Table E1. Immunologic features in patients with cartilage-hair hypoplasia. Cell counts are reported as cells x10^9/l, immunoglobulin levels and C3 and C4 complement components concentrations are given in g/l. Local laboratory or previously published reference values were adopted.[1-5].

Laboratory parameter	Normal values in adults*	Patients tested, n	Median (range)	Patients with decreased counts/levels, n (%)	Patients with increased counts/levels, n (%)
WBC	3.4-8.2	56	5.65 (1.2-12.0)	7 (13%)	7 (13%)
Neutrophils	1.5-6.7	56	3.68 (0.28-8.4)	4 (7%)	5 (9%)
Eosinophils	0.03-0.44	40	0.20 (0.01-1.45)	1 (3%)	2 (5%)
TLC	1.3-3.6	56	1.31 (0.26-3.84)	31 (55%)	0
CD3+ cells	0.85-2.28	55	0.92 (0.16-4.45)	25 (45%)	2 (4%)
CD4+ cells	0.458-1.406	55	0.55 (0.12-3.83)	24 (44%)	0
RTE cells	0.024-0.824	52	0.03 (0.00-0.56)	27 (52%)	0
CD8+ cells	0.24-0.98	55	0.28 (0.04-1.72)	25 (45%)	2 (4%)
CD19+ cells	0.12-0.43	55	0.12 (0.00-1.29)	37 (67%)	0
CD27+IgD+ cells	0.009-0.088	51	0.008 (0.000-0.044)	29 (57%)	0
CD27+IgD- cells	0.013-0.122	51	0.098 (0.000-0.064)	34 (67%)	0
CD16/56+ cells	0.08-0.57	55	0.19 (0.05-0.63)	4 (7%)	0
IgG	6.8-15.0	50	10.45 (4.20-15.90)	1 (2%)	2 (4%)
IgG2	1.50-6.40	50	1.89 (0.27-4.56)	13 (26%)	0
IgG3	0.20-1.63	50	0.44 (0.07-1.20)	8 (16%)	0
IgG4	0.08-1.40	55	0.09 (0.00-0.92)	23 (46%)	0
IgA	0.52-4.84	55	1.83 (0.00-7.49)	2 (4%)	3 (5%)
IgM	0.36-2.84	55	0.90 (0.20-3.06)	7 (13%)	1 (2%)
C3	0.5-1.5	43	1.11 (0.67-1.55)	0	1 (2%)
C4	0.12-0.42	43	0.21 (0.12-0.34)	0	0
Antibodies to tetanus toxoid	> 0.1 IU/ml	43	2.00 (0.05-6.30)	3 (7%)	not applicable

Ig immunoglobulin, n number, RTE recent thymic emigrants CD3+CD4+CD45RA+CD31+ cells, TLC total lymphocyte counts, WBC white blood cells.
* in children age-specific normative data were used.

[1] Boldt A, Borte S, Fricke S, Kentouche K, Emmrich F, Borte M, Kahlenberg F, Sack U. Eight-color immunophenotyping of T-, B-, and NK-cell subpopulations for characterization of chronic immunodeficiencies. Cytometry B Clin Cytom 2014 May;86(3):191-206.
[2] Driessen GJ, van Zelm MC, van Hagen PM, Hartwig NG, Trip M, Warris A, de Vries E, Barendregt BH, et al. B-cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency. Blood 2011 Dec 22;118(26):6814-23.3
[3] Schatorjé EJ, Gemen EF, Driessen GJ, Leuvenink J, van Hout RW, de Vries E. Paediatric reference values for the peripheral T cell compartment. Scand J Immunol 2012 Apr;75(4):436-44.
[4] Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD. et al; Pediatric AIDS Clinical Trials Group. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003 Nov;112(5):973-80.
[5] Vlug A, Nieuwenhuys EJ, van Eyk RVW, Geertzen HGM, van Houte AJ. Nephelometric measurements of human IgG subclasses and their reference ranges. Ann Biol Clin 1994;52, 561.

Table E2. Subpopulations of B and T cells in randomly selected 12 patients with cartilage-hair hypoplasia. Compared with other individuals in our cohort, this group of patients differed only by lower median number of CD16/56+ cells (0.12 vs 0.21 cells x10^9/l, p=0.002).

Flow cytometry markers	Cell group	Units	Reference values in adults[1,2]	F 16 yr	F 18 yr	F 23 yr	F 26 yr	F 26 yr	M 39 yr	M 44 yr	M 45 yr	F 45 yr	F 51 yr	M 58 yr	F 67 yr
CD3+	T cells	cells x10^9/l	0.85-2.28*	**0.60**	**0.46**	**0.56**	**0.16**	**0.76**	0.94	1.20	**0.46**	0.89	0.94	0.93	*3.01*
CD4-CD8-	DNT	% of CD3+	n/a	1.0	0.4	0.5	0.7	0.4	0.4	0.2	0.4	0.4	0.5	n/a	0.4
CD4+CD8+		% of CD3+	0.3-3.3	0.6	0.3	0.4	0.4	0.3	0.7	*5.2*	0.3	2.2	0.5	n/a	**1.0**
TCR-α/β+		% of CD3+	88.1-97.8	**81.1**	88.6	*98.3*	92.1	92.9	*99.1*	92.8	88.6	*99.1*	97.5	n/a	*99.0*
TCR-γ/δ+		% of CD3+	1.9-11.7	*18.8*	11.0	**1.6**	7.8	7.0	**0.8**	7.2	11.0	**0.9**	2.5	n/a	**0.9**
CD4+CD25^high^CD127^low^	Regulatory	% of CD3+	2.8-6.4	4.7	4.0	3.0	3.6	5.9	3.2	3.5	4.0	5.8	6.2	n/a	**2.2**
CD4+		cells x10^9/l	0.458-1.406*	**0.401**	**0.324**	**0.342**	**0.123**	0.615	**0.401**	0.625	**0.324**	0.708	0.691	0.765	1.287
CD45RA+CCR7+	Naive CD4+	% of CD4+	20.5-54.8	**16.6**	**10.5**	**9.1**	**4.9**	**9.0**	**17.2**	26.1	**10.5**	**1.3**	25.8	n/a	**2.5**
HLA-DR+CD38-	Activated CD4+	% of CD4+	2.4-9.6	*12.2*	*14.4*	*22.8*	*18.0*	*14.8*	*27.6*	*23.0*	*14.4*	*30.7*	7.6	n/a	*25.7*
CD45RA-CCR7+	CD4+ TCM	% of CD4+	8.4-32.8	*57.5*	*49.6*	*53.4*	*54.2*	*56.3*	*38.9*	*44.4*	*49.6*	*55.0*	*57.9*	n/a	*51.1*
CD45RA-CCR7-	CD4+ TEM	% of CD4+	19.9-52.4	25.2	37.4	36.6	39.3	34.4	41.8	28.5	37.4	42.3	**16.0**	n/a	42.2
CD45RA+CCR7-	Effector memory CD4+	% of CD4+	1.4-17.0	**0.7**	**2.6**	**0.9**	1.6	**0.3**	2.1	**1.0**	2.6	1.4	**0.2**	n/a	4.1
CD8+		cells x10^9/l	0.24-0.98*	**0.13**	**0.10**	**0.21**	**0.03**	**0.11**	**0.13**	0.59	**0.10**	**0.19**	**0.23**	**0.15**	*1.72*
CD45RA+CCR7+	Naive CD8+	% of CD8+	18.8-71.0	20.9	**13.8**	**6.4**	**8.4**	22.1	**10.2**	**0.7**	**13.8**	**7.0**	19.1	n/a	**2.1**
HLA-DR+CD38-	Activated CD8+	% of CD8+	3.8-32.4	11.5	20.9	15.9	5.0	15.9	23.8	17.5	20.9	21.0	24.6	n/a	30.7
CD45RA-CCR7+	CD8+ TCM	% of CD8+	1.2-7.3	*11.1*	6.1	*9.1*	*9.2*	*19.6*	4.5	**1.0**	6.1	*14.8*	*24.6*	n/a	6.8
CD45RA-CCR7-	CD8+ TEM	% of CD8+	14.6-63.0	31.0	38.6	44.4	47.2	39.0	*76.2*	22.8	38.6	26.1	45.1	n/a	45.3
CD45RA+CCR7-	Effector memory CD8+	% of CD8+	4.5-33.7	*37.1*	*41.5*	*40.2*	*35.1*	19.4	9.0	*75.4*	*41.5*	*52.1*	11.1	n/a	*45.8*
CD19+	B cells	cells x10^9/l	0.12-0.43*	0.12	**0.08**	0.12	**0.01**	0.14	0.12	0.16	**0.08**	0.14	0.14	**0.03**	**0.07**
CD27-IgD+IgM+	Naive	% of CD19+	43.2-82.4	55.7	**23.0**	n/a	*85.4*	**40.6**	61.3	*83.6*	**23.0**	**40.6**	**31.5**	60.7	**41.9**
CD21^low^CD38^low^	Activated	% of CD19+	0.8-7.7	*31.7*	*32.9*	n/a	*26.3*	*13.6*	*21.6*	*11.4*	*32.9*	*17.3*	*24.6*	*19.4*	*32.4*
CD27+IgD+	Marginal zone like	% of CD19+	7.2-30.8	25.1	9.6	n/a	5.5	9.2	11.4	**3.6**	9.6	11.5	8.2	14.9	9.2
CD27+IgD-	Switched memory	% of CD19+	6.5-29.2	6.5	*32.7*	n/a	**<2.0**	26.2	14.2	7.3	*32.7*	27.8	*41.3*	12.0	*31.2*
CD38++IgM+	Transitional	% of CD19+	0.6-3.5	2.4	1.3	n/a	*5.5*	*7.0*	2.7	3.3	1.3	1.8	1.6	2.9	1.4
CD38++IgM-	Plasmablasts	% of CD19+	0.4-3.6	0.7	*6.1*	n/a	n/a	0.8	0.4	**0.0**	*6.1*	0.9	0.6	**<0.2**	**0.3**

DNT double negative T cells, F female, M male, n/a not available, TCM central memory T cells, TCR T cell receptor, TEM effector memory T cells, yr years.

* local laboratory reference values

Numbers in **bold** and *italics* represent decreased and increased values respectively.

[1] Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood 2008 Jan 1;111(1):77-85.

[2] Boldt A, Borte S, Fricke S, Kentouche K, Emmrich F, Borte M, et al. Eight-color immunophenotyping of T-, B-, and NK-cell subpopulations for characterization of chronic immunodeficiencies. Cytometry B Clin Cytom 2014 May;86(3):191-206.

Table E3. Antibody response to Pneumovax® in eight adults with cartilage-hair hypoplasia, measured with fluoroimmunoassay[1] from samples before and 3-8 weeks after immunization.

N	Age (yrs)	Sex	S 1	S 4	S 5	S 6B	S 7F	S 9V	S 14	S 18C	S 19F	S 23F	Total number of reactive serotypes
1	23	F	<0.02 0.024	<0.024 0.058	0.47 0.27	0.047 0.033	0.12 0.26	<0.014 <0.014	0.15 0.08	<0.02 <0.02	0.065 0.087	**<0.018 2**	1/10
2	39	F	**0.23 6**	**0.043 1.7**	**0.26 13**	**2.5 13**	0.067 0.2	0.11 0.25	**0.18 5.3**	3.3 4.9	<0.036 0.13	**0.8 37**	6/10
3	40	M	**<0.02 1.6**	0.046 0.26	<0.04 0.082	<0,022 0.069	1.4 2	<0.014 0.33	**0.81 20**	<0,02 0.074	0.14 0.28	0.023 0.13	2/10
4	44	M	<0.02 0.023	**0.2 15**	**4.1 >30**	<0.022 <0.022	1.5 4	**0.91 8.6**	**2.7 24**	**1.1 14**	**0.075 1.6**	<0.018 0.11	6/10
5	46	F	**0.096 0.88**	<0.024 0.23	**<0.04 0.43**	<0.022 0.32	0.042 0.29	0.39 1	**0.21 25**	**0.11 0.61**	**0.048 0.65**	<0.018 0.15	5/10
6	58	M	<0.02 0.024	**0.047 13**	<0.04 0.26	<0.022 <0.022	1.6 2.1	**0.12 2.7**	<0.062 0.18	**0.15 0.85**	0.094 0.11	<0.018 0.23	3/10
7	65	M	**0.14 3.7**	0.07 0.22	**0.3 7.9**	**0.089 0.57**	7.9 8.6	**0.42 2.6**	**0.49 6.8**	2.3 7.5	**1.7 >30**	**0.12 0.85**	7/10
8	67	F	**<0.02 0.82**	<0.024 <0.024	<0.04 0.15	0.31 0.71	0.082 0.2	<0.014 0.12	<0.062 0.075	**0.33 6.2**	2.8 2.5	**1.3 13**	3/10

F female, M male, N patient' number, S serotype, yrs years
Numbers is **bold** represent adequate antibody response to a specific serotype, defined as a fourfold rise in antibody titers and post-immunization antibody levels ≥ 0.35 µg/ml[2]. Numbers in the serotype columns represent pre-immunization (upper number) and post-immunization (lower number) antibody titers for each patient.

[1] Timby N, Hernell O, Vaarala O, Melin M, Lönnerdal B, Domellof M. Infections in infants fed formula supplemented with bovine milk fat globule membranes. J Pediatr Gastroenterol Nutr 2015;60:384-9.
[2] Sorensen RU, Leiva LE. Specific Antibody Deficiency with Normal Immunoglobulins. In Sullivan KE, Stiehm, ER (eds) 2014, Stiehm's Immune Deficiencies, 1st edition, Academic Press, London. Page 413.

Table E4. Comparison of selected laboratory parameters in patients with cartilage-hair hypoplasia. Comparisons were made separately between age three age groups and between patients with and without CID symptoms. Only comparisons with significant differences between the subgroups are presented. Cell counts are reported as cells x10^9/l, immunoglobulin levels are given in g/l.

Laboratory parameter	Children <18 yr (n=15)*	Adults 18-45 yr (n=23)*	Adults >45 yr (n=18)*	Patients with symptoms of CID*	Patients without symptoms of CID*	p value
Neutrophils	2.45^	3.91	4.36^			0.005
Naïve thymic T cells**				0.009	0.036	0.022
CD3+ cells				0.66	0.93	0.048
CD4+ cells	0.514	0.487^	0.699^			0.038
CD8+ cells				0.22	0.33	0.018
CD19+ cells	0.150^	0.085^	0.120			0.014
IgA	1.42^	1.80	2.75^			0.006
IgG	9.2^	10.5	11.7^			0.027
IgG2	1.25^	1.98	2.19^			0.034
Antibodies to tetanus toxoid (IU/ml)	0.35^	3.50^	1.50			0.002

* Numbers in columns represent median counts/levels in the defined group of patients

** CD3+CD4+CD45RA+CD31+ cells

^ Variables for which the p-value is given (pairwise comparison in the Kruskall-Wallis test)

CID combined immunodeficiency, Ig immunoglobulin, n number, yr years

Neutrophil, CD4+ and CD19+ cell counts, IgA, IgG and IgG2 levels, as well as antibody titers to tetanus toxoid varied significantly among patients by age group, whereas naïve thymic T cell, CD3+ and CD8+ cell counts were significantly lower among patients with symptoms of CID compared with other patients.