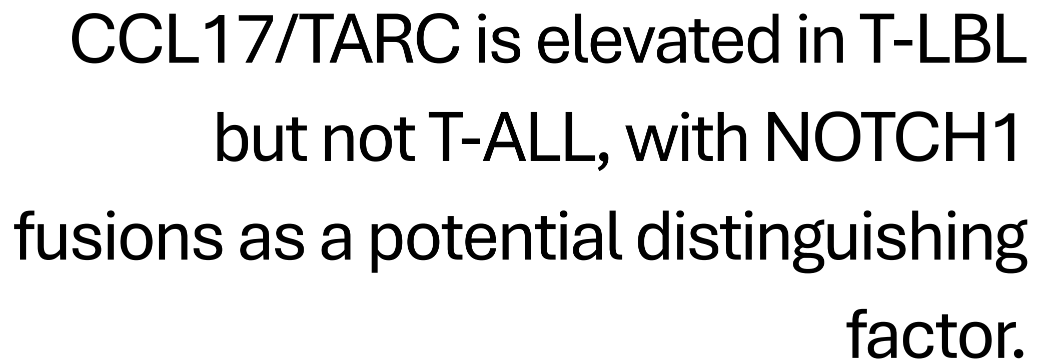

CCL17/TARC is elevated in T-LBL but not T-ALL, with NOTCH1 fusions as a potential distinguishing factor.

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Abbreviations

AML	Acute myeloid leukemia
ASCT	Allogeneic stem cell transplant
CCL17	C-C motif chemokine ligand 17
CCR4	C-C chemokine receptor type 4
CNS	Central nervous system
ELISA	Enzyme-linked immunosorbent assay
FCM	Flow cytometry
HR	High risk
IR	Intermediate risk
MR	Medium risk
MRD	Minimal residual disease
RNAseq	RNA sequencing
T-ALL	T-cell acute lymphoblastic leukemia
TARC	Thymus and activation-regulated chemokine
T-LBL	T-cell lymphoblastic lymphoma

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Abstract

T-cell acute leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are regarded as a spectrum of the same disease. 80% of patients survive, but only 15-25% of relapsed patients can be rescued. Risk stratification is based on MRD (in T-ALL) and/or high risk genetics. However, additional biomarkers are needed to early identify high-risk patients who could benefit from more intensive or alternative treatment strategies. T-LBL patients with NOTCH1 gene fusions have recently been identified as a high-risk group with CCL17 as a possible biomarker. This study investigates CCL17 levels in patients with T-LBL as well as T-ALL.
We included all children diagnosed with T-ALL between 2018-2024 in the Netherlands. Blood samples of 38 T-ALL patients were available for retrospective CCL17 measurements. Additionally, we included 36 T-LBL patients diagnosed between 2018-2024 for whom CCL17 values at time of diagnosis were available. CCL17 levels were measured by enzyme-linked immunosorbent assay.
CCL17 was not elevated in T-ALL patients (range 38-593 pg/mL). 9 T-LBL patients had elevated levels of CCL17, which was strongly correlated with the presence of NOTCH1 fusions ($p<0{,}001$). Additionally, the incidence of relapse was significantly higher for T-LBL patients with elevated CCL17, compared to patients with normal CCL17 ($p=0{,}002$). Elevated CCL17 was not correlated with mediastinal enlargement in T-ALL ($r=0{,}28$) or T-LBL ($r=0{,}14$).
These results suggest that CCL17 is a high risk biomarker for T-LBL but not T-ALL, with NOTCH1 fusions as a possible discriminator. Further research is necessary to translate these findings into clinical applications.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are malignancies of immature T-cells. The survival rate of children with T-ALL or T-LBL is approximately 80%, (1-5) but outcome after relapse is dismal, with salvage rates reaching only 15-25% (2, 3, 6-8). Considering the high toxicity of treatment and the poor prognosis after relapse, there is an urgent need for improved risk stratification by identifying new prognostic biomarkers.

T-ALL represents approximately 15% of ALL cases in children, whereas T-LBL accounts for approximately 20% of non-Hodgkin lymphomas in children (5, 8-10). In the current classification of hematolymphoid tumors, the World Health Organization regards T-ALL and T-LBL as the same disease, based on the great similarities in molecular genetics and immunophenotype (11). The origin of these immature T-cell malignancies lies in the accumulation of genetic aberrations in developing T-cells in the thymus, leading to differentiation arrest and leukemic transformation (1-4, 9). This malignant clone may subsequently infiltrate the bone marrow, peripheral blood, lymph nodes and central nervous system (CNS) (2, 3, 9). By definition, a diagnosis of T-ALL is

made in case of ≥25% bone marrow involvement, whereas patients with T-LBL have <25% involvement of the bone marrow (12, 13). T-ALL is often characterized by extensive bone marrow involvement and leukocytosis, resulting in symptoms such as bone pain, fever and thrombopenia (2). Patients with T-LBL typically present with large mediastinal masses and infiltration in the lymph nodes. As a result, symptoms include pleural or pericardial effusion, respiratory difficulties due to airway compression, and superior vena cava syndrome (14, 15). There is, however, overlap in clinical presentation.

Treatment of patients with immature T-cell malignancies consists of steroids, multiagent chemotherapy regimens and, in case of high-risk patients, stem-cell transplantation (1, 10, 14). Currently, some high-risk genetic biomarkers (e.g. ABL-class fusions) and minimal residual disease (MRD) measurements are used for risk stratification of patients with T-ALL. Other factors, such as immunophenotype and the majority of genetic aberrations found in T-ALL, have not conclusively been shown to have prognostic value beyond MRD-based stratification (2, 3, 10). For patients with T-LBL, MRD-based stratification has not been proven to be useful. Stratification of patients with T-LBL is currently based on the presence of NOTCH1 and/or FBXW7 mutations and CNS status (13, 14).

Considering the poor prognosis after relapse and the challenges of genetic diagnostics, additional and accessible parameters are needed for an early identification of high-risk patients who need more intensive treatment, as opposed to lower-risk patients for whom less intensive treatment is feasible.

A recent study by Kroeze et al. has identified a subgroup of T-LBL patients with NOTCH1 gene fusions and a high rate of relapse (67%)(13). All of these patients had elevated levels of C-C motif chemokine ligand 17 (CCL17), also called Thymus and activation-regulated chemokine (TARC)(13). Based on these results, CCL17 is a potential high-risk biomarker for patients with T-LBL.

CCL-17, which is a ligand for C-C chemokine receptor type 4 (CCR4), is highly expressed in the thymus and plays an important role in the development and function of T-cells (16, 17). Though mainly associated with T-helper 2 cells, CCL17 is secreted by various cell types (e.g. leukocytes, epithelial cells, platelets), and plays a part in several diseases, including autoinflammatory and autoimmune diseases, and different types of cancer (18-21). Currently, CCL-17 is used as a diagnostic and follow-up biomarker in children with classical Hodgkin lymphoma (16).

In this study, we present CCL17 levels in a larger cohort of T-LBL patients to investigate the extent to which NOTCH1 fusions are associated with elevated CCL17. In addition, we report CCL17 values in a cohort of children with T-ALL, and compare these data to the CCL17 values found in children with T-LBL.

Method

This retrospective cohort study included children who were diagnosed with T-ALL and treated in the Princess Máxima Center for Pediatric Oncology between 2018-2024. Patients with T-ALL who received part of their initial treatment abroad were excluded. For 80 out of 88 eligible patients, written informed consent was obtained from the patients and/or their legal guardians. In addition, we included 36 children who were diagnosed with T-LBL between 2018 and 2024 and for whom CCL17 levels at time of diagnosis were available. Clinical characteristics, stage of disease, central nervous system (CNS) status and hematological values were retrieved from

patient files. Organ involvement was confirmed by imaging (e.g. ultrasound). For patients with a mediastinal mass, the extent of mediastinal enlargement was quantified by measuring the ratio of the mass to the thoracal diameter above the fifth vertebra on chest X-ray. Flow cytometry (FCM) data were used to quantify the involvement of the bone marrow and peripheral blood. The presence of NOTCH1 gene fusions was determined with RNA sequencing (RNAseq) data. Patients diagnosed with T-ALL were treated according to the ALL Together protocol or its predecessor, the ALL-11 protocol. Treatment of T-LBL patients was in accordance with the EuroLB02 protocol or its successor, the EuroLB2018 protocol. Relapses and/or mortality were recorded. Length of follow-up was calculated from date of primary diagnosis. This study was performed in accordance with the Declaration of Helsinki and approved by all institutional ethic committees.

CCL17 measurements

For 38 T-ALL patients, frozen blood samples taken at time of diagnosis were available for CCL17 measurements and collected from the Princess Máxima Center Biobank. CCL17 measurements were performed by standard enzyme-linked immunosorbent assay (ELISA), using the Quantikine ELISA Human CCL17/TARC (Bio-Techne R&Dsystems). Standardized cut-off values of 848 pg/mL for serum and 285 pg/mL for plasma were used to determine whether CCL17 was elevated. CCL17 levels were measured either in plasma or serum, depending on the available samples. Additional details on the timing and source of samples used for CCL17 measurements are provided in supplementary table 1.

For 36 T-LBL patients, CCL17 values were determined at time of diagnosis and retrieved from patient files.

Statistical analysis

T-test or Fisher's exact test were used to determine the statistical significance of differences in CCL17 levels between patient groups. Pearson's correlation coefficient was calculated to assess the correlation between CCL17 levels and continuous variables.

Results

Patient characteristics

The clinical characteristics of 80 T-ALL patients are summarized in table 1. Mean age at time of diagnosis was 10 years (range 0-19). 74% of patients were male, 26% were female. The majority of patients presented with a mediastinal mass (70%) and/or lymphadenopathy (71%). Other symptoms included hepatomegaly (43%), splenomegaly (48%), renal infiltration (24%) and testicular infiltration (5%). For 45 patients, FCM on bone marrow was available, which revealed a median lymphoblastic involvement of 72% (range 6-98%). FCM on peripheral blood was available for 69 patients, showing a median involvement of 87% (range 6-98%). RNAseq was available for 61 patients, none of whom had NOTCH1 gene fusions. 30 patients were treated according to the ALL-11 protocol, of whom 60% were stratified to the medium risk (MR) group and 30% to the high risk (HR) group. 49 patients were treated according to the ALL Together protocol, of which the majority was stratified to the Intermediate Risk (IR) high group (67%). Fewer patients (14%) were stratified to the HR group compared to the ALL-11 cohort. 9 patients (11%) relapsed. 14 patients (18%) did not survive, of whom 7 died after a relapse. Median follow-up was 36 months (range 1-72).

		Complete cohort (n=80)			CCL17 cohort (n=38)		
Age, year (mean, range)		9,7 (1-19)			10,5 (1-19)		
Sex (number, %)	Male	59 (73,8)			30 (78,9)		
	Female	21 (26,3)			8 (21,1)		
CNS[a] status (number, %)	1	26 (32,5)			12 (31,6)		
	2	37 (46,3)			22 (57,9)		
	3	8 (10,0)			3 (7,9)		
	TLP+[b]	5 (6,3)			1 (2,6)		
	Unknown	4 (5,0)			0 (0)		
Symptoms (number, %)	Mediastinal mass	56 (70,0)			26 (68,4)		
	Lymphadenopathy	57 (71,3)			31 (81,6)		
	Hepatomegaly	34 (42,5)			13 (34,2)		
	Splenomegaly	38 (47,5)			19 (50,0)		
	Renal infiltration	19 (23,8)			8 (21,1)		
	Testicular infiltration	4 (5,0)			4 (10,0)		
Hematological (median, range)	Hemoglobin	6,2 (2,6-10,4)			6,5 (2,7-10,4)		
	Thrombocytes	54 (4-393)			58 (11-289)		
	Leukocytes	114 (0,8-765)			88,0 (0,9-581)		
	Lactate dehydrogenase	2455 (164-15075)			2304 (414-10400)		
Flow cytometry (median, range)	Bone marrow (n=45)[c]	72% (6-98%)			71% (6-98%)		
	Peripheral blood (n=69)[c]	87% (6-98%)			81% (6-97%)		
NOTCH1 fusion (number, %)	Yes	0 (0)			0 (0)		
	No	61 (76,3)			33 (91,7)		
	Unknown	19 (23,8)			3 (8,3)		
Protocol (number, %)	ALL 11	30	MR[d]	18 (60,0)	11	MR[d]	8 (72,7)
			HR[d]	11 (36,7)		HR[d]	3 (27,3)
	ALL Together	49	IR low[d]	6 (12,2)	27	IR low[d]	4 (14,8)
			IR high[d]	33 (67,3)		IR high[d]	18 (66,7)
			HR[d]	7 (14,3)		HR[d]	4 (14,8)
	Other	1			0		
Relapse (number, %)	Yes	9 (11,3)			2 (5,3)		
	No	71 (88,8)			36 (94,7)		
Status (number, %)	Alive	66 (82,5)			35 (92,1)		
	Deceased	14 (17,5)			3 (7,9)		

Table 1. Clinical characteristics for 80 T-ALL patients.
The complete cohort was comprised of 80 children with T-ALL, whose characteristics are shown in the left column. For 38 patients, CCL17 values at time of first diagnosis were retrospectively measured. Their characteristics are presented in the right column.

a. *CNS = central nervous system*
b. *TLP+ = traumatic lumbar puncture containing lymphoblasts.*
c. *For some patients, flow cytometry bone marrow and/or peripheral was not available at time of diagnosis, e.g. due to clinical emergency.*
d. *MR = medium risk; IR = intermediate risk; HR = high risk.*

		n=36		
Age, year (mean, range)		9,9 (1-17)		
Sex (number, %)	Male	22 (61,1)		
	Female	14 (38,9)		
Stage (number, %)	III	25 (69,4)		
	IV	11 (30,6)		
CNS[a] status (number, %)	1	21 (58,3)		
	2	3 (8,3)		
	3	1 (2,8)		
	TLP+[b]	1 (2,8)		
	Unknown	10 (27,8)		
Symptoms (number, %)	Mediastinal mass	34 (94,4)		
	Lymphadenopathy	28 (77,8)		
	Hepatomegaly	10 (27,8)		
	Splenomegaly	5 (13,9)		
	Renal infiltration	9 (25,0)		
	Testicular infiltration	1 (2,8)		
Hematological (median, range)	Hemoglobin	8,0 (5,6-10,1)		
	Thrombocytes	333 (40-617)		
	Leukocytes	8,8 (2,8-336,6)		
	Lactate dehydrogenase	546,5 (188-2573)		
Flow cytometry (median, range)	Bone marrow (n=25)[c]	0,6% (0%-14%)		
	Peripheral blood (n=25)[c]	0,3% (0%-22%)		
NOTCH1 fusion (number, %)	Yes	7 (19,4)		
	No	23 (63,9)		
	Unknown	8 (22,2)		
Protocol (number, %)	EuroLB02	12		
	EuroLB2018	24	SR[d]	15 (62,5)
			HR[d]	8 (33,3)
Relapse (number, %)	Yes	4 (11,1)		
	No	32 (88,9)		
Status (number, %)	Alive	31 (86,1)		
	Deceased	5 (13,9)		

Table 2. Clinical characteristics for 36 T-LBL patients.
Clinical parameters for T-LBL patients for whom CCL17 levels at time of first diagnosis were available.

a. *CNS = central nervous system*
b. *TLP+ = traumatic lumbar puncture containing lymphoblasts.*
c. *For some patients, flow cytometry on bone marrow and/or peripheral blood was not available at time of diagnosis, e.g. due to clinical emergency.*
d. *SR = standard risk; HR = high risk.*

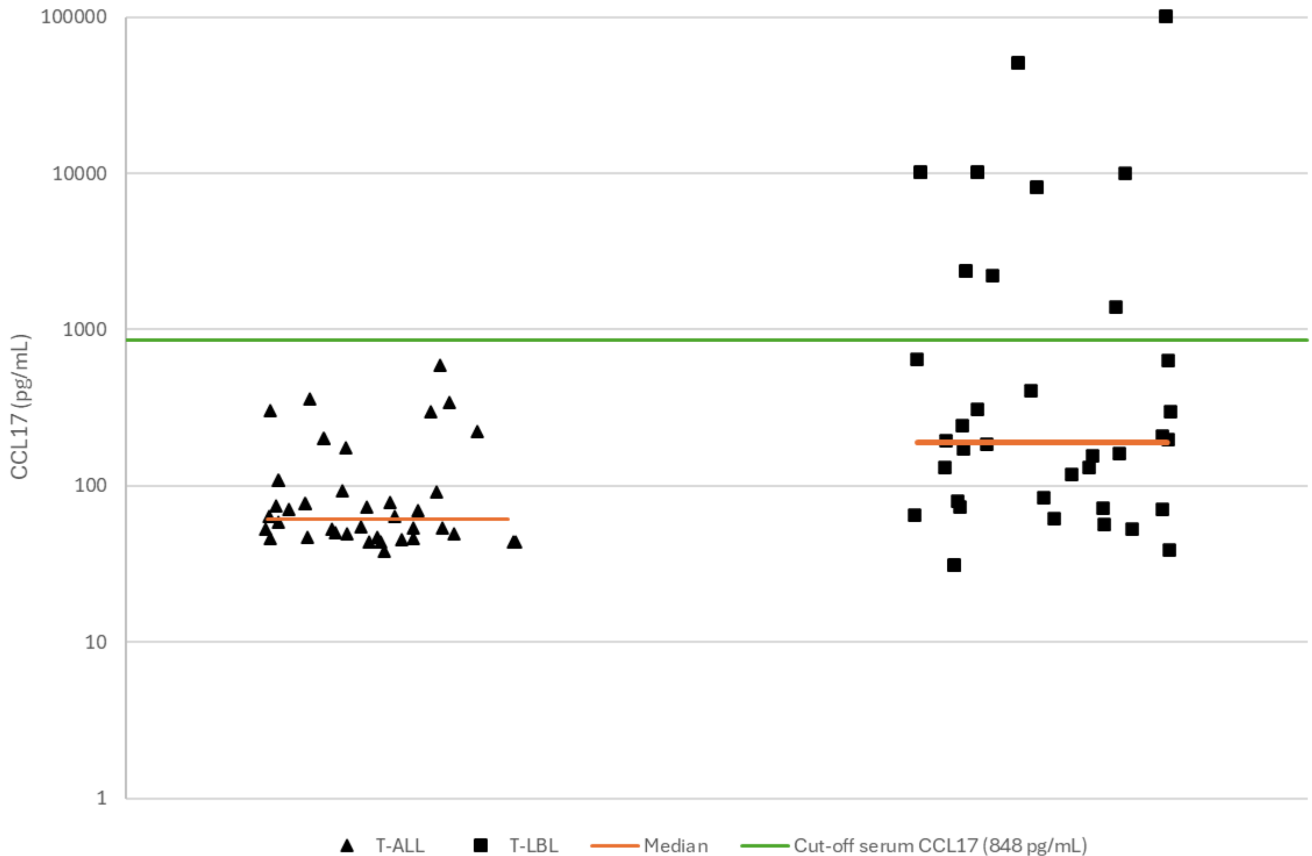


Figure 1. CCL17 in T-ALL and T-LBL.

CCL17 in plasma and serum from newly diagnosed T-ALL and T-LBL patients. Median CCL17 was 61,5 pg/mL (38-593) in T-ALL, compared to 189 pg/mL (31-100000) for T-LBL.

Retrospective CCL17 measurements at time of diagnosis were performed for 38 patients (table 1). Distribution of sex and age were comparable to the complete cohort, as well as clinical presentation. However, this group only contained 2 patients who developed a relapse. 3 patients did not survive, of whom 2 died after suffering a relapse. Mean length of follow-up was 33 months (range 4-58).

The T-LBL cohort is comprised of 36 patients, whose clinical characteristics are provided in Table 2. Mean age at time of diagnosis was 10 years (range 1-17). This cohort included 22 males (61%) and 14 females (39%). The majority of patients presented with stage III T-LBL (69%), compared to 31% who presented with stage IV disease. Mediastinal enlargement was present in the majority of patients (94%), as well as lymphadenopathy (78%). Other symptoms were less frequent. FCM on bone marrow was performed for 25 patients, revealing a median bone marrow infiltration of 0,6% (range 0-14%). FCM on peripheral blood was available for 25 patients, which showed a median involvement of 0,3% (range 0-22%). NOTCH1 gene fusions were found in 7 patients, including all 4 relapsed patients. 1 patient with a NOTCH1 gene fusion developed a therapy-related acute myeloid leukemia (AML) during maintenance treatment of T-LBL. 5 patients did not survive, of whom 4 died with relapsed T-LBL. The fifth patient died due to therapy-related pancreatitis and did not have a NOTCH1 gene fusion.

CCL17 values

Median CCL17 in T-ALL patients was 61,5 pg/mL (range 38-593) (figure 1). CCL17 was measured in plasma for 6 patients (range 44-305 pg/mL). For 2 of these patients, CCL17 was only mildly elevated above the cut-off of 285 pg/mL for plasma CCL17 (298 and 305 pg/mL respectively). CCL17 was measured in serum for 32 patients (range 38-593 pg/mL). In all serum samples, CCL17 levels were lower than the cut-off for serum CCL17 (848 pg/mL).

In comparison, median CCL17 in T-LBL patients was 189 pg/mL (range 31-100000) (figure 1). For 9 patients, CCL17 was elevated (range 1399-100000 pg/mL). It was unknown whether these measurements were performed in plasma or serum. Therefore, we used the serum cut-off value to determine if CCL17 was elevated.

CCL17 and NOTCH1 fusions

For 61 out of 80 T-ALL patients, RNAseq was available. In none of these patients, NOTCH1 gene fusions were found (figure 2). RNAseq was available for 35 out of 38 T-ALL patients for whom CCL17 measurements could be performed. For 28 out of 36 T-LBL patients, RNAseq was available, which revealed rearranged NOTCH1 in 7 patients (figure 2). These 7 patients all showed elevated levels of CCL17 (range 2196-100000 pg/mL). In the T-LBL group without NOTCH1 fusions, only one patient had elevated CCL17 (1399 pg/mL). These results

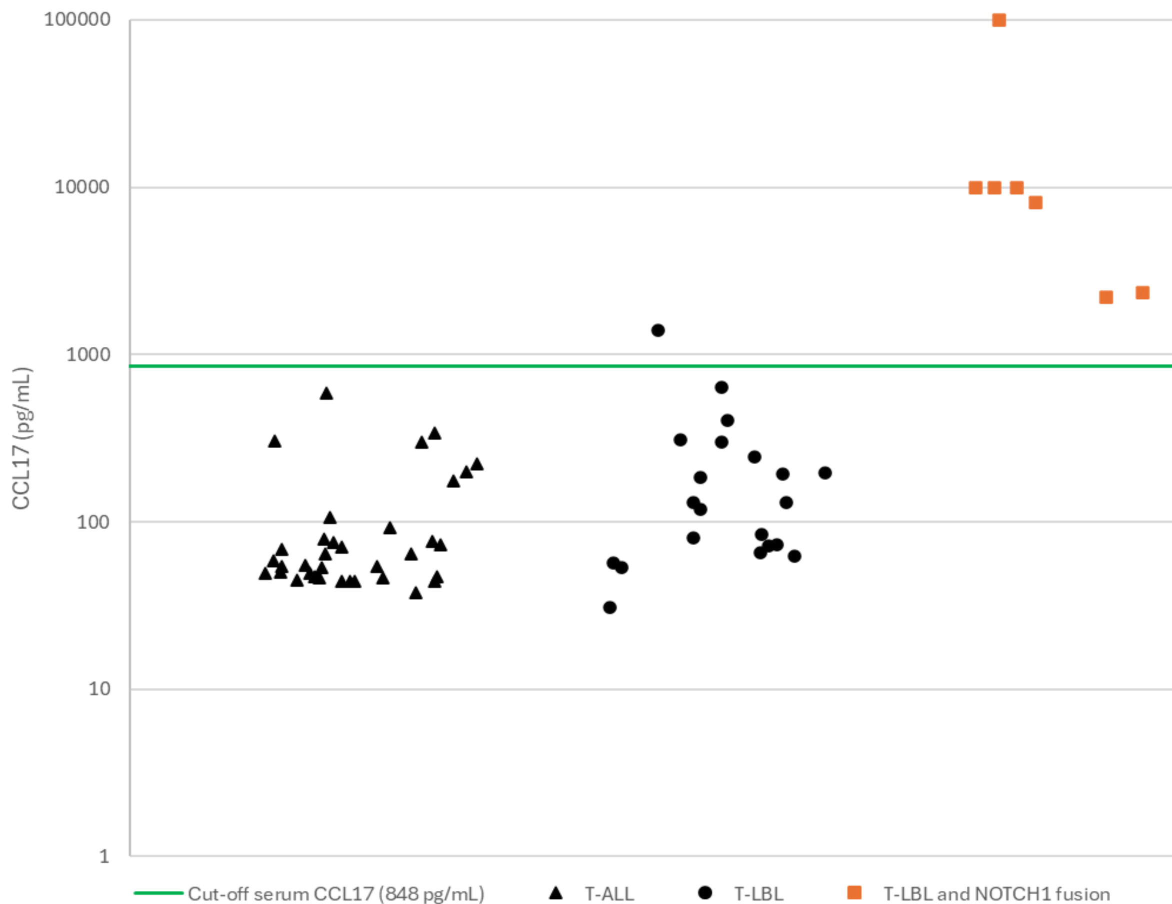


Figure 2. CCL17 values and NOTCH1 gene fusions.

Patients with T-ALL and T-LBL for whom CCL17 levels and RNAseq were available. Elevated CCL17 is correlated to the presence of NOTCH1 fusions (p<0,001). NOTCH1 gene fusions were not found in the T-ALL cohort, whereas 7 T-LBL patients had rearranged NOTCH1, all of whom had elevated CCL17.

suggest that elevated CCL17 is correlated to the presence of NOTCH1 gene fusions ($p<0,001$).

Prognostic value of CCL17

T-ALL patients who were stratified to the high risk treatment arm had median CCL17 values of 71 pg/mL (range 44-224 pg/mL) compared to 57 pg/mL (38-593 pg/mL) for patients who were stratified as intermediate or medium risk. There was no statistically significant difference between these two groups ($p=0,56$).

CCL17 values at time of primary diagnosis for T-ALL patients who developed a relapse (n=2) were 77 and 199 pg/mL, respectively (figure 3). One deceased patient who did not suffer a relapse had a CCL17 value of 107 pg/mL. There was no significant difference in CCL17 values between patients who developed a relapse and those who did not relapse ($p=0,76$).

Out of 9 T-LBL patients with elevated CCL17, 4 patients developed a relapse (figure 3). All 4 patients had highly elevated CCL17 at time of first diagnosis (range 2345-10.000). One patient who had CCL17 levels of 100.000 pg/mL at diagnosis did not relapse. However, this patient developed a secondary AML during maintenance therapy for T-LBL, for which he received allogeneic stem cell transplantation (ASCT). The ASCT possibly prevented an early relapse of T-LBL in this patient. 4 patients with elevated

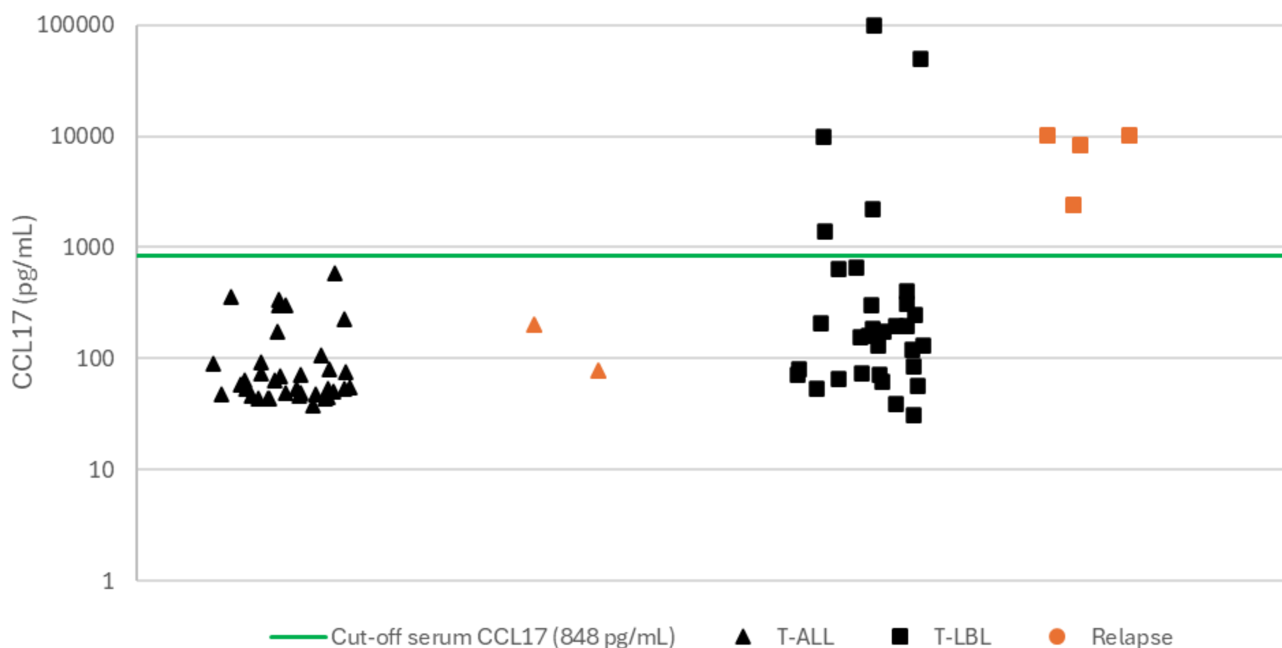
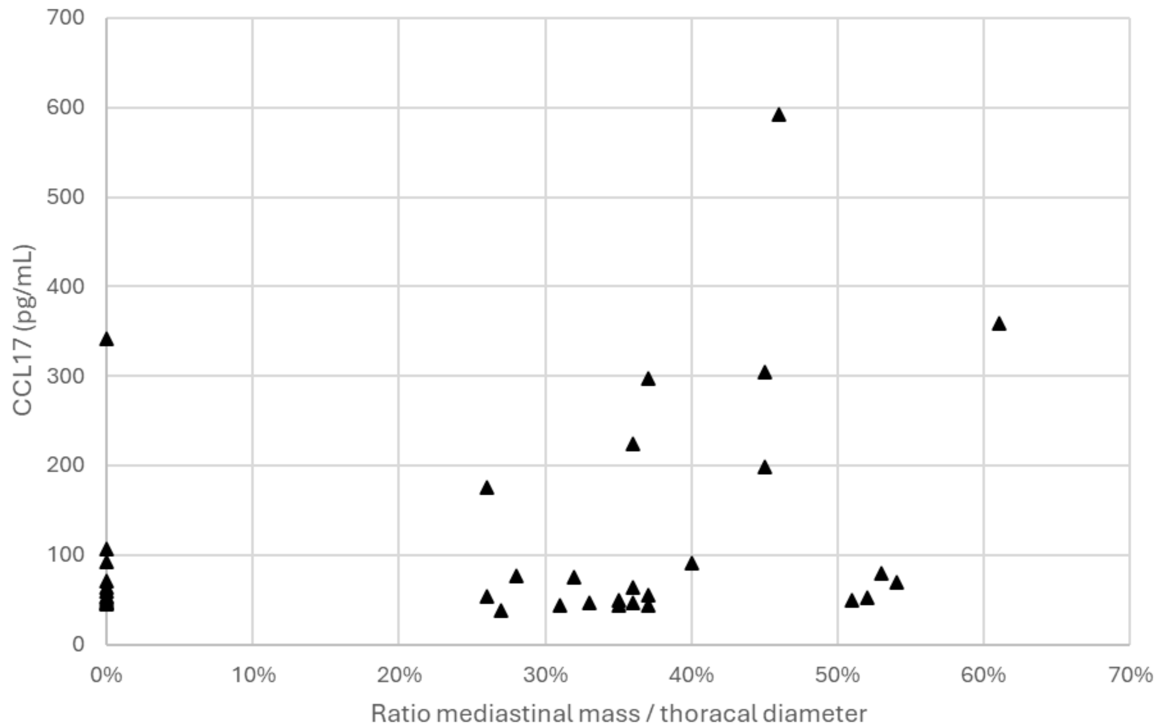


Figure 3. CCL17 in patients who developed a relapse.

CCL17 values at time of first diagnosis for patients who developed a relapse, compared to patients who did not relapse. There was no statistically significant difference in CCL17 values between relapsed T-ALL patients and patients who did not relapse ($p>0,5$). In contrast, there was a significant correlation between elevated CCL17 and incidence of relapse in patients with T-LBL ($p=0,002$).

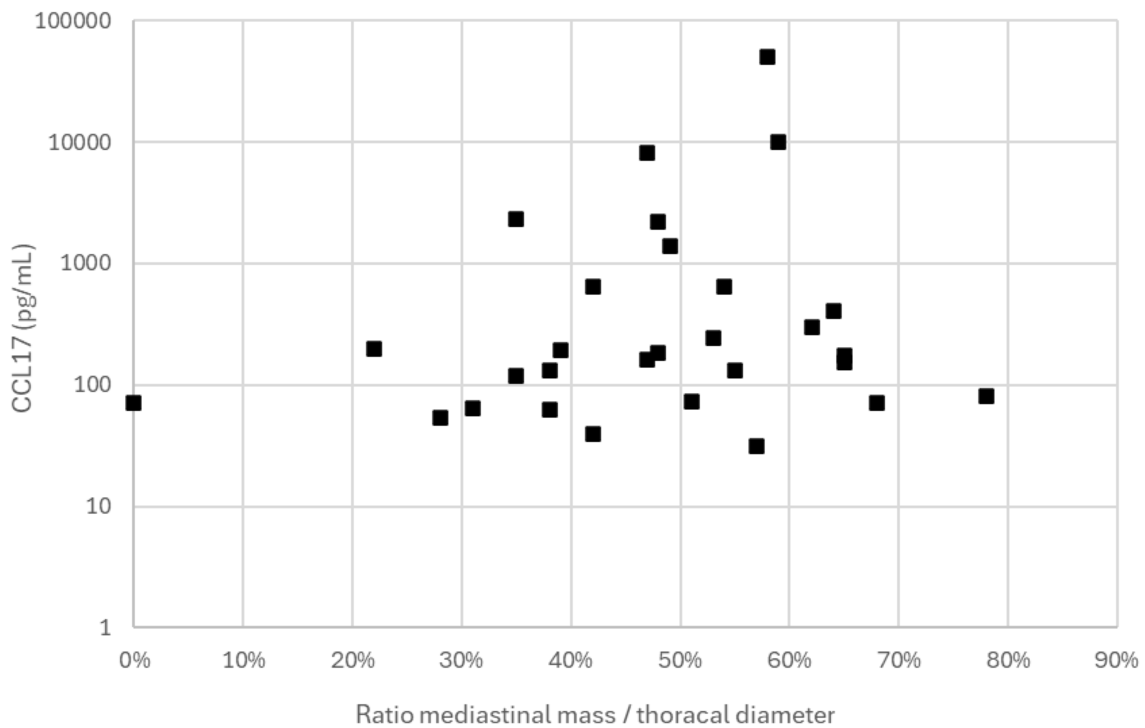
Figure 4 CCL17 and mediastinal enlargement.



A. Mediastinal enlargement in patients with T-ALL.

For patients with T-ALL, extent of mediastinal enlargement did not correlate with CCL17 values (r=0,28, p=0,09).

Patients with no mediastinal enlargement are shown with a ratio of 0%.



B. Mediastinal enlargement in patients with T-LBL.

For patients with T-LBL, extent of mediastinal enlargement did not correlate with CCL17 values (r=0,14, p=0,4). Patients with no mediastinal enlargement are shown with a ratio of 0%.

CCL17 values (range 1399-50144 pg/mL) did not develop a relapse. 3 of these patients are still receiving treatment for T-LBL. Length of follow-up for the fourth patient is now 3 years after end of treatment for T-LBL. In contrast, none of the 27 patients with normal CCL17 developed a relapse. The incidence of relapse was significantly higher in the elevated CCL17 group compared to the group with normal CCL17 (p=0,002).

CCL17 and mediastinal mass

For T-ALL patients, CCL17 levels did not correlate to the extent of mediastinal enlargement (r=0,28, p=0,09) (figure 4). For 2 T-ALL patients, the mediastinal mass could not be quantified due to either absence of chest X-ray or extensive pleural effusion.

Accordingly, we did not find a correlation between mediastinal mass and CCL17 values in T-LBL patients (r=0,14, p=0,4) (figure 4). In 7 T-LBL patients, the extent of mediastinal enlargement could not be quantified due to extensive pleural effusion.

Discussion

In this study, we measured CCL17 values in a cohort of patients with immature T-cell malignancies, and investigated whether there is a connection between elevated CCL17 and clinical parameters, risk groups or NOTCH1 gene fusions. In this cohort of T-ALL patients, no elevated values of CCL17 were found and CCL17 could not be used to discriminate between high-risk and lower-risk patients. In contrast, the T-LBL cohort shows a statistically significant correlation between elevated CCL17 and NOTCH1 gene fusions, and between elevated CCL17 and the occurrence of relapses. In both patient groups, we did not find a relation between the extent of mediastinal enlargement and elevated values of CCL17.

These results demonstrate a potential difference between the pathogenesis of T-ALL and T-LBL. A possible explanation for this distinction is the occurrence of NOTCH1 fusions in T-LBL, which were not detected in T-ALL (13). 7 out of 9 T-LBL patients with elevated CCL17 had NOTCH1 fusions. For one patient with elevated CCL17, it was unknown whether a NOTCH1 gene fusion was present. NOTCH1 fusions lead to strongly increased NOTCH1 signaling, which may in turn result in upregulated CCL17 (13). This could explain why CCL17 is elevated in T-LBL, but not in T-ALL.

Elevated levels of CCL17 are also present in patients with atopic dermatitis, and accumulating evidence shows CCL17 to be a reliable biomarker for disease severity (22, 23). Furthermore, elevated levels of CCL17 have been reported in several allergic and auto-inflammatory disorders(18, 20). We checked clinical records of all patients with elevated CCL17 for allergic and/or auto-inflammatory disease and found a history of eczema and asthmatic complaints in 2 NOTCH1-rearranged patients. However, these symptoms were not present at time of diagnosis. For one patient with elevated CCL17 but no NOTCH1 gene fusions, we did not find evidence of allergic or auto-inflammatory disease. Therefore, the presence of allergic or auto-inflammatory disease is not a likely explanation for the elevation of CCL17 in our cohort.

There are several mechanisms by which CCL17 could play a role in immature T-cell malignancies. CCL17 is constitutively expressed in the thymus and plays an important role in the maturational process of developing T-cells by guiding them through the thymus (17). Therefore, CCL17 might play a role in the homing of malignant lymphoblasts, explaining why T-LBL lymphoblasts remain predominantly in the

thymus, whereas T-ALL cells invade the bone marrow and peripheral blood. In this cohort, a mediastinal mass was found in 56 out of 80 T-ALL patients, and in 34 out of 36 T-LBL patients. However, we did not find a significant correlation between elevated CCL17 and mediastinal enlargement.

CCL17 has been reported to play various roles in the pathogenesis of cancer. In solid tumors, CCL17 attracts regulatory T-cells to the tumor microenvironment, thereby suppressing immune responses by cytotoxic T-cells (24-26). In classical Hodgkin lymphoma, tumor cells communicate with surrounding T-helper 2 cells using CCL17. These cells, in turn, secrete stimulatory cytokines to the Reed-Sternberg cells, resulting in a positive feedback loop (16). In mature T-cell malignancies (cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma), CCR4 is expressed strongly in tumor cells, which in turn respond highly to CCL17 (27). Via this mechanism, CCL17 plays a role in the trafficking and homing of these malignant cells.

This leaves us with a number of hypotheses of the role CCL17 could play in immature T-cell malignancies. Clarifying this mechanism has several potential applications. First of all, this may contribute to defining the difference between T-ALL and T-LBL. In addition, further research into the role of CCL17 in T-LBL may clarify why NOTCH1 fusions and CCL17 are associated with a poor prognosis. Finally, CCL17 and its receptor CCR4 are potential therapeutic targets. CCR4 antagonists have been shown to be effective in mature T-cell malignancies, although with notable side effects (28). Furthermore, modulating the tumor microenvironment with CCR4 blockage has been shown to increase efficacy of immunotherapy (29) .

To translate our data into improved risk stratification and possible therapeutic targets, further research is necessary. In this study, CCL17 measurements were performed for only 2 out of 9 T-ALL patients who developed a relapse. To confirm that CCL17 is a high-risk biomarker for T-LBL but not for T-ALL, with NOTCH1 fusions as a likely explanation, CCL17 measurements should be obtained for the remaining 7 patients who developed a relapse. In addition, immunohistochemistry staining for CCR4 and regulatory T-cells in T-LBL biopsies would provide information about the mechanism CCL17 plays in T-LBL and thereby offer starting points for possible therapeutic applications.

Limitations to this study include the small sample size. CCL17 measurements at time of primary diagnosis were available for only 2 out of 9 T-ALL patients who developed a relapse. Additionally, follow-up was in some cases as short as 4 months. This limited our ability to define CCL17 as a high-risk biomarker in patients with T-ALL. Additionally, due to the retrospective nature of this study, CCL17 measurements were in some cases performed in samples collected several days after start of steroids, which possibly influenced our results. Furthermore, the measurements are not entirely uniform, considering these were performed in either plasma or serum for both cohorts. Validating our findings in a larger cohort of patients with immature T-cell malignancies will strengthen these findings as a potential distinction between T-ALL and T-LBL, and pave the way for clinical application of CCL17 and NOTCH1 fusions as high-risk biomarkers.

Conclusion

This study does not show elevated levels of CCL17 in patients with T-ALL. In contrast, elevated CCL17 is significantly linked to NOTCH1 gene fusions and the occurrence of relapses in patients with T-LBL. These data illustrate a possible difference between T-ALL and T-LBL. Further research

is needed to strengthen these findings and to clarify the role of CCL17 in immature T-cell malignancies. This could lead to clinical applications such as the use of CCL17 as a high-risk biomarker or as a therapeutic target.

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Supplementary table 1

Origin of samples that were used for CCL17 measurements.

Patient ID	Origin of sample	Taken >2 days after start of treatment?*	CCL17 (pg/mL)
TALL001	Plasma (blood)	No	298
TALL002	Serum (bone marrow)	No	342
TALL003	Serum (bone marrow)	No	50
TALL004	Serum (bone marrow)	No	93
TALL005	Serum (bone marrow)	No	38
TALL006	Plasma (blood)	No	44
TALL007	Serum (bone marrow)	No	47
TALL008	Plasma (blood)	No	305
TALL009	Serum (bone marrow)	No	59
TALL010	Serum (bone marrow)	No	77
TALL011	Serum (bone marrow)	Yes	64
TALL012	Serum (blood)	No	593
TALL013	Serum (bone marrow)	Yes	79
TALL014	Serum (blood)	No	107
TALL015	Serum (blood)	No	54
TALL016	Serum (blood)	No	75
TALL017	Serum (bone marrow)	No	47
TALL018	Serum (bone marrow)	No	44
TALL019	Serum (blood)	No	49
TALL020	Serum (blood)	No	359
TALL021	Serum (bone marrow)	Yes	91
TALL022	Serum (blood)	No	44
TALL023	Serum (bone marrow)	No	71
TALL024	Serum (bone marrow)	No	224
TALL025	Serum (bone marrow)	No	45
TALL026	Serum (bone marrow)	No	53
TALL027	Serum (bone marrow)	No	69
TALL028	Serum (bone marrow)	No	176
TALL029	Serum (bone marrow)	No	44
TALL030	Serum (bone marrow)	No	54
TALL031	Serum (blood)	No	199
TALL032	Plasma (blood)	No	73
TALL033	Serum (bone marrow)	No	46
TALL034	Plasma (blood)	No	55
TALL035	Serum (bone marrow)	Yes	64
TALL036	Serum (bone marrow)	No	46
TALL037	Plasma (bone marrow)	No	49
TALL038	Serum (bone marrow)	No	53

** Treatment with steroids and/or chemotherapy.*