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Analysis of Clinical and Immunologic Phenotype in a Large Cohort of Children and Adults with Cartilage-hair Hypoplasia

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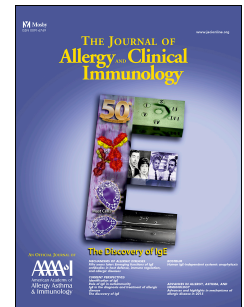
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**Analysis of clinical and immunologic phenotype in a large cohort of children and adults with cartilage-hair hypoplasia.**

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**Conflicts of interest**

The authors declare no conflicts of interest.

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**Capsule summary**

In our cohort of individuals with cartilage-hair hypoplasia 1) over 25% of patients manifest clinical combined immunodeficiency and they have lower CD3+, CD8+ and recent thymic emigrant counts; 2) specific antibody deficiency may exist; 3) low IgG2 levels associate with severe varicella.

**Key words**

Cartilage-hair hypoplasia; combined immunodeficiency; humoral immunodeficiency; immunoglobulin subclasses; memory B cells; *RMRP*; specific antibody deficiency; varicella.

To the Editor:

Cartilage-hair hypoplasia (CHH) is a chondrodysplasia with variable immunodeficiency. Patients' immunologic and clinical phenotypes are highly variable and correlate weakly. Most previous studies have involved only children, and the quality of immune function in CHH adults remains largely unknown. In addition, paucity of data exists regarding T and B cell subpopulations and specific antibody responses in CHH. Here we report on the clinical and immunological phenotype in a large group of Finnish children and adults with CHH.

We invited all 110 living Finnish patients with genetically confirmed CHH. Fifty-six individuals (19 males, 37 females, median age 34, range 0.7-68 years) agreed to participate, with informed consent. The study protocol was approved by the Institutional Research Ethics Committee. Clinical data were collected, and laboratory tests performed (see *Appendix E1* in the *Online Repository* for methods). Correlations between laboratory parameters and clinical manifestations were analyzed with the Fisher's exact test, the Mann-Whitney or the Kruskal-Wallis tests and regression analysis, as appropriate.

All patients were homozygous (n=43) or compound heterozygous (n=13) for the *RMRP* g.70A>G mutation. *Table I* describes clinical features. We grouped patients as having: 1) no symptoms/signs of immunodeficiency (n=15, 27%), 2) features of humoral immunodeficiency only (n=26, 46%) and 3) features of combined immunodeficiency (CID, n=15, 27%) (see *Table I* for definitions).

Laboratory findings are presented in *Figure I* and *Tables E1* and *E2* in the *Online Repository*. Common pattern of abnormalities in the B and T cell subpopulations (*Table E2* in the *Online Repository*) included: 1) decreased CD3+CD4+CD45RA+CD31+ recent thymic emigrants (RTE), naive CD4+ and CD8+ cells; 2) increased activated CD4+, central memory CD4+ and effector memory CD8+ cells; 3) normal CD4+CD25<sup>high</sup>CD127<sup>low</sup> regulatory T cells; 4) decreased naive, transitional and CD19+CD27+ memory B cells; 5) increased activated

CD21<sup>low</sup>CD38<sup>low</sup>CD19<sup>+</sup> cells. The reduced counts of rapidly proliferating cells (naïve T, RTE, and B) support a disturbed cell cycle as an important pathogenic mechanism<sup>1</sup>. The pattern of abnormalities in B cell subpopulations indicate B cell production and germinal center defect, while decreased CD27<sup>+</sup> B cells suggest defective maturation or survival<sup>2</sup>.

Pre-existing tetanus toxoid antibody levels were below 0.1 IU/ml in only 3/43 patients (*Table E1* in the *Online Repository*). Seven out of eight (88%) patients who agreed to receive Pneumovax® vaccine demonstrated specific antibody deficiency (SAD) (see *Table E3* in the *Online Repository*). Clinical features of the seven subjects with SAD included rhinosinusitis (n=6), OM (n=5), warts (n=3), malignancies (n=3; lymphoma, basal cell carcinoma, uterus carcinoma), severe varicella requiring hospitalization (n=2) and recurrent herpes simplex virus infection (n=1). Patients with SAD showed clinical signs of humoral (n=2) and combined (n=4) immunodeficiency. SAD may therefore be a marker of more severe disease and vaccine responses should be routinely studied after infancy.

Patients with no clinical signs of immunodeficiency showed no specific pattern of laboratory abnormalities. No correlation was observed between history of pneumonia or rhinosinusitis and immunologic defects. Genotype did not correlate with any clinical or laboratory parameter.

Patients with clinically suggested CID showed decreased median CD3<sup>+</sup>, CD8<sup>+</sup> and RTE counts (see *Table E4* in the *Online Repository*). Of these parameters, RTE counts demonstrated the strongest trend towards significance in multiple regression analysis (p=0.094, B coefficient - 15.628). Lymphocyte proliferative responses were not performed, since previous studies have demonstrated that they are abnormal in the majority of patients and show no correlation with clinical manifestations in CHH<sup>1</sup>.

Lower median IgG2 concentrations (1.08 vs 1.96 g/l, p=0.016) were observed in patients who required hospitalization for VZV infection. Four out of five hospitalized patients (80%) were IgG2 deficient (IgG2 range 0.55-1.47 g/l) compared with 9 out of 37 (24%) non-hospitalized

patients ( $p=0.011$ ). In multiple regression analysis, only low IgG2 showed a trend towards significance ( $p=0.055$ , B coefficient  $-1.734$ ). The association between IgG2 deficiency and severe varicella has been described previously<sup>3</sup>, suggesting that CHH patients with low IgG2 should be regarded at risk for severe VZV infection. However, these findings require validation in further studies, as low IgG2 may only represent a marker of a more profound immunodeficiency.

We then analyzed laboratory results in various age groups: children <18 years ( $n=15$ ), young adults 18-44 years ( $n=23$ ) and adults >45 years ( $n=18$ ) (see *Table E4* in the *Online Repository*). Patients aged >45 years demonstrated higher neutrophil and CD4+ cell counts, as well as higher median IgA, IgG and IgG2 levels than younger patients. In addition, children showed higher CD19+ cell counts and lower median concentrations of antibodies to tetanus toxoid than adult patients. While lower immunoglobulin and vaccine antibody levels in pediatric patients may reflect slow maturation of immune system, lower CD19+ and higher CD4+ cell numbers in CHH adults deserve further studies. As mortality due to infections and malignancies is increased in CHH<sup>4</sup>, patients surviving into adulthood represent individuals with milder disease. Furthermore, while some *RMRP* gene mutations induce a more severe immunodeficiency<sup>5</sup>, the predominance of g.70A>G mutation in Finnish patients can result in milder presentations.

Consistent with previous reports<sup>6</sup>, measured laboratory values correlated poorly with any assessed symptoms and despite clearly abnormal laboratory indices, few patients developed serious complications. Worldwide, of the 30 subjects with CHH and hematopoietic stem cell transplantation (HSCT) whose genotype has been reported, only six patients were homozygous for g.70A>G mutation<sup>6,7,8,9</sup>. Only one Finnish child with CHH, homozygote for g.70A>G mutation, has required HSCT for severe hypoplastic anemia - not for immunodeficiency. Thus selection of patients who would benefit from HSCT based on routinely available laboratory parameters is highly cumbersome in a cohort carrying predominantly *RMRP* g.70A>G mutations.

The major limitation of this study is the retrospective nature of clinical data. Inclusion of only living patients left out those with CHH who had died of cancer or severe infections. Another

limitation is the use of a single laboratory measurement per patient. Results of immunologic tests fluctuate over time and predicting any clinical course based on cross-sectional laboratory values remains challenging.

The observed high number of asymptomatic patients and individuals with clinical signs of humoral immunodeficiency only should be interpreted with caution, as clinical features may occur and cumulate with time. Follow-up studies should assess the applicability of clinical and immunological phenotype correlations, including the potential to predict a more severe course (CID) in patients with lower CD3+, CD8+ and RTE counts.

In summary, we demonstrated that approximately one fourth of the surviving Finnish patients with CHH included in this study manifested clinical signs of CID, while another fourth showed no signs of immunodeficiency despite laboratory immunologic abnormalities. This is the first report describing high prevalence of SAD and a specific pattern of abnormalities in B and T cell compartments in patients with CHH.

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182 Immunodeficiencies Associated with RMRP Gene Mutations. *J Clin Immunol* 2015;35:147–57.

183 **Table I.** Clinical features of the 56 patients with cartilage-hair hypoplasia.

| Clinical feature                                               | Proportion of patients                            | Comments                                                                                                                                                                        |
|----------------------------------------------------------------|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clinical humoral immunodeficiency                              | 26/56 (46%)                                       | Defined as otitis media and/or rhinosinusitis requiring surgical interventions, sepsis, pneumonia and/or bronchiectasis.                                                        |
| Clinical combined immunodeficiency                             | 15/56 (27%)                                       | Defined as a history of severe warts, recurrent and/or severe herpes virus infections, malignancy and/or autoimmunity, with clinical signs of humoral immunodeficiency.         |
| Pneumonia<br>recurrent<br>hospitalization                      | 11/56 (20%)<br>4/11 (36%)<br>5/11 (45%)           |                                                                                                                                                                                 |
| Bronchiectasis                                                 | 10/34 (29%)                                       | Diagnosed on lung high-resolution computed tomography.                                                                                                                          |
| Sinusitis<br>sinus surgery                                     | 33/56 (59%)<br>21/33 (64%)                        |                                                                                                                                                                                 |
| Otitis media<br>tympanostomy                                   | 41/56 (73%)<br>16/41 (39%)                        |                                                                                                                                                                                 |
| Sepsis                                                         | 3/56 (5%)                                         |                                                                                                                                                                                 |
| History of warts<br>warts at the time of visit<br>severe warts | 19/56 (34%)<br>14/19 (74%)<br>2/19 (11%)          | Out of two patients with severe warts, one underwent partial amputation of the toe with warts for suspected malignancy and another had protracted history of wide-spread warts. |
| Varicella<br>hospitalization                                   | 42/56 (75%)<br>5/42 (12%)                         | History of varicella or positive serology for varicella-zoster virus.                                                                                                           |
| Boils<br>recurrent                                             | 3/56 (5%)<br>2/3 (67%)                            |                                                                                                                                                                                 |
| Mucocutaneous herpes<br>simplex virus infection                | 2/56 (4%)                                         | One patient had required continuous acyclovir prophylaxis for 15 yrs for recurrent skin herpes simplex virus infections.                                                        |
| Allergy                                                        | 23/56 (41%)                                       | History of allergic rhinoconjunctivitis.                                                                                                                                        |
| Malignancy<br>lymphoma<br>basal cell carcinoma only<br>other   | 9/56 (16%)<br>2/9 (22%)<br>5/9 (56%)<br>2/9 (22%) | Other malignancies included uterus carcinoma in one patient and vocal cord carcinoma in another.                                                                                |
| Autoimmunity                                                   | 1/56 (2%)                                         | Juvenile idiopathic arthritis.                                                                                                                                                  |
| Prophylactic antibiotics                                       | 7/56 (13%)                                        | Sulfamethoxazole/trimethoprim or azithromycin, commenced for recurrent infections or lymphopenia.                                                                               |
| Immunoglobulin substitution                                    | 7/56 (13%)                                        | In four patients treatment was still ongoing at the time of the study.                                                                                                          |

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**Figure legends**

*Figure I.* Selected immunologic features in 56 patients with cartilage-hair hypoplasia. All laboratory values are shown as medians. Patients are grouped by age and the number of subjects is shown in each age group.

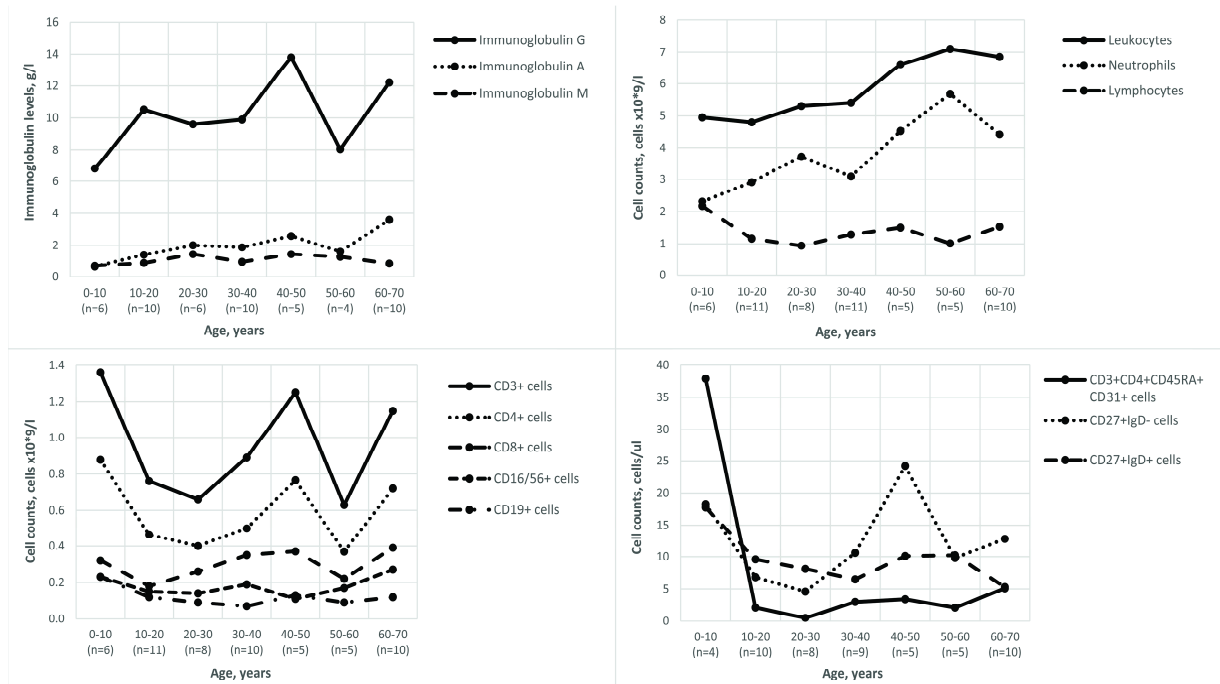


**Table E1.** Immunologic features in patients with cartilage-hair hypoplasia. Cell counts are reported as cells  $\times 10^9/l$ , immunoglobulin levels and C3 and C4 complement components concentrations are given in g/l. Local laboratory or previously published reference values were adopted.<sup>1-5</sup>.

| 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.

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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  $\times 10^9/l$ ,  $p=0.002$ ).

| Flow cytometry markers                        | Cell group           | Units                 | Reference values in adults <sup>1,2</sup> | 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 $\times 10^9/l$ | 0.85-2.28*                                | <b>0.60</b>  | <b>0.46</b>  | <b>0.56</b>  | <b>0.16</b>  | <b>0.76</b> | 0.94         | 1.20       | <b>0.46</b>  | 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          | <b>1.0</b>  |
| TCR- $\alpha/\beta$ +                         |                      | % of CD3+             | 88.1-97.8                                 | <b>81.1</b>  | 88.6         | 98.3         | 92.1         | 92.9        | 99.1         | 92.8       | 88.6         | 99.1        | 97.5        | n/a          | 99.0        |
| TCR- $\gamma/\delta$ +                        |                      | % of CD3+             | 1.9-11.7                                  | 18.8         | 11.0         | <b>1.6</b>   | 7.8          | 7.0         | <b>0.8</b>   | 7.2        | 11.0         | <b>0.9</b>  | 2.5         | n/a          | <b>0.9</b>  |
| CD4+CD25 <sup>high</sup> CD127 <sup>low</sup> | 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          | <b>2.2</b>  |
| CD4+                                          |                      | cells $\times 10^9/l$ | 0.458-1.406*                              | <b>0.401</b> | <b>0.324</b> | <b>0.342</b> | <b>0.123</b> | 0.615       | <b>0.401</b> | 0.625      | <b>0.324</b> | 0.708       | 0.691       | 0.765        | 1.287       |
| CD45RA+CCR7+                                  | Naive CD4+           | % of CD4+             | 20.5-54.8                                 | <b>16.6</b>  | <b>10.5</b>  | <b>9.1</b>   | <b>4.9</b>   | <b>9.0</b>  | <b>17.2</b>  | 26.1       | <b>10.5</b>  | <b>1.3</b>  | 25.8        | n/a          | <b>2.5</b>  |
| 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        | <b>16.0</b> | n/a          | 42.2        |
| CD45RA+CCR7-                                  | Effector memory CD4+ | % of CD4+             | 1.4-17.0                                  | <b>0.7</b>   | <b>2.6</b>   | <b>0.9</b>   | 1.6          | <b>0.3</b>  | 2.1          | <b>1.0</b> | 2.6          | 1.4         | <b>0.2</b>  | n/a          | 4.1         |
| CD8+                                          |                      | cells $\times 10^9/l$ | 0.24-0.98*                                | <b>0.13</b>  | <b>0.10</b>  | <b>0.21</b>  | <b>0.03</b>  | <b>0.11</b> | <b>0.13</b>  | 0.59       | <b>0.10</b>  | <b>0.19</b> | <b>0.23</b> | <b>0.15</b>  | 1.72        |
| CD45RA+CCR7+                                  | Naive CD8+           | % of CD8+             | 18.8-71.0                                 | 20.9         | <b>13.8</b>  | <b>6.4</b>   | <b>8.4</b>   | 22.1        | <b>10.2</b>  | <b>0.7</b> | <b>13.8</b>  | <b>7.0</b>  | 19.1        | n/a          | <b>2.1</b>  |
| 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          | <b>1.0</b> | 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 $\times 10^9/l$ | 0.12-0.43*                                | 0.12         | <b>0.08</b>  | 0.12         | <b>0.01</b>  | 0.14        | 0.12         | 0.16       | <b>0.08</b>  | 0.14        | 0.14        | <b>0.03</b>  | <b>0.07</b> |
| CD27-IgD+IgM+                                 | Naive                | % of CD19+            | 43.2-82.4                                 | 55.7         | <b>23.0</b>  | n/a          | 85.4         | <b>40.6</b> | 61.3         | 83.6       | <b>23.0</b>  | <b>40.6</b> | <b>31.5</b> | 60.7         | <b>41.9</b> |
| CD21 <sup>low</sup> CD38 <sup>low</sup>       | 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         | <b>3.6</b> | 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          | < <b>2.0</b> | 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          | <b>0.0</b> | 6.1          | 0.9         | 0.6         | < <b>0.2</b> | <b>0.3</b>  |

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.

<sup>1</sup> 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.

<sup>2</sup> 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<sup>1</sup> 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<br>0.024                 | <0.024<br>0.058            | 0.47<br>0.27                   | 0.047<br>0.033              | 0.12<br>0.26  | <0.014<br><0.014          | 0.15<br>0.08              | <0.02<br><0.02             | 0.065<br>0.087              | <b>&lt;0.018</b><br><b>2</b> | 1/10                               |
| 2 | 39        | F   | <b>0.23</b><br><b>6</b>        | <b>0.043</b><br><b>1.7</b> | <b>0.26</b><br><b>13</b>       | <b>2.5</b><br><b>13</b>     | 0.067<br>0.2  | 0.11<br>0.25              | <b>0.18</b><br><b>5.3</b> | 3.3<br>4.9                 | <0.036<br>0.13              | <b>0.8</b><br><b>37</b>      | 6/10                               |
| 3 | 40        | M   | <b>&lt;0.02</b><br><b>1.6</b>  | 0.046<br>0.26              | <0.04<br>0.082                 | <0.022<br>0.069             | 1.4<br>2      | <0.014<br>0.33            | <b>0.81</b><br><b>20</b>  | <0.02<br>0.074             | 0.14<br>0.28                | 0.023<br>0.13                | 2/10                               |
| 4 | 44        | M   | <0.02<br>0.023                 | <b>0.2</b><br><b>15</b>    | <b>4.1</b><br><b>&gt;30</b>    | <0.022<br><0.022            | 1.5<br>4      | <b>0.91</b><br><b>8.6</b> | <b>2.7</b><br><b>24</b>   | <b>1.1</b><br><b>14</b>    | <b>0.075</b><br><b>1.6</b>  | <0.018<br>0.11               | 6/10                               |
| 5 | 46        | F   | <b>0.096</b><br><b>0.88</b>    | <0.024<br>0.23             | <b>&lt;0.04</b><br><b>0.43</b> | <0.022<br>0.32              | 0.042<br>0.29 | 0.39<br>1                 | <b>0.21</b><br><b>25</b>  | <b>0.11</b><br><b>0.61</b> | <b>0.048</b><br><b>0.65</b> | <0.018<br>0.15               | 5/10                               |
| 6 | 58        | M   | <0.02<br>0.024                 | <b>0.047</b><br><b>13</b>  | <0.04<br>0.26                  | <0.022<br><0.022            | 1.6<br>2.1    | <b>0.12</b><br><b>2.7</b> | <0.062<br>0.18            | <b>0.15</b><br><b>0.85</b> | 0.094<br>0.11               | <0.018<br>0.23               | 3/10                               |
| 7 | 65        | M   | <b>0.14</b><br><b>3.7</b>      | 0.07<br>0.22               | <b>0.3</b><br><b>7.9</b>       | <b>0.089</b><br><b>0.57</b> | 7.9<br>8.6    | <b>0.42</b><br><b>2.6</b> | <b>0.49</b><br><b>6.8</b> | 2.3<br>7.5                 | <b>1.7</b><br><b>&gt;30</b> | <b>0.12</b><br><b>0.85</b>   | 7/10                               |
| 8 | 67        | F   | <b>&lt;0.02</b><br><b>0.82</b> | <0.024<br><0.024           | <0.04<br>0.15                  | 0.31<br>0.71                | 0.082<br>0.2  | <0.014<br>0.12            | <0.062<br>0.075           | <b>0.33</b><br><b>6.2</b>  | 2.8<br>2.5                  | <b>1.3</b><br><b>13</b>      | 3/10                               |

F female, M male, N patient' number, S serotype, yrs years

Numbers in **bold** represent adequate antibody response to a specific serotype, defined as a fourfold rise in antibody titers and post-immunization antibody levels  $\geq 0.35 \mu\text{g/ml}^2$ . Numbers in the serotype columns represent pre-immunization (upper number) and post-immunization (lower number) antibody titers for each patient.

<sup>1</sup> Timby N, Hernell O, Vaarala O, Melin M, Lönnnerdal B, Domellof M. Infections in infants fed formula supplemented with bovine milk fat globule membranes. J Pediatr Gastroenterol Nutr 2015;60:384-9.

<sup>2</sup> 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  $\times 10^9/l$ , 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 <sup>^</sup>       | 3.91                    | 4.36 <sup>^</sup>     |                                |                                   | 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 <sup>^</sup>      | 0.699 <sup>^</sup>    |                                |                                   | 0.038   |
| CD8+ cells                           |                         |                         |                       | 0.22                           | 0.33                              | 0.018   |
| CD19+ cells                          | 0.150 <sup>^</sup>      | 0.085 <sup>^</sup>      | 0.120                 |                                |                                   | 0.014   |
| IgA                                  | 1.42 <sup>^</sup>       | 1.80                    | 2.75 <sup>^</sup>     |                                |                                   | 0.006   |
| IgG                                  | 9.2 <sup>^</sup>        | 10.5                    | 11.7 <sup>^</sup>     |                                |                                   | 0.027   |
| IgG2                                 | 1.25 <sup>^</sup>       | 1.98                    | 2.19 <sup>^</sup>     |                                |                                   | 0.034   |
| Antibodies to tetanus toxoid (IU/ml) | 0.35 <sup>^</sup>       | 3.50 <sup>^</sup>       | 1.50                  |                                |                                   | 0.002   |

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

\*\* CD3+CD4+CD45RA+CD31+ cells

<sup>^</sup> Variables for which the p-value is given (pairwise comparison in the Kruskal-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.