

## Blood cytokine concentrations in pediatric patients with anaplastic lymphoma kinase-positive anaplastic large cell lymphoma

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# **Blood cytokine concentrations of pediatric ALK-positive anaplastic large cell lymphoma patients**

## **Supplementary material**

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**Table S1: Clinical characteristics of the study patients**

Clinical characteristics of ALCL patients and control patients. Bone marrow (BM) and CNS involvement were considered positive if malignant cells were seen on smears, irrespective of cell numbers.

	ALK+ALCL		B-NHL		ALK+ALCL in Remission	
	n = 119		n = 11		n = 15	
<b>Sex</b>						
m	69	58.0%	7	63.6%	9	60.0%
w	50	42.0%	4	36.4%	6	40.0%
<b>Age</b>						
≤ 9 years	41	34.5%	4	36.4%	4	26.7%
> 9 years	78	65.5%	7	63.6%	11	73.3%
<b>Stage</b>						
I	8	6.7%	0	0.0%	1	6.7%
II	20	16.8%	3	27.3%	3	20.0%
III	71	59.7%	7	63.6%	11	73.3%
IV	17	14.3%	0	0.0%	0	0.0%
n. a.	3	2.5%	11	100.0%	0	0.0%
<b>Histology</b>						
common	50	57.5%	-	-	10	66.7%
non-common	37	42.5%	-	-	5	33.3%
n. a.	32		-	-	0	0.0%
<b>B symptoms</b>						
yes	63	52.9%	2	18.2%	5	33.3%
no	56	47.1%	9	81.8%	10	66.7%
<b>General condition</b>						
unimpaired	29	24.4%	3	27.3%	6	40.0%
Slight impairment	52	43.7%	7	63.6%	7	46.7%
Strong impairment	24	20.2%	1	9.1%	1	6.7%
bed-ridden	12	10.1%	0	0.0%	1	6.7%
Seriously ill	2	1.7%	0	0.0%	0	0.0%
<b>Organ involvement</b>						
CNS	2	1.7%	0	0.0%	0	0.0%
Bone marrow	16	13.4%	0	0.0%	0	0.0%
Skin	30	25.2%	0	0.0%	1	6.7%
Mediastinal	59	49.6%	0	0.0%	7	46.7%
Liver	11	9.2%	1	9.1%	0	0.0%
Spleen	18	15.1%	0	0.0%	3	20.0%
Lung	24	20.2%	0	0.0%	3	20.0%
<b>Relapse</b>						
yes	38	31.9%	0	0.0%	0	0.0%
no	81	68.1%	11	100.0%	15	100.0%

## CYTOKINE LEVELS IN ALCL PATIENTS

**Table S2: Cytokine concentrations in ALCL patients compared to controls**

This shows the percentage cytokine concentrations above the detection limit and ranges for the cytokine levels. (IP-10, 118 measurements available).

	ALCL (diagnosis)		B-NHL		ALCL (remission)		B-NHL vs. ALCL in remission p-Value
	Detectable	Median value (range) [pg/ml]	Detectable	Median value (range) [pg/ml]	Detectable	Median value (range) [pg/ml]	
	n = 119		n = 11		n = 15		
<b>IL-1<math>\beta</math></b>	14/119 (11.8%)	0 (0 - 543.2)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-2</b>	14/119 (11.8%)	0 (0 - 679)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>sIL-2R</b>	119/119 (100%)	94163.8 (629 - 353044.8)	11/11 (100%)	10839 (6041.6 - 38131)	15/15 (100%)	6399.7 (3088.3 - 11250.1)	.006
<b>IL-4</b>	3/119 (2.5%)	0 (0 - 466)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-5</b>	3/119 (2.5%)	0 (0 - 155.3)	1/11 (9.1%)	0 (0 - 34.5)	3/15 (20%)	0 (0 - 9.6)	n.s.
<b>IL-6</b>	56/119 (47.1%)	0 (0 - 1326.6)	4/11 (36.4%)	0 (0 - 161.5)	1/14 (7.1%)	0 (0 - 137.3)	n.s.
<b>IL-9</b>	60/119 (50.4%)	4.5 (0 - 25207.4)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-10</b>	53/119 (44.5%)	0 (0 - 8693.7)	1/11 (9.1%)	0 (0 - 283.1)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-12p70</b>	1/119 (0.8%)	0 (0 - 2033.1)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-13</b>	25/119 (21%)	0 (0 - 239.6)	1/11 (9.1%)	0 (0 - 67)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-17a</b>	43/119 (36.1%)	0 (0 - 2623.3)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-22</b>	17/119 (14.3%)	0 (0 - 2322.5)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IL-23</b>	46/119 (38.7%)	0 (0 - 12102.7)	6/11 (54.5%)	36.4 (0 - 2970.6)	9/15 (60%)	143.2 (0 - 8450)	n.s.
<b>TNF-<math>\alpha</math></b>	28/119 (23.5%)	0 (0 - 224)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>IFN-<math>\gamma</math></b>	29/119 (24.4%)	0 (0 - 5068.1)	0/11 (0%)	0 (0 - 0)	0/15 (0%)	0 (0 - 0)	n.s.
<b>HGF</b>	119/119 (100%)	2716.8 (77.5 - 42569.8)	11/11 (100%)	989.4 (319.5 - 2358.4)	15/15 (100%)	1061.9 (449.5 - 10965.3)	n.s.
<b>IL-8</b>	96/119 (80.7%)	49.5 (0 - 16580.9)	10/11 (90.9%)	34.3 (0 - 2495.7)	13/15 (86.7%)	41 (0 - 402.3)	n.s.
<b>MIG</b>	119/119 (100%)	299 (35.1 - 5010.9)	11/11 (100%)	472.6 (111.1 - 1528.6)	15/15 (100%)	186.1 (97.2 - 1322.8)	n.s.
<b>G-CSF</b>	51/119 (42.9%)	0 (0 - 4731.4)	3/11 (27.3%)	0 (0 - 1061.9)	7/15 (46.7%)	0 (0 - 1847.4)	n.s.
<b>MIP-1<math>\alpha</math></b>	110/119 (92.4%)	139.3 (0 - 49650.4)	8/11 (72.7%)	13.3 (0 - 668.3)	11/15 (73.3%)	149.4 (0 - 4983)	n.s.
<b>MIP-1<math>\beta</math></b>	119/119 (100%)	89.7 (20 - 4174.6)	11/11 (100%)	87.6 (51.7 - 230.2)	15/15 (100%)	90.5 (30 - 150.3)	n.s.
<b>MCP-1</b>	119/119 (100%)	511.8 (151.8 - 40720)	11/11 (100%)	653.7 (231 - 1175.6)	15/15 (100%)	340.2 (201.9 - 622.4)	0.018
<b>IP-10</b>	118/118 (100%)	138.1 (37.9 - 7357.4)	11/11 (100%)	121.2 (36.5 - 548)	15/15 (100%)	120.8 (70.1 - 298.3)	n.s.
<b>sCD30</b>	119/119 (100%)	79788.9 (678.6 - 584894.4)	11/11 (100%)	1993.1 (1111.1 - 6117.3)	15/15 (100%)	1568.7 (1012.7 - 3639.2)	n.s.
<b>VEGF</b>	117/119 (98.3%)	979.7 (0 - 63031.2)	11/11 (100%)	644 (161.3 - 1144.5)	15/15 (100%)	1870.6 (240.9 - 3182.2)	.005

## CYTOKINE LEVELS IN ALCL PATIENTS

**Table S3: Correlations between cytokine concentrations in ALK-positive ALCL patients at the time of diagnosis**

Spearman correlation and p values is given. Only correlations > .5 are shown.

IL-1 $\beta$  correlated with TNF- $\alpha$  (R=.56, p<.001)

sIL-2R correlated with IL-10 (R=.55, p<.001) and sCD30 (R=.7, p<.001)

IL-4 correlated with IL-5 (R=.66, p<.001) and IL-12p70 (R=.58, p<.001)

IL-5 correlated with IL-4 (R=.66, p<.001) and IL-12p70 (R=.57, p<.001)

IL-6 correlated with IL-10 (R=.51, p<.001), IL-17a (R=.52, p<.001), and HGF (R=.56, p<.001)

IL-10 correlated with sIL-2R (R=.55, p<.001) and IL-6 (R=.51, p<.001)

IL-12p70 correlated with IL-4 (R=.58, p<.001) and with IL-5 (R=.57, p<.001)

IL-17a correlated with IL-6 (R=.52, p<.001)

TNF- $\alpha$  correlated with IL-1 $\beta$  (R=.56, p<.001)

MIG correlated with IP-10 (R=.59, p<.001)

IP-10 correlated with MIG (R=.59, p<.001)

MIP-1 $\beta$  correlated with IL-8 (R=.57, p<.001)

IL-8 correlated with MIP-1 $\beta$  (R=.57, p<.001)

HGF correlated with IL-6 (R=.56, p<.001)

sCD30 correlated with sIL-2R (R=.7, p<.001)

## CYTOKINE LEVELS IN ALCL PATIENTS

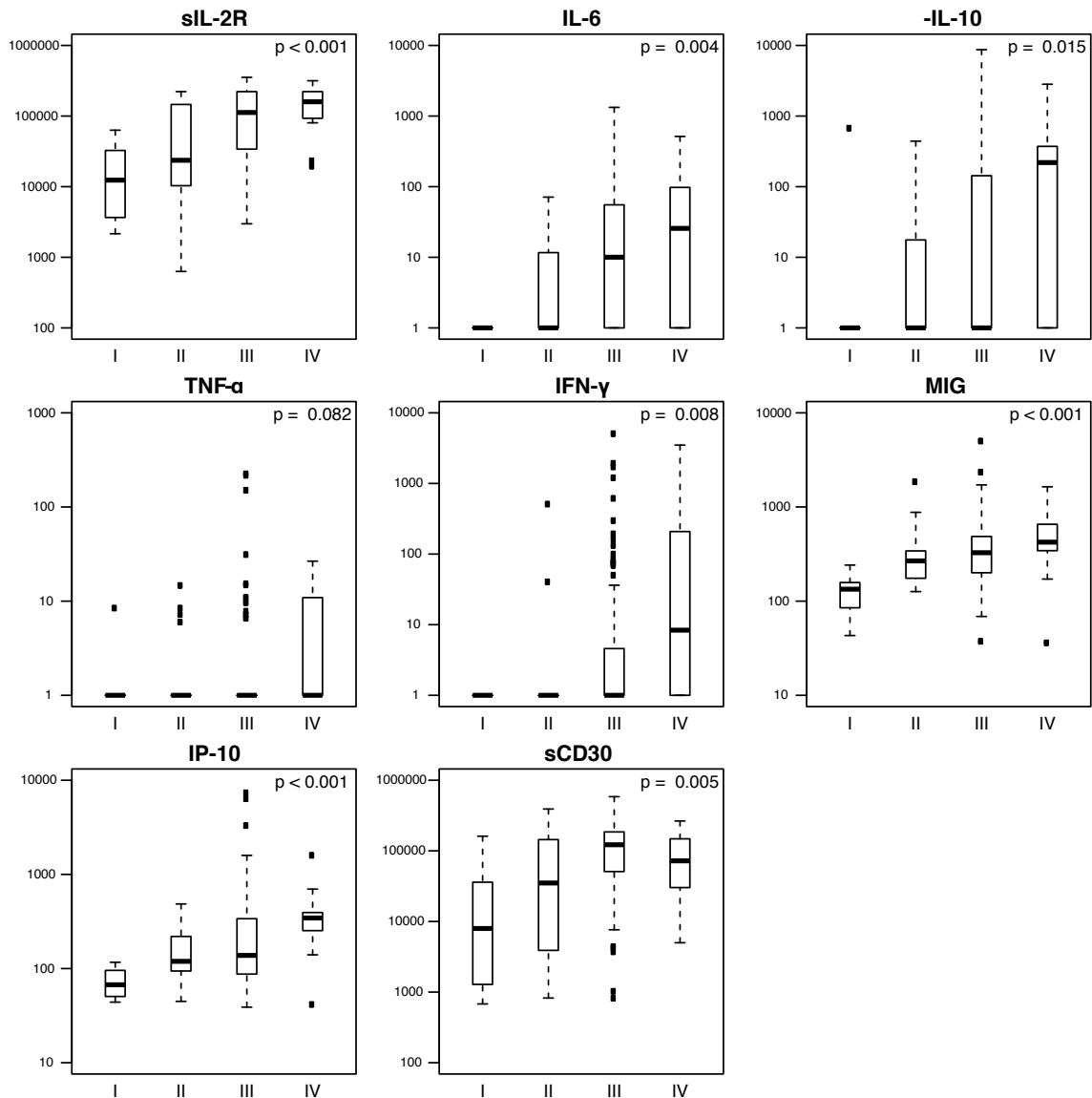
**Table S4: Cytokine concentrations and organ involvement**

This table shows median values of cytokine concentrations according to organ involvement of 119 ALCL patients at time of diagnosis. P values are given where significant differences were found between subgroups. (IP-10, 118 measurements are available).

	Spleen			Skin			Mediastinum			Lung			Liver		
	no n = 91	yes n = 28	p	no n = 92	yes n = 27	p	no n = 59	yes n = 60	p	no n = 94	yes n = 25	p	no n = 99	yes n = 20	p
IL-1	0	0	n.s.	0	0	<.001	0	0	n.s.	0	0	n.s.	0	0	.034
IL-2	0	0	n.s.	0	0	0.05	0	0	n.s.	0	0	n.s.	0	0	n.s.
sIL-2R	74861	190649.2	.001	85770.3	125776.9	n.s.	44953	128327.7	<.001	81917.7	159428.4	.030	82570.5	190649.2	.026
IL-4	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
IL-5	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
IL-6	0	60.9	<.001	0	14.3	n.s.	0	14.3	.006	0	34.6	.017	0	44.3	.002
IL-9	0	8	n.s.	0	33.6	n.s.	4.5	2.6	n.s.	4.8	0	n.s.	10.9	0	n.s.
IL-10	0	198.9	<.001	0	17.6	n.s.	0	15.4	n.s.	0	13.4	n.s.	0	134.5	.036
IL-12P70	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	.026
IL-13	0	0	.023	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
IL-17A	0	26.2	.005	0	2.2	.030	0	0	n.s.	0	0	n.s.	0	26.2	.019
IL-22	0	0	.016	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	.03
IL-23	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
TNF $\kappa$	0	0	<.001	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	.005
IFN $\gamma$	0	0	.016	0	0	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
MIG	286.1	338.6	n.s.	293.1	334.4	n.s.	251.7	404.5	.002	304.9	296.1	n.s.	278.8	404.5	n.s.
IP10	118.3	276	.017	122.3	246.7	n.s.	116.2	187.8	.022	118.3	188.3	n.s.	133.2	229.3	n.s.
MIP1 $\kappa$	139.3	129.6	n.s.	136.9	142.5	n.s.	142.5	121.5	n.s.	131	158.3	n.s.	148	50.6	n.s.
MIP1	87.3	104.5	n.s.	92	86	n.s.	81.2	96.4	n.s.	88.9	99.3	n.s.	89.7	91.2	n.s.
MCP1	481.7	748.1	.018	512.9	491.1	n.s.	543.1	501.4	n.s.	495.3	518.9	n.s.	491.1	550	n.s.
IL-8	45	63.3	n.s.	41.8	81.1	n.s.	42.1	63.5	n.s.	47.3	77.4	n.s.	45	63.3	n.s.
GCSF	0	0	n.s.	0	6.7	n.s.	0	0	n.s.	0	0	n.s.	0	0	n.s.
HGF	2406.4	4669.2	<.001	2675.9	3279.1	n.s.	2159.4	3099.3	.012	2488.7	4725.2	.033	2716.8	2814.1	n.s.
VEGF	979.7	1044.2	n.s.	977.3	1087.4	n.s.	790.2	1230.7	n.s.	918.9	1392.4	n.s.	942.4	1306.9	n.s.
sCD30	91090.9	69444.8	n.s.	93346.4	64578.2	n.s.	72195.7	102768	n.s.	79160.8	126514	n.s.	79788.9	84825.9	n.s.

**Figure S1: Cytokine concentrations according to stage**

Logarithmic representation of serum cytokine concentrations in pg/ml according to the St. Jude stage of the patients. In 3 patients stage could not be determined because a lumbar puncture was not done or not conclusive. Number of patients: Stage I – 8, Stage II – 20, Stage III – 71, Stage IV – 17. Only cytokines with significant differences are shown.



**Figure S2: Cytokine concentrations according to general condition**

Logarithmic representation of serum cytokine concentrations in pg/ml according to general condition among the 119 ALCL patients. Categories: 1 – no impairment, 2 – slight impairment, 3 – considerate impairment, 4 – severely ill, 5 – bedridden. Only cytokines with significant differences are shown.

