostic groups of children with haemato-oncological diseases, at diagnosis. We found that children with diseases that impair myelopoiesis, such as acute leukaemia, aplastic anaemia, or myelodysplastic syndrome, presented with low hCAP-18 levels, whereas patients with non-haematological malignancies displayed serum hCAP-18 levels in the same range as healthy children. Children with chronic myeloid leukaemia presented with high circulating levels of hCAP-18, probably reflecting the high number of all differentiation stages of myeloid cells. We suggest that analysis of serum hCAP-18 provides additional information regarding myelopoiesis in children with haemato-oncological diseases, which may have future implications in assessment of myelopoiesis in clinical management.

KEYWORDS
aplastic anaemia, cathelicidin, hCAP-18, leukaemia, myelopoiesis, paediatric cancer

Katrin Pütsep and Arja Harila-Saari contributed equally.

INTRODUCTION

Children with haemato-oncological diseases are often immunocompromised due to bone marrow suppression caused by the disease and/or the cytotoxic treatment. Severe infections during immunosuppression are associated with significant morbidity and mortality.[1–4] Even among patients who receive an identical treatment, the individual risk for myelosuppression and infections can vary significantly. Absolute neutrophil count (ANC) is used to evaluate myelopoiesis in patients with haemato-oncological diseases.[5–7]

Approximately two-thirds of the haematopoiesis in the bone marrow is devoted to myelopoiesis, with about 10^9 neutrophils/kg body weight being generated every day in healthy humans.[8] Neutrophils are distributed in proliferative, circulating, and marginating pools.[9] The measurement of neutrophils in the peripheral blood estimates the circulating pool, which may be influenced by various factors, such as autoimmunity, infections, inflammation, and treatment with glucocorticoids, and thus does not necessarily reflect myelopoiesis in the bone marrow. Therefore, specific markers of myelopoietic bone marrow function are needed.

The human cathelicidin hCAP-18 (human cationic antimicrobial protein, pro-LL-37),[10] is the pro-protein of the antimicrobial peptide LL-37.[11] hCAP-18 in blood stems from the bone marrow,[12] where it is produced during granule formation at the myelocyte to metamyelocyte stage of neutrophil differentiation.[13] hCAP-18 may be a more specific marker of myelopoiesis than ANC as the differentiating neutrophil precursors in the bone marrow are the main source of hCAP-18. hCAP-18 levels in blood are low in congenital neutropenia with bone marrow maturation arrest, but normal levels are reported in auto-immune neutropenia with normal bone marrow function.[14,15] A decreased level of plasma hCAP-18 was associated with recurrent periodontal infections in patients with severe congenital neutropenia[16] and with oral mucositis in children with leukaemia.[17] Vitamin D has the capacity to induce hCAP-18/LL-37 in leukocytes and epithelial cells,[18,19] but it is not known whether vitamin D status affects blood hCAP-18 levels. Reference values for hCAP-18 in adults have been published,[20] but are lacking for children.

In light of these findings, it is of interest to study serum hCAP-18 levels in children with haemato-oncological diseases at diagnosis and in healthy children. We aimed to establish the reference interval of serum hCAP-18 in a population of healthy children, investigate hCAP-18 levels in children with haemato-oncological diseases at diagnosis, and to assess the usefulness of hCAP-18 as a marker of myelopoiesis by investigating the association between serum hCAP-18, clinical characteristics, vitamin D levels and laboratory values related to myelopoiesis.

METHODS

Patients, controls and study design

This retrospective cross-sectional cohort study included samples from 198 children (121 males, 61%), that were routinely collected at diagnosis from children with haemato-oncological diseases between May 1989 and November 2016 and stored at −80°C in Uppsala Biobank. Altogether 1480 children were diagnosed with haemato-oncological diseases during this time at University Uppsala Children's Hospital; biobanked samples were available for research use from 945 of them (64%).

The cohort included 87 patients with acute leukaemia, 80 patients with non-haematological malignancies and 31 patients with rare diseases including chronic myeloid leukaemia (CML), juvenile myelomonocytic leukaemia (JMML), myelodysplastic syndrome (MDS), aplastic anaemia, Fanconi anaemia (FA), Langerhans cell histiocytosis (LCH), and haemophagocytic lymphohistiocytosis (HLH). Among the 11 patients with aplastic anaemia, four patients displayed very severe aplastic anaemia (with a neutrophil count of $<0.2 \times 10^9$/l), three, severe aplastic anaemia (with a neutrophil count of $<0.5 \times 10^9$/l), and four, aplastic anaemia (with a neutrophil count of $>0.5 \times 10^9$/l) (Table 1).

The median age at the time of diagnosis was 7.5 years (range 0.1–17.8 years).

The control group was a school-based study cohort that was originally recruited for a cross-sectional study assessing vitamin D status in healthy children and adolescents in Helsinki, Finland[21] and included 175 healthy children (68 males, 39%), with a median age of 13.2 years (range 7.4–18.8 years). The serum samples in the control cohort were collected between November and March in 2006–2008 and stored at −80°C.

All samples were thawed only once and then frozen in aliquots to avoid rounds of freezing–thawing.

The use of biobank samples and associated clinical data of patients was approved by the Regional Ethical Review Board of Uppsala, Sweden (2014/511). The original study from which the controls were retrieved was approved by the Ethics committee at Helsinki University Hospital, Helsinki, Finland (2005/212).

Clinical data

Clinical data, collected from the Swedish Childhood Cancer Registry and from medical records, included age, sex, diagnosis and laboratory parameters: ANC, white blood cell count (WBC) and C-reactive protein (CRP). In addition, for the children with acute leukaemia we recorded the percentage of blasts in the bone marrow in the morphological analyses. In three children with T-cell acute lymphoblastic leukaemia (T-ALL) and very high WBC (410, 216 and 320×10^9/l) and peripheral blast count (86%, 95%, and 96%, respectively) the value was lacking. In these cases, percentages of peripheral blood blasts were used instead of bone marrow blasts. Data on WBC were available for 196/198 (99%) children, ANC for 185/198 (93%) children, CRP for 190/198 (96%) children, and bone marrow blasts for 85/87 (98%) children with acute leukaemia.

TABLE 1 Patient characteristics and laboratory findings according to sex and diagnosis

Groups	*N*	Age, years median (IQR)	hCAP-18 (ng/ml) median (IQR)	ANC × 10^9/l median (IQR)	WBC × 10^9/l median (IQR)	25(OH)D nmol/l (IQR)
All patients	198	7.5 (3.0–13.5)	259 (59–580)	2.3 (0.6–4.7)	7.9 (4.6–16.6)	51 (35–69)
Sex						
Male	121	9.2 (3.9–13.9)	267 (70–581)	2.4 (0.7–4.6)	7.9 (5.0–20.8)	48 (32–64)
Female	77	5.5 (2.2–12.1)	250 (46–583)	1.9 (0.5–5.0)	7.9 (3.4–13.5)	55 (43–74)
Non-haematological malignancies	80	7.8 (3.0–14.5)	567 (388–759)	3.5 (2.5–5.2)	8.1 (5.7–10.4)	53 (31–70)
Nephroblastoma	8	3.1 (1.0–6.1)	398 (281–664)	3.3 (2.6–4.0)	7.8 (5.3–12.7)	67 (25–81)
Soft-tissue sarcoma	5	9.3 (7.5–14.2)	563 (309–813)	2.3 (1.9–3.1)	5.4 (4.6–6.5)	55 (43–73)
Neuroblastoma	16	2.1 (1.1–3.2)	442 (301–536)	3.7 (2.3–5.4)	8.7 (7.0–10.4)	62 (32–82)
Osteosarcoma	10	13.9 (11.0–15.8)	720 (639–858)	3.9 (2.9–5.1)	6.6 (5.5–8.6)	34 (27–62)
Ewing sarcoma	4	9.9 (6.5–14.0)	601 (401–927)	3.8	8.4 (6.3–9.4)	46
Other solid tumours	5	5.1 (1.6–11.8)	725 (361–992)	3.5 (2.3–6.9)	8.8 (5.0–11.2)	70 (59–77)
Brain tumour	10	6.7 (2.5–13.7)	474 (360–729)	6	10.4 (8.8–13.8)	51 (29–68)
HD	11	15.2 (12.4–17.3)	725 (578–1074)	4.6 (2.2–9.0)	5.9 (4.6–12.3)	34 (23–63)
NHL	11	8.5 (4.0–16.4)	569 (445–753)	3.8 (2.9–7.2)	6.8 (5.6–15.0)	45 (27–66)
Acute leukaemia	87	7.4 (3.3–13.4)	68 (26–156)	1.0 (0.2–2.8)	9.1 (3.8–63.6)	51 (39–69)
B-ALL	37	4.0 (2.7–7.7)	42 (18–78)	0.4 (0.2–1.2)	6.0 (3.1–17.8)	63 (46–76)
Ph^+ ALL	8	10.9 (9.4–13.4)	85 (36–179)	1.6 (0.7–4.0)	36.3 (6.9–78.6)	55 (41–82)
T-ALL	13	13.2 (5.0–14.0)	135 (95–285)	9.1 (3.0–11.5)	216.0 (88.8–301.0)	32 (20–61)
AML	29	11.8 (4.6–15.0)	80 (34–168)	1.4 (0.3–2.3)	8.8 (2.4–51.8)	50 (37–56)
CML	5	8.5 (5.9–10.7)	2883 (1937–5881)	149.6 (104.8–177.4)	296 (169.0–326.0)	42 (33–54)
JMML	3	1.2/1.9/3.6	277/364/555	7.8/9.7	25.6/46.0/21.0	34/44/83
MDS	3	3.1/16.0/14.0	144/221/45	1.2/0.8/0.3	5.0/3.8/2.0	43/44/65
Aplastic anaemia	11	10.7 (5.1–13.8)	15 (6–68)	0.3 (0.1–0.7)	2.1 (1.3–2.5)	46 (36–62)
FA	2	10.0/10.1	71/586	0.6/0.7	3.2/2.0	35/54
LCH	3	0.9/2.2/13.5	536/856/552	6.9/4.3	10.4/6.7	58/64
HLH	4	1.5 (0.5–2.9)	820 (169–1790)	2.3 (0.2–9.3)	4.9 (2.0–17.6)	47.2

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AML, acute myeloid leukaemia; ANC, absolute neutrophil count; B-ALL, precursor B-cell acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; FA, Fanconi anaemia; hCAP-18, human cationic antimicrobial protein; HD, Hodgkin lymphoma; HLH, haemophagocytic lymphohistiocytosis; IQR, interquartile range; JMML, juvenile myelomonocytic leukaemia; LCH, Langerhans cell histiocytosis; MDS, myelodysplastic syndrome; NHL, Non-Hodgkin lymphoma; Ph^+ ALL, Philadelphia chromosome-positive B-ALL; T-ALL, T-cell ALL; WBC, white blood cells.

hCAP-18 enzyme-linked immunosorbent assay

For quantification of hCAP-18 in blood serum, a recently developed enzyme-linked immunosorbent assay (ELISA) validated for clinical use was utilized. For assay details, see Mörtberg *et al.*[20] In short, the hCAP-18 ELISA is a sandwich ELISA with a monoclonal mouse-anti-hCAP-18 as capture antibody (clone H7, Thermo Fisher Scientific, Stockholm, Sweden) and a polyclonal rabbit anti-hCAP-18 detection antibody (OSC0000W, Thermo Fisher Scientific). Recombinant hCAP-18 protein (cAMP, 34–173 aa, Nordic Biosite, Täby, Sweden) was used to construct a calibration curve. For analysis of cancer patient samples, pooled human sera (Sigma-Aldrich, #H4522, Stockholm, Sweden) diluted 1:100 was included as an internal control. Patient samples were diluted 1:100, except for patients with CML for whom a dilution of 1:300 was used. Absorbance was read on a SpectraMax 190 plate reader (Molecular Devices, Wokingham, Berkshire, UK). A four-parameter logistic function was used to fit the curve and determine unknown concentrations with the software SoftMaxPro v. 6.2.2.

Serum 25-hydroxyvitamin D

Serum 25-hydroxyvitamin D (25[OH]D) was measured in patients with the direct competitive immunochemiluminescent assay LIAISON 25-OH Vitamin D TOTAL Assay CLIA DiaSorin (Stillwater, MN, USA) and in the control group, with high-performance liquid chromatography. Both methods were standardized according to the Vitamin D External Quality Assessment Scheme. Details of the measurements and the results have been reported elsewhere.[21–23] Vitamin D deficiency was defined as a 25(OH)D level of less than 50 nmol/l.

Statistical analyses

Statistical analyses were performed using SPSS, version 27 (IBM, New York, NY, USA). Parameters are presented as median and interquartile range (IQR). $p < 0.05$ was regarded statistically significant. Since serum hCAP-18 level was not normally distributed across samples, we used the non-parametric independent-samples Kruskal–Wallis test with Bonferroni adjustment for multiple testing to compare serum hCAP-18 levels across diagnostic groups with more than five participants, and healthy children. The diagnostic groups encompassed children with acute leukaemia, aplastic anaemia and non-haematological malignancies. To further compare serum hCAP-18, ANC and WBC values between patients with acute leukaemia across diagnostic groups (precursor B-cell acute lymphoblastic leukaemia, B-ALL; Philadelphia chromosome-positive B-ALL, Ph^+ ALL; T-ALL; and acute myeloid leukaemia, AML) we used the non-parametric Kruskal–Wallis test with Bonferroni adjustment for all pair-wise comparisons, as Levene's test for homogeneity of variance showed that all these three variables were heterogenic ($p = 0.04$ for hCAP-18, $p = 0.03$ for ANC, and $p < 0.001$ for WBC).

Simple and multiple linear regression analyses with backward elimination were performed to assess the association between clinical and laboratory parameters and hCAP-18 in children with acute leukaemia and in children with non-haematological malignancies. Because residuals in regression models including hCAP-18 were not normally distributed, we used the logarithm of hCAP-18 (log-hCAP-18) to obtain normally distributed residuals. The following candidate predictors were included: age, sex, ANC, WBC, CRP, 25(OH)D for both groups, and additionally, diagnosis (B-ALL, Ph^+ ALL, T-ALL, and AML) and percentage of bone marrow blasts for the acute leukaemia group. We used separate regression models for these two diagnostic groups because the two variables, acute leukaemia diagnosis and blasts in the bone marrow, were included in the acute leukaemia group only and because significant interaction was observed between ANC and diagnosis ($p = 0.005$) in the ANOVA test.

We used the Spearman correlation test to explore the association between hCAP-18 and age, sex and 25(OH)D levels in healthy controls; between hCAP-18 and 25(OH)D levels in the patients group; and between percentage of leukaemic blasts in the bone marrow and hCAP-18 or ANC in children with acute leukaemia. Regarding 25(OH)D, statistical analyses were done for the whole groups and then separately for the participants who were presented with 25(OH)D levels below 50 nmol/l or below 75 nmol/l.

RESULTS

Healthy children

The serum hCAP-18 levels in the control group of healthy children were not normally distributed. The reference range was set to 2.5–97.5 percentiles which corresponds to 260–1260 ng/ml and is similar to the reference interval for plasma concentrations obtained for healthy adult blood donors (223–1276 ng/ml).[20] Serum 25(OH)D measurements were available for 174 participants and among these, 71% were vitamin D-deficient. None of the tested variables, age, sex, or 25(OH)D levels, correlated with the hCAP-18 serum levels in healthy children (data not shown).

Children with haemato-oncological diseases

hCAP-18 in blood serum and plasma has been shown to be stable over long time, stored at −80°C, as evident from previous work.[14–16,20] In order to investigate a possible impact of long-term storage on hCAP-18 stability in serum, we performed Spearman correlation tests between hCAP-18 and sample age. There was no correlation for children with acute leukaemia ($p = 0.4$), nor for children with non-haematological malignancies ($p = 0.7$), indicating that hCAP-18 levels in this study were stable following long-term storage.

The median serum hCAP-18 in the patient group was 259 ng/ml, with a range between 6 and 6288 ng/ml (Table 1). In 50% of the children in the entire cohort, hCAP-18 values were below the reference interval and in 4% values were above the reference interval. Of the children with non-haematological malignancies, 93% presented with normal hCAP-18 levels (Table 1). Serum 25(OH)D levels were available for 183 patients, 46% of whom were vitamin D-deficient. There was no correlation between serum hCAP-18 and 25(OH)D levels, neither within the whole group of patients, nor in the group that presented with 25(OH)D levels below 50 nmol/l or below 75 nmol/l ($p = 0.3$, $p = 0.07$ and $p = 0.5$ respectively).

Comparison of serum hCAP-18 levels among different diagnostic groups and healthy children

No differences could be discerned between healthy children and children with non-haematological malignancies, while the serum hCAP-18 level was significantly lower in children with acute leukaemia and aplastic anaemia as compared to the control group ($p < 0.001$ for both) or to the group of children with non-haematological malignancies ($p < 0.001$ for both), (Figure 1A). For children with rare haemato-oncological diseases there seemed to be differences between diagnostic groups, and especially the patients with CML displayed high hCAP-18 values above reference levels (Figure 1B). However, no statistical analysis could be performed due to the small group sizes. Upon stratification of the acute leukaemias into subgroups, only the difference between B-ALL and T-ALL was found to be statistically significant ($p = 0.002$) (Figure 2). Patients with T-ALL also presented with higher ANC and WBC than children with B-ALL ($p < 0.001$ for both) and children with AML ($p = 0.004$ and $p < 0.001$, respectively).

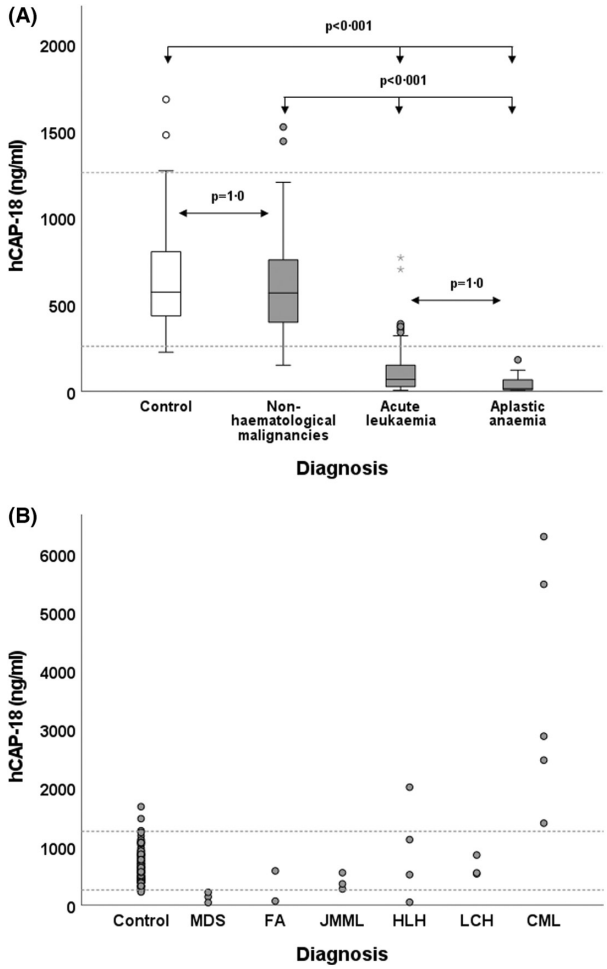

FIGURE 1 Serum hCAP-18 levels in paediatric patients with haemato-oncological diseases compared to healthy children (control). Dotted lines show the reference interval obtained from healthy controls. (A) Healthy controls ($n = 175$) and patient diagnosis groups with more than five participants; non-haematological malignancies ($n = 80$), acute leukaemia ($n = 87$), aplastic anaemia ($n = 11$). Statistical analyses were done by Kruskal–Wallis test with Bonferroni adjustment. Bullets and stars represent outliers. $p < 0.05$ was considered statistically significant. (B) hCAP-18 levels in diagnostic groups with five or less participants. MDS: myelodysplastic syndrome ($n = 3$); FA: Fanconi anaemia ($n = 2$); JMML: juvenile myelomonocytic leukaemia ($n = 3$); HLH: haemophagocytic lymphohistiocytosis ($n = 4$); LCH: Langerhans cell histiocytosis ($n = 3$); CML: chronic myeloid leukaemia ($n = 5$).

Factors associated with log-hCAP-18

We assessed whether age, sex, ANC, WBC, CRP, 25(OH) D, bone marrow blasts or type of diagnosis had an impact on log-hCAP-18 in children with acute leukaemia. Using simple linear regression, age ($p = 0.044$), ANC ($p < 0.001$), WBC ($p = 0.02$), and diagnosis (T-ALL, $p < 0.001$ and AML, $p = 0.007$, compared with B-ALL), were positively associated with log-hCAP-18, whereas bone marrow blasts ($p = 0.043$) were negatively associated with log-hCAP-18. When using multiple linear regression with backward elimination for all possible predictors, only age ($p = 0.045$) and ANC ($p < 0.001$) were positively associated with log-hCAP-18 in the acute leukaemia group of patients (Table 2 and Table S1).

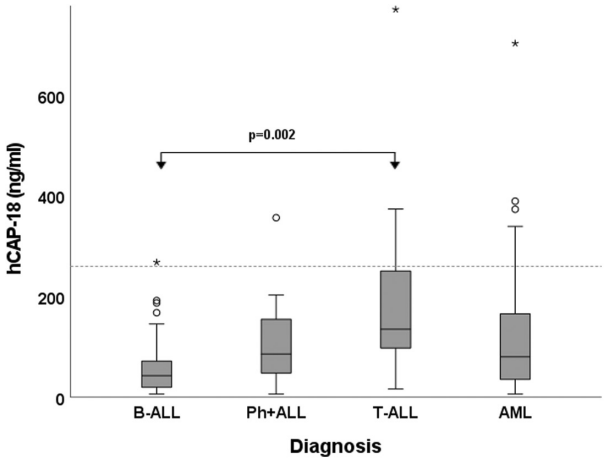

FIGURE 2 Serum hCAP-18 levels in patients with acute leukaemia ($n = 87$), stratified into different diagnosis groups: precursor B-cell acute lymphoblastic leukaemia, (B-ALL, $n = 37$); Philadelphia chromosome-positive B-ALL, (Ph^+ ALL, $n = 8$); T-cell ALL, (T-ALL, $n = 13$); acute myeloid leukaemia (AML, $n = 29$). The dotted line shows the lower reference interval obtained from healthy controls. Bullets and stars represent outliers and $p < 0.05$ was considered statistically significant (Table S2). The statistical analyses were performed by Kruskal–Wallis test with Bonferroni adjustment.

We analysed whether age, sex, ANC, WBC, CRP or 25(OH)D had an impact on log-hCAP-18 in children with non-haematological malignancies and found that age ($p = 0.004$), CRP ($p = 0.043$) and ANC ($p = 0.012$) were positively associated with log-hCAP-18 in the simple linear regressions, whereas none of the tested variables was associated with log-hCAP-18 using multiple linear regression with backward elimination (Table 2 and Table S1). Although six patients with neuroblastoma and two patients with non-Hodgkin lymphoma presented with bone marrow metastases, they displayed hCAP-18 levels between 311 and 753 ng/ml, which is within the reference interval.

Relation between leukaemic infiltration of the bone marrow and hCAP-18 or ANC

Next, we investigated which parameters, serum hCAP-18 level or ANC, most consistently mirrored the extent of bone marrow infiltration with blasts in patients with acute leukaemia. Percentage of leukaemic blasts in the bone marrow correlated negatively with serum hCAP-18 (correlation coefficient −0.223, $p = 0.04$), but not with ANC ($p = 0.2$). Among children with more than 75% blasts in the bone marrow, 93% had low serum hCAP-18 levels, whereas ANC below 1.5×10^9/l was only observed in 60% of these children (Figure 3).

DISCUSSION

In the present study, we established a reference interval for serum hCAP-18 in healthy children and assessed serum

TABLE 2 Factors influencing log-hCAP-18 in children with acute leukaemia (87) and in children with non-haematological malignancies (80) using simple linear regression (unadjusted) and multiple linear regression (adjusted) with backward elimination analyses. Only the last models of the backward elimination analyses are shown (complete analyses are presented in the Table S1)

	B	Beta	*p*	R^2
Factors influencing log-hCAP-18 in children with acute leukaemia				
Simple linear regression				
Age (years)	0.049	0.216	0.044*	0.047
Sex	−0.255	−0.104	0.3	0.011
CRP	−0.001	−0.048	0.6	0.002
ANC	0.137	0.492	<0.001*	0.242
WBC	0.003	0.249	0.02*	0.062
25(OH)D	−0.007	−0.126	0.2	0.016
Blasts in bone marrow (%)	−0.010	−0.220	0.043*	0.048
Diagnosis				0.173
B-ALL	Reference			
Ph^+ ALL	0.653	0.159	0.1	
T-ALL	1.372	0.413	<0.001*	
AML	0.754	0.300	0.007*	
				0.601
Multiple regression with backward elimination (last model)				
Age (years)	0.043	0.192	0.045*	0.262
ANC	0.133	0.485	<0.001*	
Factors influencing log-hCAP-18 in children with non-haematological malignancies				
Simple linear regression				
Age (years)	0.026	0.315	0.004*	0.099
Sex	0.211	0.212	0.059	0.045
CRP	0.003	0.230	0.043*	0.053
ANC	0.039	0.300	0.012*	0.090
WBC	0.013	0.120	0.2	0.014
				0.301
Multiple regression with backward elimination (last model)				
Age (years)	0.020	0.245	0.053	0.070
CRP	0.003	0.226	0.073	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AML, acute myeloid leukaemia; ANC, absolute neutrophil count; B-ALL, precursor B-cell acute lymphoblastic leukaemia; CRP, C-reactive protein; Ph + ALL, Philadelphia chromosome positive B-ALL; T-ALL, T-cell ALL; WBC, white blood cells.

*$p < 0.05$.

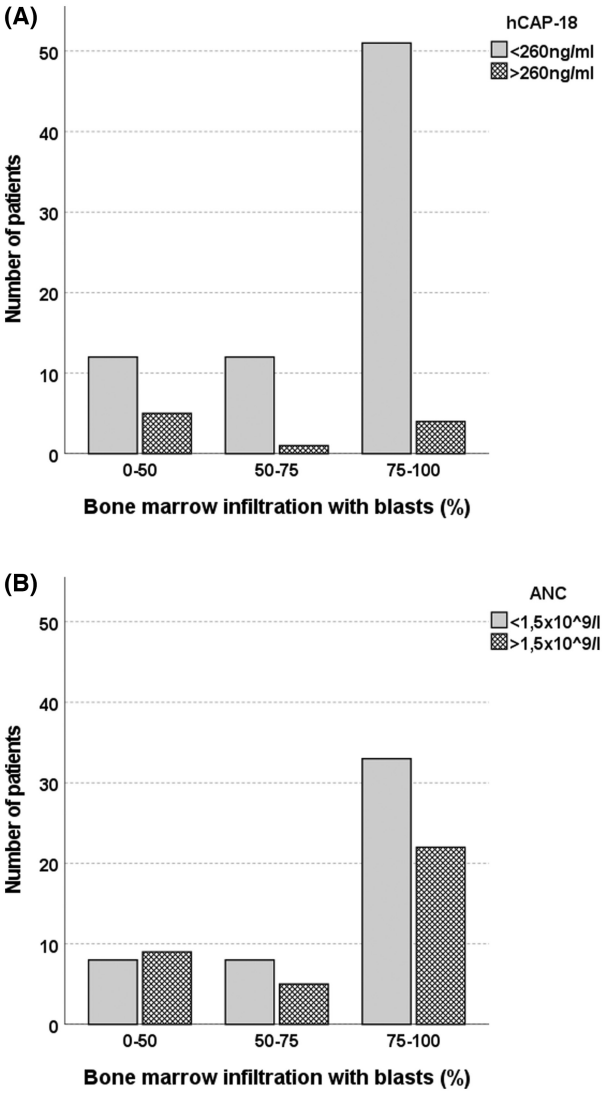

FIGURE 3 Serum hCAP-18 levels (A) and absolute neutrophil count (ANC) (B) in 85 children with acute leukaemia in relation to leukaemic infiltration of the bone marrow within the ranges of 0%–50%, 50%–75% and 75%–100% of blasts.

levels of hCAP-18 in children with haemato-oncological diseases. Our results indicate that hCAP-18 mirrors the myelopoietic function of the bone marrow and is thus low in children with depressed myelopoiesis, such as in children with acute leukaemia, MDS or aplastic anaemia. In contrast, serum hCAP-18 was within the reference interval in children with non-haematological malignancies, including those with bone marrow metastases.

The relationship between circulating hCAP-18 levels and bone marrow myelopoiesis has been demonstrated previously in individuals subjected to haematopoietic stem cell transplantation.[12] hCAP-18 disappeared from the circulation with pretransplantation conditioning, in parallel to cessation of bone marrow activity. After transplantation and resumed bone marrow activity, hCAP-18 reappeared in the circulation. This was contrary to glucocorticoid treatment, after which the hCAP-18 concentration in blood was unaffected while ANC increased as neutrophils from the marginating pool entered the circulation.[12] Together these observations suggest that it is the bone marrow neutrophil precursors and not the circulating neutrophils that give rise to hCAP-18 in blood. In accordance with these results are studies of patients with severe congenital neutropenia whose bone marrow neutrophil precursors go into apoptosis before the stage

at which hCAP-18 is produced and who therefore present with low plasma levels of hCAP-18.[15]

In the present study, we demonstrate that children with acute leukaemia and children with aplastic anaemia present with low levels of hCAP-18 compared with children with non-haematological malignancies or with healthy children. In a previous study of mucositis in children with cancer, patients with acute leukaemia revealed lower levels of hCAP-18 in their blood plasma compared to patients with solid tumours or lymphoma.[17] This concurs with our findings and shows that similar results are obtained using either serum or plasma for hCAP-18 analysis.

The development of bone marrow failure in children with acute leukaemia and aplastic anaemia involves different mechanisms. Leukaemic cells may deplete the bone marrow of haematopoietic cells through displacement, inhibit production of downstream haematopoietic cells by impeding differentiation at the haematopoietic stem cell progenitor transition, or alter the bone marrow microenvironment.[24,25] In patients with aplastic anaemia, immunomediated destruction of haematopoietic stem cells results in a hypocellular bone marrow with a low proportion of CD34-positive cells.[26] Here, we observed that low hCAP-18 levels in patients with these diseases mirrored the impaired myelopoietic bone marrow function irrespective of underlying mechanisms.

Previous studies showed that the hyperleukocytosis in patients with ALL is associated with the T-cell phenotype.[27] According to our study, patients with T-ALL presented levels of hCAP-18 below the reference range, but higher as compared with patients with B-ALL, and with higher ANC and WBC as compared with patients with B-ALL and AML. The higher hCAP-18 level in these patents may imply that the myelopoietic activity in T-ALL patients is less affected. In the adjusted model, log-hCAP-18 was positively associated with age and ANC in children with acute leukaemia, but not in children with non-haematological malignancies. The fact that children with non-haematological malignancies displayed hCAP-18 levels in the same range as healthy children who were older, indicates that age does not influence hCAP-18 levels in individuals with unaffected myelopoiesis.

Not surprisingly, all patients with CML displayed high levels of hCAP-18, which probably reflects the high numbers of all differentiation stages of myeloid cells present in the bone marrow and peripheral blood. There are no previously published data regarding hCAP-18 in patients with CML available for comparison. Moreover, patients with MDS presented with low hCAP-18 levels similar to the patients with aplastic anaemia. This concurs with what has been reported on hCAP-18 in cases of marrow failure syndromes and impaired myelopoiesis, such as Barth syndrome and Schwachman–Diamond syndromes.[15]

Previous studies have shown that the *CAMP* gene encoding hCAP-18 has several vitamin D-binding elements in its promoter region and the active form of vitamin D, 1,25-dihydroxyvitamin D, enhances the expression of hCAP-18.[18,28] A positive correlation has been found between hCAP-18 and vitamin D levels in healthy adults, but only among individuals with vitamin D deficiency.[29] In our study, there was no correlation between serum hCAP-18 and 25(OH)D either in children with haemato-oncological diseases or in healthy controls, regardless of vitamin D status, excluding 25(OH)D as a confounding factor. Discrepancies between these findings may reflect differences in age of study cohorts and study design.

Finally, we demonstrated that the proportion of bone marrow blasts in children with acute leukaemia correlated negatively with serum hCAP-18 level, but not ANC, also indicating that hCAP-18 may more accurately reflect the myelopoiesis in the bone marrow than ANC.

Study limitations and strengths

The major limitations of the study are the use of a retrospective cross-sectional study design and the limited numbers of patients in rare diagnostic subgroups. Further limitations include the lack of control samples from younger subjects and differences between the patient group and the group of healthy children regarding age, sex, and season of the sample collection (all seasons of the year in the study group and November to March in the control group). Previous studies have shown a seasonal variation in vitamin D levels in the Nordic countries[22,30] and this may explain the different proportions of vitamin D deficiency in the patients and controls. Furthermore, the limited cohort size for the rare diagnostic groups may have prevented us from observing differences in hCAP-18 levels between them.

A major strength of our study is that we present novel data on serum hCAP-18 levels in several different groups of children with haemato-oncological diseases with varying bone marrow involvement, and also hCAP-18 levels in healthy children. To our knowledge this is the largest group of children with no disease or known underlying condition, for which serum hCAP-18 data have been determined and thus should prove useful in future research.

CONCLUSION

Taken together, our results demonstrate that patients with diseases that impair myelopoiesis, such as acute leukaemia, MDS, or aplastic anaemia, present with reduced serum hCAP-18 levels, whereas patients with non-haematological malignancies display hCAP-18 levels comparable to those of healthy children. Children with CML present with high levels of hCAP-18, probably reflecting the high numbers of all differentiation stages of myeloid cells present in the bone marrow and the peripheral blood. Based on our data, we suggest that assessment of serum hCAP-18 levels may provide complementary information regarding myelopoiesis in patients with haemato-oncological diseases, which may have future clinical implications, for example in the assessment of therapy toxicity on myelopoiesis and bone marrow recovery.

Further studies are needed to evaluate the value of hCAP-18 in the diagnosis and follow-up of children with haemato-oncological diseases.

AUTHOR CONTRIBUTIONS

Arja Harila-Saari and Katrin Pütsep conceived the study; Natalja Jackmann, Arja Harila-Saari, Per Frisk, Outi Mäkitie, Pauliina Utriainen and Katrin Pütsep designed the study; Birgitta Henriques-Normark and Katrin Pütsep provided essential laboratory resources and reagents; Natalja Jackmann, Per Frisk, Outi Mäkitie and Pauliina Utriainen collection the data; Natalja Jackmann, Katrin Pütsep, Arja Harila-Saari, Per Frisk, Sofia Englund, Outi Mäkitie, Pauliina Utriainen and Anette Mörtberg analysed and interpreted the data; Natalja Jackmann wrote the first draft of the manuscript; Natalja Jackmann, Katrin Pütsep, Arja Harila-Saari, Per Frisk, Pauliina Utriainen, Outi Mäkitie, Sofia Englund, Anette Mörtberg and Birgitta Henriques-Normark critically revised the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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