Shorter birth length and decreased T-cell production and function predict severe infections in children with non–severe combined immunodeficiency cartilage–hair hypoplasia

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Background: Cartilage–hair hypoplasia (CHH) is a syndromic inborn error of immunity caused by variants in the *RMRP* gene. Disease manifestations vary, and their ability to predict outcome is uncertain. The optimal management of infants with CHH who do not fulfill classical severe combined immunodeficiency (SCID) criteria is unknown.

Objective: We described longitudinal changes in lymphocyte counts during childhood and explored correlations of early childhood clinical and laboratory features with clinical outcomes on long-term follow-up of CHH patients.

Methods: Immunologic laboratory parameters, birth length, the presence of Hirschsprung disease, and severe anemia correlated to the primary end points of respiratory and severe infections. We implemented traditional statistical methods and machine learning techniques.

Results: Thirty-two children with CHH were followed up for 2.7 to 22.1 years (median, 8.2 years, in total 331.3 patient-years). None of the patients had classical SCID. Median lymphocyte subclass counts, apart from $CD16^{+}/56^{+}$ cells, were subnormal throughout childhood, but did not show age-related decline seen in healthy children. Low immunoglobulin levels were uncommon and often transient. Respiratory and/or severe infections developed in 14 children, 8 of whom had low naive T-cell counts, absent T-cell receptor excision circles, and/or partial "leaky" SCID–level lymphopenia. Shorter birth length correlated with lower lymphocyte counts and the occurrence of infections. Of the laboratory parameters, decreased naive T-cell counts and abnormal lymphocyte proliferation responses contributed most to the development of severe infections. In addition, all participants with absent T-cell receptor excision circles developed severe infections. Opportunistic infections occurred only in children with leaky SCID–level lymphopenia.

Conclusions: Shorter birth length and a combination of laboratory abnormalities can predict the development of severe infections in children with CHH. (J Allergy Clin Immunol Global 2024;3:100190.)

Key words: *Immunodeficiency, inborn error of immunity, lymphopenia, machine learning,* RMRP, *TREC*

Cartilage–hair hypoplasia (CHH) is an autosomal-recessive metaphyseal chondrodysplasia and a syndromic inborn error of immunity. This rare disease is enriched in the Amish (1-2 in 1000 births) and Finnish (1 in 23,000 births) populations.[1] CHH is caused by pathogenic variants in the *RMRP* gene, which encodes the untranslated RNA molecule of the mitochondrial RNA-processing endoribonuclease.[2] *RMRP* dysfunction induces a generalized abnormality in cell cycle, which in lymphocytes leads to defective proliferation and increased apoptosis, resulting in immunodeficiency.[3,4]

Clinical and laboratory manifestations of immune dysfunction in CHH demonstrate remarkable variability.[5-8] Immunodeficiency-related mortality is increased, and major causes of death include infections in childhood, malignancies in early adulthood, and lung disease in adulthood.[9] The most uniform laboratory features are abnormal lymphocyte proliferation responses and lymphopenia, particularly low counts of naive T and B cells, recent thymic emigrants (RTEs), and T-cell receptor excision circles (TRECs).[3,5,10]

It remains unknown whether lymphopenia in CHH progresses over time and correlates with clinical features. There are currently no criteria available to predict which infants will develop severe immunodeficiency and need early hematopoietic stem cell transplantation (HSCT), as opposed to those who will live into late adulthood without any immunologic issues.[9,11] This uncertainty is particularly problematic in the era of newborn screening for severe combined immunodeficiency (SCID) when patients with CHH test positive.[10] We therefore collected a unique data set of longitudinal clinical and laboratory characteristics of 32 children with CHH. We analyzed long-term trends in lymphocyte counts and evaluated whether early childhood laboratory indices and clinical features at birth correlated with disease severity.

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Abbreviations used

CHH: Cartilage–hair hypoplasia
HSCT: Hematopoietic stem cell transplantation
IGRT: Immunoglobulin replacement therapy
Leaky-SCID: Partial "leaky" SCID (according to PIDTC 2022 criteria)
NK: Natural killer
PHA: Phytohemagglutinin
PIDTC: Primary Immune Deficiency Treatment Consortium
RTE: Recent thymic emigrant
SCID: Severe combined immunodeficiency
TREC: T-cell receptor excision circle

METHODS

Study cohort and research permits

All children with CHH in Finland are followed at 5 university hospitals, and most of the patients visit Helsinki and/or Turku University hospitals. Our study included 32 children with CHH from these 2 centers, involving almost all the children with CHH in Finland (Fig 1). The inclusion criteria were as follows: biallelic pathogenic variants in *RMRP* gene, availability of data on lymphocyte subclasses, availability of clinical data to evaluate disease course, and birth in the year 2000 or later (when T-cell–subset measurements became routine). Of 3 children excluded because of lack of any clinical or laboratory information, 2 are alive, and for the third, the diagnosis of CHH was made after death, but the cause of death remained unknown. Clinical data and laboratory parameters were obtained from medical records until HSCT had been performed, or, for nontransplanted patients, until December 31, 2022.

The study was approved by the research ethics committee of Helsinki and Uusimaa Hospital District (HUS/836/2018). Data were collected from medical records, and therefore no patient consent was required under Finnish laws. However, 10 children have been recruited and consented during our previous research activities.[5]

Immunologic laboratory parameters

Total lymphocyte counts were acquired from complete blood counts. Lymphocyte subclasses ($CD3^+$, $CD4^+$, and $CD8^+$ T cells; $CD19^+$ B cells; and $CD16/56^+$ natural killer [NK] cells) and T-cell subsets ($CD3^+CD4^+CD45RA^+CCR7^+$ naive CD4 cells, $CD3^+CD8^+CD45RA^+CCR7^+$ naive CD8 cells, and $CD45RA^+CD62L^+CD31^+$ RTEs) were counted by flow cytometry, and pediatric reference values were applied.[12,13] IgA, IgM, and IgG levels were quantified by enzyme-linked immunosorbent assay.[14] Individuals were considered to have low immunoglobulin levels if at least 1 measurement was low. TRECs were quantified by PCR to amplify target sequence of TRECs. TREC values of >20 TRECs/μL were considered normal. In case of repeated TREC measurements, we reported the highest value. Lymphocyte proliferation responses to mitogen stimulation were analyzed with 2 methods: (1) ^{3}H-thymidine incorporation assay (used in Helsinki University Hospital before 2015 and in Turku University Hospital throughout the study duration) and (2) flow-cytometric assay for specific cell-mediated immune response in activated whole blood (aka FASCIA) (used in Helsinki University Hospital since 2015).[15] We used only T-cell proliferation responses to phytohemagglutinin (PHA) for our analyses because they were most consistently reported in our study participants. Reference values for proliferation tests used in Finland are not standardized, and numerical values of different tests cannot be compared. Rather, the results are interpreted on an individual basis, and we classified them as normal or abnormal. The results are thus not applicable for Primary Immune Deficiency Treatment Consortium (PIDTC) 2014 SCID criteria.[16,17] Therefore, PIDTC 2022 criteria were used to define SCID or partial "leaky" SCID (Leaky-SCID).[18] In patients who had contradictory results in multiple measurements, we classified laboratory parameters as normal or low/abnormal (for RTE [n = 2], T [n = 3], B [n = 3], and naive $CD4^+$ [n = 1] cell counts and for lymphocyte proliferation responses [n = 9]) based on the more prevalent results.

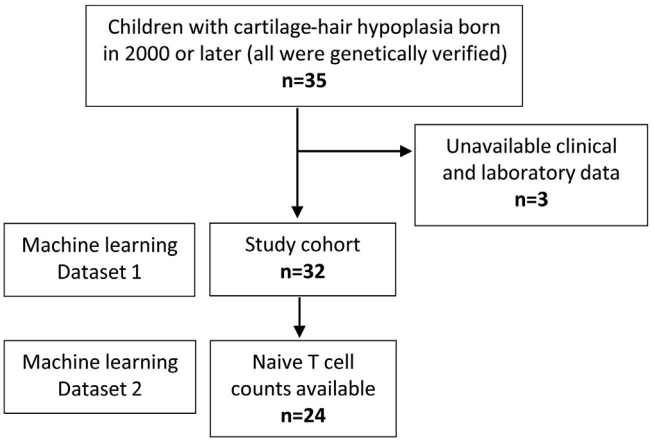

FIG 1. Flowchart demonstrating inclusion of patients with cartilage hypoplasia to study, as well as 2 cohorts used for machine learning approach.

Statistical analyses

We evaluated respiratory infections (recurrent otitis media and/or pneumonia) and severe infections (sepsis, recurrent pneumonia, and/or opportunistic infections) as primary outcome variables. Sepsis diagnoses were made by hospital pediatricians, and we verified the compliance with sepsis criteria when detailed data were available.[19] Recurrent pneumonia was defined as at least 3 radiologically confirmed pneumonias. Recurrent otitis media was defined as at least 3 episodes of acute otitis media within 6 months, at least 4 within a year, or at least 10 ever. We explored whether clinical course correlated with laboratory immunologic parameters or with birth length (adjusted for gestational age[20]), Hirschsprung disease, or severe anemia, which we defined as anemia requiring red blood cell transfusions. We used the Mann-Whitney *U* test, Spearman correlation test, and Fisher exact test, as appropriate. Statistical analyses and visualization of the results were performed by SPSS Statistics v25 and v27 (IBM) and GraphPad Prism v9.2.0 (GraphPad Software).

Machine learning analyses

We used the random forest method for identification of the relative importance of each feature compared to the target outcome. For the study, we used 2 data sets. Data set 1 comprised all 32 patients. Data set 2 comprised 24 patients with additional naive T-cell counts, with 2 different target values: target 1,

TABLE I. Clinical and laboratory characteristics and risk factors for infections in 32 children with CHH

Characteristic	All patients (n = 32)	Severe infection†			Respiratory infection‡		
		Yes (n = 6)	No (n = 26)	OR (95% CI)	Yes (n = 14)	No (n = 18)	OR (95% CI)
Male sex	16/32 (50%)	4/6 (67%)	12/26 (46%)	2.33 (0.36-15.05)	7/14 (50%)	9/18 (50%)	1.00 (0.25-4.04)
RMRP n.71A>G/n.71A>G variant	25/32 (78%)	4/6 (67%)	21/26 (81%)	0.48 (0.07-3.37)	10/14 (71%)	15/18 (83%)	0.50 (0.09-2.73)
Birth length below −4 SDS§	12/32 (38%)	3/6 (50%)	9/26 (35%)	1.89 (0.31-11.34)¶	7/14 (50%)	5/18 (28%)	2.60 (0.60-11.31)
Anemia requiring blood transfusions	10/32 (31%)	3/6 (50%)	7/26 (27%)	2.71 (0.44-16.75)	5/14 (36%)	5/18 (28%)	1.44 (0.32-6.49)
Hirschsprung disease	8/32 (25%)	1/6 (17%)	7/26 (27%)	0.54 (0.05-5.50)	0/14 (0%)	8/18 (44%)	0.04 (0.002-0.82)*
Recurrent otitis media	13/32 (41%)	5/6 (83%)	8/26 (31%)	11.25 (1.13-112.54)*	NA	NA	NA
Antibiotic prophylaxis	13/32 (41%)	5/6 (83%)	8/26 (31%)	11.25 (1.13-112.54)*	9/14 (64%)	4/18 (22%)	6.30 (1.33-29.95)*
IGRT	6/32 (19%)	3/6 (50%)	3/26 (12%)	7.67 (1.04-56.77)*	2/14 (14%)	4/18 (22%)	0.58 (0.09-3.76)
HSCT	8/32 (25%)	4/6 (67%)	4/26 (15%)	11.00 (1.48-81.61)*	6/14 (43%)	2/18 (11%)	6.00 (0.98-36.72)
Low IgG levels‖	6/32 (19%)	3/6 (50%)	3/26 (12%)	7.67 (1.04-56.77)*	3/14 (21%)	3/18 (17%)	1.36 (0.23-8.08)
Low IgM levels‖	4/32 (13%)	2/6 (33%)	2/26 (8%)	6.00 (0.65-55.66)	1/14 (7%)	3/18 (17%)	0.38 (0.04-4.16)
Low IgA levels‖	8/32 (25%)	2/6 (33%)	6/26 (23%)	1.67 (0.24-11.45)	1/14 (7%)	7/18 (39%)	0.12 (0.01-1.14)
Low in first year of life	8/21 (38%)	2/4 (50%)	6/17 (35%)	1.83 (0.20-16.51)	1/8 (13%)	7/13 (54%)	0.12 (0.01-1.30)
Low $CD19^+$ counts††	23/32 (72%)	5/6 (83%)	18/26 (69%)	2.22 (0.22-22.23)	9/14 (64%)	14/18 (78%)	0.51 (0.11-2.44)
$CD19^+$ counts below 0.20 × 10^9/L	10/32 (31%)	4/6 (67%)	6/26 (23%)	6.67 (0.97-45.80)	6/14 (43%)	4/18 (22%)	2.63 (0.57-12.18)
Low $CD3^+$ counts‡‡	15/32 (47%)	5/6 (83%)	10/26 (38%)	8.00 (0.81-78.83)	7/14 (50%)	8/18 (44%)	1.25 (0.31-5.07)
Low RTE§§	15/25 (60%)	3/3 (100%)	12/22 (55%)	5.88 (0.27-127.27)	4/9 (44%)	11/16 (69%)	0.36 (0.07-1.97)
Low naive $CD4^+$ counts¶¶	10/24 (42%)	3/3 (100%)	7/21 (33%)	13.53 (0.61-297.90)	6/8 (75%)	4/16 (25%)	9.00 (1.27-63.89)*
Abnormal lymphocyte proliferation	8/32 (25%)	4/6 (67%)	4/26 (15%)	11.00 (1.48-81.61)*	5/14 (36%)	3/18 (17%)	2.78 (0.53-14.50)
Combinations of T-cell abnormalities‖‖							
Any 2 of 3	14/30 (47%)	5/6 (83%)	9/24 (38%)	8.33 (0.84-83.17)	6/12 (50%)	8/18 (44%)	1.25 (0.29-5.41)
Low $CD3^+$ and low naive $CD4^+$ counts/low TRECs (definition of Leaky-SCID by PIDTC 2022)	11/30 (37%)	4/5 (80%)	7/25 (28%)	10.29 (0.97-108.81)	4/12 (33%)	7/18 (39%)	0.79 (0.17-3.63)
Low naive $CD4^+$ counts/low TRECs and abnormal proliferation	5/30 (17%)	3/5 (60%)	2/25 (8%)	17.25 (1.73-172.02)*	3/12 (25%)	2/18 (11%)	2.67 (0.37-19.06)
Low $CD3^+$ counts and abnormal proliferation	6/32 (19%)	4/6 (67%)	2/26 (8%)	24.00 (2.59-222.65)**	4/14 (29%)	2/18 (11%)	3.20 (0.49-20.81)
All 3	5/31 (16%)	4/5 (80%)	1/26 (4%)	100.00 (5.15-1941.45)**	3/13 (23%)	2/18 (11%)	2.40 (0.34-16.97)

CI, Confidence interval; *OR,* odds ratio; *SDS,* standard deviation score.
†Severe infections were defined as sepsis, recurrent pneumonia, and/or opportunistic infection.
‡Respiratory infections included recurrent otitis media and/or pneumonia.
§Birth length was corrected for gestational age.[20]
¶OR 12.5 (95% CI 1.92-60.00) for birth length below −6.5 SDS when using bootstrapping.
‖Individuals were considered to have low immunoglobulin levels if at least 1 measurement was subnormal.
††$CD19^+$ cell counts were considered low if they were repeatedly and consistently below 10th percentile for age compared to healthy children's reference values.[12]
‡‡Low $CD3^+$ cells were defined according to the PIDTC 2022 criteria: <0.6 × 10^9/L (any age), <0.8 × 10^9/L if aged 2-4 years, or <1.0 × 10^9/L if younger than 2 years.[18]
§§Low RTE was defined as count below 10th percentile for age compared to healthy children's reference values.[13]
¶¶Low naive $CD4^+$ cells were defined according to the PIDTC 2022 criteria: less than 20% of $CD4^+$ cells are naive.[18]
‖‖Laboratory T-cell abnormalities: (1) low $CD3^+$ cell counts (by PIDTC 2022 definitions), (2) low naive $CD4^+$ cell counts (by PIDTC 2022 definitions) *or* abnormal TRECs, (3) abnormal lymphocyte proliferation responses to PHA.
*$P < .05$, **$P < .01$, exact 2-sided P values with Fisher exact test; statistically significant odds ratios are indicated.

development of respiratory infections; and target 2, development of severe infections (Fig 1).

For all model trainings, we used sample division of 80% of samples for model training and 20% of samples for model validation. Estimation accuracy of the models with library 'sklearn' accuracy_score was as follows: for data set 1, respiratory infections accuracy was 0.93 and severe infections 0.93, and for data set 2, respiratory infections accuracy was 1.0 and severe infections 1.0 (maximum value, 1.0). These values indicated that the tested parameters did predict the outcome in the validation model, so we continued analysis.

Training models were done with Python v.3.10.6 'sklearn' v1.2.2 library RandomForestClassifier object, using parameters: n_estimator = 2000, random_state = 42 for all data set and target outcomes. Results of the training model represent the relative importance of each feature with the given data set and target outcome. The importance value was acquired from inbuilt method feature_importances of the RandomForest classifier.

RESULTS

Clinical characteristics of cohort

Our study included 16 girls and 16 boys with CHH aged 2.7 to 22.1 years (median, 8.2 years) at last follow-up, comprising a total of 331.3 patient-years. Children were usually followed up by the immunologist 1 to 3 times during the first year of life and yearly thereafter. Most of the participants were homozygous (25/32) or heterozygous (5/32) for the Finnish founder n.71A>G *RMRP* variant. Other variants included n.263G>T, n.-26_-4dup, and n.-22_-13dup. Clinical characteristics of the cohort are presented in Table I, and the clinical course of each patient is demonstrated in Fig 2.

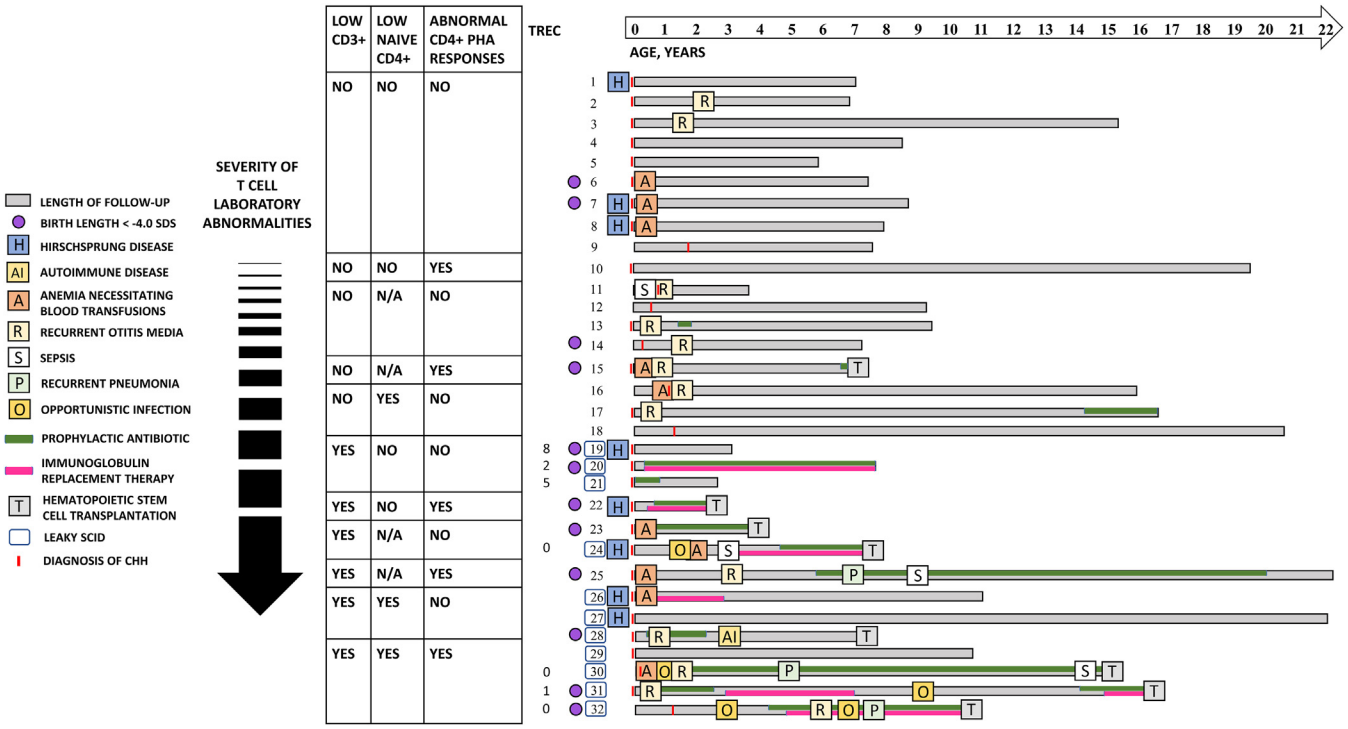

FIG 2. Clinical course of 32 children with CHH. Patients are arranged according to severity of T-cell abnormalities. Within each laboratory subgroup, patients are arranged in order of decreasing $CD3^+$ median counts. Low $CD3^+$ T cells, low naive $CD4^+$ T cells, and Leaky-SCID were all defined according to PIDTC 2022 criteria.[19] TRECs are shown as copies/μL. Autoimmune disease in a single patient was hemolytic anemia. *N/A,* Data not available.

Unexpectedly, the prevalence of birth length below −4.0 standard deviation score, severe anemia, and Hirschsprung disease were all significantly higher in our cohort compared to the previously reported prevalence in Finnish patients with CHH born before 2000 (12/32, 38% vs 13/80, 16%, Fisher exact test P = .023, 10/32, 31% vs 7/114, 6%, P < .001, and 8/32, 25% vs 8/108, 7%, P = .011, respectively).[21,22] These clinical characteristics did not correlate with sex, genotype, or each other (see Table E1 in this article's Online Repository at www.jaci-global.org).

In line with previously reported prevalence of clinically asymptomatic immunodeficiency in the Finnish CHH population,[9] 18 (56%) of 32 study participants did not have increased number of infections during a median of 7.7 years of follow-up (168.8 patient-years). However, 5 (28%) of these 18 had received prophylactic antibiotics and/or immunoglobulin replacement therapy (IGRT). Fourteen children fulfilled our criteria for increased incidence of infections (respiratory and/or severe), all early during follow-up, at a median age of 0.9 years (range, 0.4-4.2 years). They developed recurrent otitis media (n = 13), single (n = 3) or multiple (n = 3) episodes of pneumonia, sepsis (n = 4), and/or opportunistic infections (n = 4). Opportunistic infections were not observed in the first 6 months of life and included chronic norovirus gastroenteritis (n = 2), human bocavirus hepatitis (n = 1),[23] refractory warts (n = 1), and vaccine-strain rubella virus–induced skin granulomas (n = 1). The length of follow-up for 14 symptomatic patients was similar to that of children with no increased incidence of infections (median 10.2 years [Mann-Whitney U test P = .283], total of 162.5 patient-years).

For the majority of children (21/32, 66%), the causative organisms of infections either have never been searched for thanks to mild manifestations or the performed investigations have yielded negative results. Apart from the abovementioned pathogens of opportunistic infections, children were able to clear (after a mild illness not requiring hospitalization) norovirus (n = 1), enterovirus (n = 1), influenza A virus (n = 1), severe acute respiratory syndrome coronavirus 2 (n = 1), rhinovirus (n = 2), and respiratory syncytial virus (n = 2). However, 2 of the most severely affected patients (patients 31 and 32, Fig 2) required hospitalization for respiratory syncytial virus–induced bronchiolitis and pneumonia. In addition, common bacterial pathogens —*Staphylococcus aureus, Haemophilus influenzae*, and/or *Pseudomonas aeruginosa*—were detected in middle-ear discharge samples from 2 patients.

Antimicrobial prophylaxis (n = 13) was administered mostly to patients with Leaky-SCID–level lymphopenia, and in at least 3 individuals, it failed to prevent recurrent pneumonia or sepsis episodes. Six patients were treated with IGRT, and of these, 1 experienced respiratory and severe infections while receiving therapy. All patients were alive at the time of writing, and 8 had received curative HSCT. The indications for HSCT included transfusion-dependent anemia (n = 2) and profound lymphopenia alone (n = 4) or in combination with severe infections (n = 2). Both children with infections were transplanted in the settings of chronic norovirus diarrhea; others were free of infections at the time of HSCT. Seven patients were receiving antibiotic prophylaxis before transplantation, and 4 were also receiving

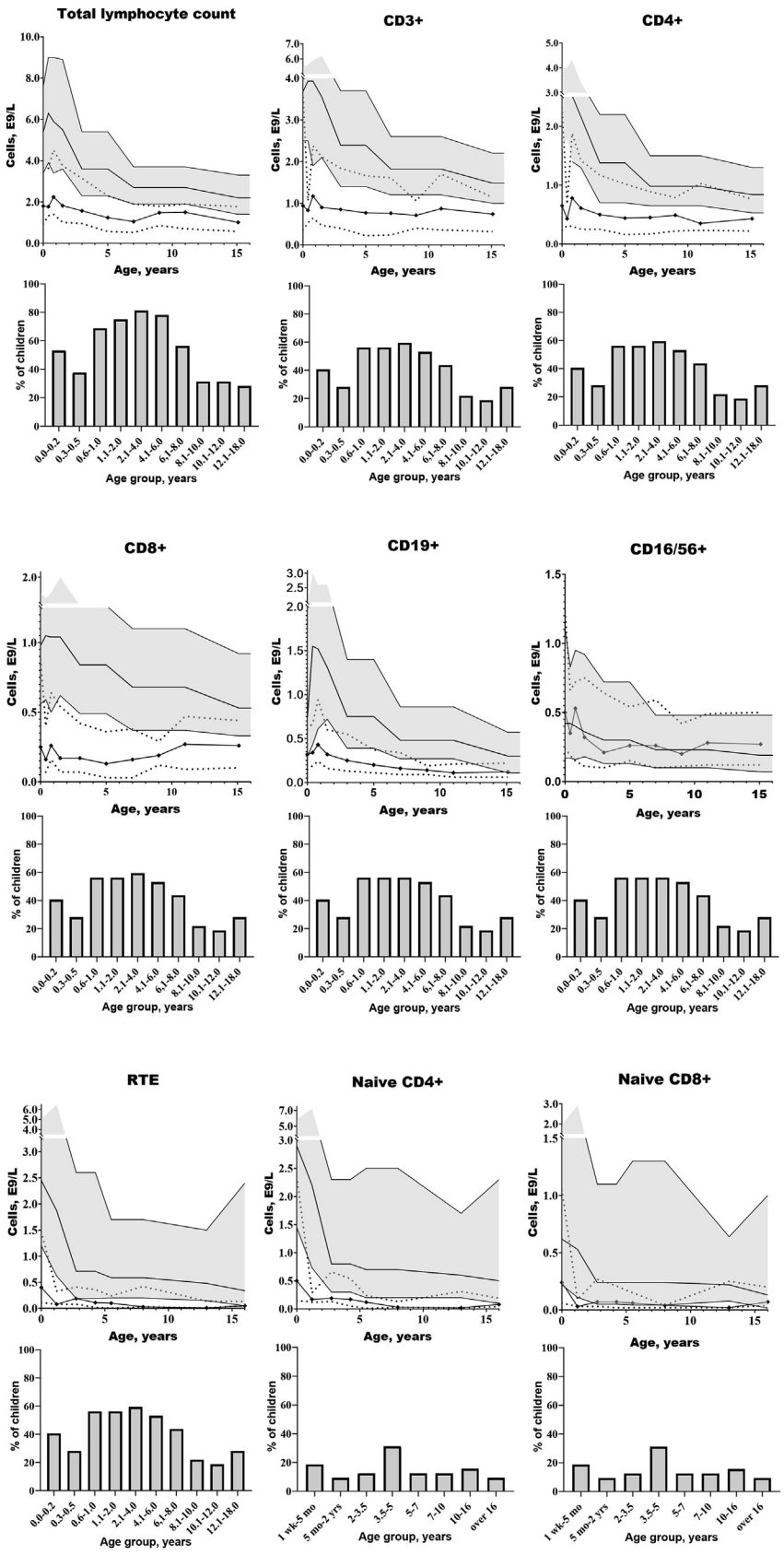

FIG 3. Lymphocyte subclass counts in children with CHH *(dotted lines)* and healthy children *(gray-shaded area)* are depicted as medians *(bold lines)* with 10th to 90th percentile range.

IGRT. Before HSCT, T-cell counts ranged from 0.13 to 0.66 $\times$ 10^9/L (median 0.4 $\times$ 10^9/L).

All lymphocyte counts, except NK cells, are low in CHH, but do not decline significantly during childhood

The median total lymphocyte, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, naive $CD4^+$ cell, and RTE counts in children with CHH were consistently below the 10th percentile of age-appropriate reference values (see Fig E3 and Table E2 in the Online Repository at www.jaci-global.org). Median naive $CD8^+$ cell counts varied below and above the normative 10th percentile values. As a notable exception, NK cells counts were mostly normal, consistent with previous reports.[5,6,8] None of the children in our cohort had very low (classical SCID level) lymphocyte counts, but 11 children had disease that fulfilled Leaky-SCID definitions.

Lymphocyte subclass counts in CHH patients were low at birth but did not decline further with age as markedly as in healthy children (Fig 3; and see Fig E1 in the Online Repository at www.jaci-global.org). Five children had $CD3^+$ cell counts over 2.5 $\times$ 10^9/L (from 2.6 to 4.5 $\times$ 10^9/L) at first measurement at age 1 week to 8 months. In all of them, $CD3^+$ counts decreased progressively with age but never reached Leaky-SCID values.

On the basis of our longitudinal data, lymphopenia was maintained in patients who survived longer without HSCT. In 18 participants aged over 6 years, lymphocyte counts behaved similarly before and after 6 years of age. NK cell counts remained normal throughout follow-up. Total lymphocyte and T-cell counts and subclasses remained consistently low (n = 11), fluctuated below and above the 10th percentile of healthy children's reference values (n = 6), or were consistently normal (n = 1). $CD19^+$ cell counts followed the same pattern, with the exception of 2 patients who had normal number of $CD19^+$ cells despite low T-cell counts.

Of 6 patients aged over 16 years, lymphocyte counts remained low in 3, continued to fluctuate in 1, and normalized in 2 patients who had previously demonstrated fluctuating counts. However, after these 6 patients turned 16 years old, the number of repeated blood samples was low, and the follow-up time was short. Therefore, the normalization of lymphocyte counts in 2 individuals may have been transient.

The lowest T-cell counts in children with CHH were observed at the age of 4 to 6 months, presenting as a decline in the median and the 90th percentile of $CD3^+$, $CD4^+$, and $CD8^+$ cell counts. However, there were only 8 patients in this age group, and 5 of them had Leaky-SCID–level decreased T-cell counts on prior measurements; in the other 3, T cells were not evaluated before 4 months of age. Therefore, a selection bias occurred because T cells were not measured at age 4 to 6 months in another 6 children with normal or slightly decreased T-cell counts on prior measurements.

Of clinical characteristics at birth, only short birth length correlated with lower total lymphocyte counts and lower $CD3^+$ cell counts at 0 to 2 years (Spearman correlation R = 0.566, P = .002, and R = 0.170, P = .045, respectively; see Fig E2 in the Online Repository at www.jaci-global.org). Accordingly, low T-cell counts at any time during follow-up were more common in patients with birth length below −4.0 standard deviation score (see Table E3 in the Online Repository).

Hypoglobulinemia is uncommon and often transient in children with CHH

Immunoglobulin levels were assessed repeatedly in all 32 patients, but measurements in the first year of life were only available in 21 children. The trends in immunoglobulin levels over time, as well as comparison of immunoglobulin levels of study participants with healthy children, are demonstrated in Fig E3. Decreased IgA levels were detected in 8 patients, and only in samples obtained during the first year of life; all 8 children demonstrated normalization of IgA levels in follow-up samples. Four of these patients showed transiently decreased IgM levels, also exclusively when they were under 1 year of age.

Our data were insufficient to evaluate the prevalence of primary hypogammaglobulinemia in children with CHH. First measurements of IgG levels at a median of 10 months demonstrated normal levels in all patients. Decreased IgG levels were detected in 6 participants during follow-up. In 2 of these children, IgG levels were repeatedly normal after the single sample with decreased IgG level. In another 3 patients, IGRT was commenced on the basis of a single subnormal IgG value. One of those 3 patients had concurrent hypoalbuminemia and short bowel syndrome; therefore, secondary hypogammaglobulinemia due to intestinal protein loss cannot be excluded. In another child, IgG levels remained normal after cessation of IGRT of 2 years' duration. Definite hypogammaglobulinemia was observed in only 1 patient, who demonstrated repeatedly low IgG levels before IGRT. Noteworthily, this child developed a drop in $CD19^+$ cell counts and hypogammaglobulinemia at the age of 4 years and had the most severe short stature and immunodeficiency phenotype of all the study patients (patient 32, Fig 2). Despite the uncertainty of the hypogammaglobulinemia entity in our cohort, low IgG levels (but not low $CD19^+$ cell counts) associated with the development of severe infections (Table I).

Antibodies to toxoid vaccines (tetanus and/or diphtheria) were assessed in 24 patients and were detectable in all of them. Consistent with our previous report,[24] a significant proportion of patients had received live vaccines and were able to mount serologic responses to at least 1 of the components of measles–mumps–rubella vaccine (n = 13) and/or varicella vaccine (n = 9). Polysaccharide pneumococcal vaccine responses were adequately assessed in 2 children, and both demonstrated normal development of antibodies. Serologic responses were normal despite the presence of Leaky-SCID–level T-cell lymphopenia or low B cells (n = 10 and n = 19 for toxoid vaccines, n = 3 and n = 8 for live vaccines, and n = 1 and n = 2 for polysaccharide pneumococcal vaccine, respectively).

Degree of T-cell abnormalities associates with severe infections

We evaluated the impact of T-cell deficiency on the incidence of infections by correlating TREC (measured in 7 children), $CD3^+$ (measured in all), and naive $CD4^+$ cell counts (available in 24 children) and T-cell proliferation responses (tested in all), alone and in various combinations, with the occurrence of infections (Table I).

Of 7 patients with TREC values available (measured at age varying from 1 day to 14 years), 4 had low (1-9 copies) and 3 had absent (0 copies) TREC values (Fig 2). Of 4 patients who developed opportunistic infections in our cohort, 3 had absent TRECs. The fourth child, with recalcitrant warts, had a TREC count of 1,

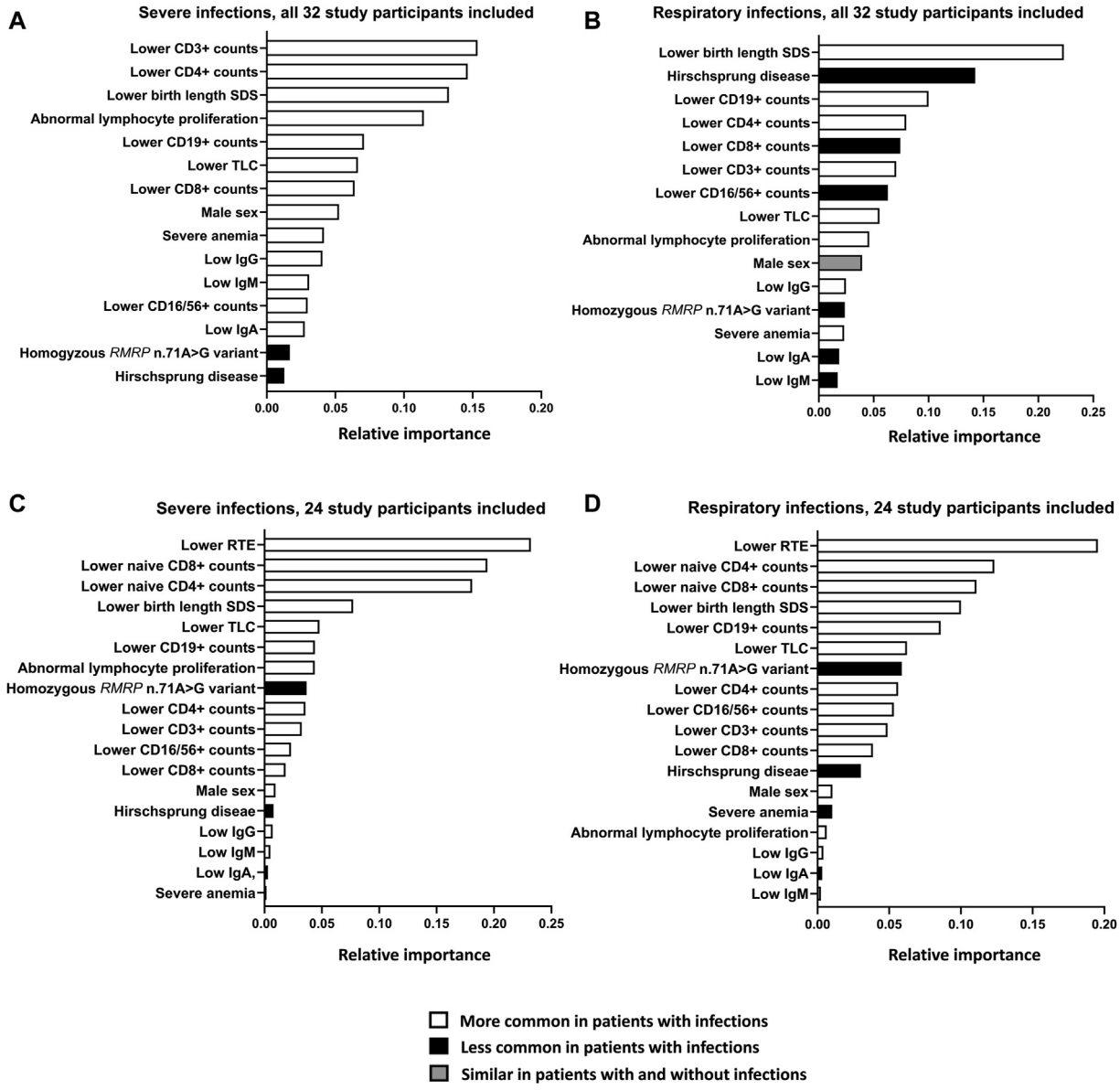

FIG 4. Random forest classification demonstrating relative importance of clinical and laboratory features in development of severe (**A** and **C**) and respiratory (**B** and **D**) infections in all 32 (*A* and *B*) study participants (naive cell counts not included) and in 24 study participants (*C* and *D*) for whom naive cell counts were available. X-axis indicates relative importance of each feature from 0 to 1. This can be interpreted as percentage scale, where 1 = 100%. Bars are colored according to prevalence of feature: *white, black,* and *gray* for features more common, less common, or similar, respectively, in patients with infections.

measured at the age of 14 years. None of the 4 of 7 7 patients with >1 TREC presented with Leaky-SCID–level decreased naive $CD4^+$ cell counts or developed significant infections. However, in 3 patients with T-cell lymphopenia and unavailable naive $CD4^+$ T-cell counts, TREC counts had not been measured. Low naive $CD4^+$ cell count was the only risk factor for respiratory infections of all clinical and laboratory variables tested. Low naive cell counts did not correlate with the incidence of severe infections; however, they were measured in 24 children out of the entire cohort and in only 3 of 6 children in this group.

In 12 patients, $CD3^+$ cell counts were measured during the first 3 months of life. We evaluated the differences in the incidence of infections within this group according to various $CD3^+$ cutoff counts (from 0.3 to 1.0 $\times$ 10^9/L, which was the median $CD3^+$ cell count in this patient group). The correlation with severe infections could not be established, as only 1 of 12 children developed severe infection. Early $CD3^+$ cell counts did not correlate with the development of respiratory infections. The small number of patients and the low incidence of infections affect the reliability of these results.

We then compared patients with (n = 15) and without (n = 17) consistently decreased $CD3^+$ cell counts measured at any point during follow-up, applying PIDTC 2022 definitions of low T-cell counts. Opportunistic infections occurred only in children with Leaky-SCID–level lymphopenia. Two patients with the lowest $CD3^+$ cell counts (below 0.3 $\times$ 10^9/L on multiple occasions)

both developed severe infections. However, low T-cell count alone did not predict infections in the entire cohort. Importantly, patients with low $CD3^+$ cell counts were treated more aggressively, receiving IGRT, antimicrobial prophylaxis, and HSCT more often, which could have affected their infection susceptibility. Among the 18 children with no increased incidence of infections, 8 demonstrated low T cells. Intriguingly, 3 of these profoundly lymphopenic patients (patients 19, 27, and 29, Fig 2) managed well without any interventions for 3, 10, and over 20 years.

Abnormal lymphocyte proliferation responses were less common in our cohort (8/32, 25%) than in previously reported case series (69-88%).[22,25] Decreased proliferation alone was a risk factor for severe infections. Among 24 patients with normal proliferation, 8 also had low T cells, but only 1 (a patient with absent TREC) developed severe infections. Abnormal responses were more common in patients with low T cells, either reflecting a more profound T-cell defect or a technical issue of measuring proliferation in a lymphopenic sample (Table E3).

We next implemented Leaky-SCID criteria that consisted of low $CD3^+$ combined with low naive $CD4^+$ cell counts or decreased TRECs. A third (11/30, 37%) of the participants fulfilled Leaky-SCID definitions, but only 4 of them developed severe infections. Both low T-cell counts alone and Leaky-SCID laboratory abnormalities associated with low IgG levels and profoundly low $CD19^+$ cells (Table E3).

Finally, we examined various combinations of T-cell abnormalities as a risk factor for the development of infections (Table I). None of the combinations was found to be associated with respiratory infections. Only combinations that included abnormal proliferation responses were associated with increased risk of severe infections, and the highest risk was observed in patients with all abnormalities present. We note that a single patient (patient 29) with all the T-cell abnormalities combined had remained asymptomatic for over a decade without receiving any therapies (Fig 2).

Machine learning identifies birth length and naive T-cell counts as important features associated with development of infection

After applying traditional statistical analyses to our data, we proceeded to machine learning techniques to overcome the issues of small sample size and inter- and intraindividual differences in the availability and distribution of laboratory data. We implemented random forest classification on 2 data sets. The first covered data from all 32 patients, but naive T-cell counts were not included in the analysis. The second one included 24 patients for whom naive T-cell counts were available (Fig 4). For our previous analyses, we were forced to transform the majority of laboratory measurements into categorical variables (eg, cell counts low vs not low) to enable data comparison among individuals of different ages. However, for machine learning, we could use the numerical counts for all lymphocyte subclass measurements. Also, it allowed us to include multiple values from the same individuals measured repeatedly at various ages. This analysis was therefore not restricted by application of reference values. In addition, in contrast to the basic statistical methods, the random forest approach compensates for the low number of patients. We chose a high number (2000) of prediction trees, each of which was a unique analysis of random combinations of patients and variables.

For the development of severe infections, of all clinical variables, shorter birth length was the most contributing feature. Interestingly, for respiratory infection risk, shorter birth length was even more contributing than the absence of Hirschsprung disease. Of the laboratory features, all naive T cells ($CD4^+$, $CD8^+$, and especially RTE) contributed most to infection risk.

DISCUSSION

We described clinical course and laboratory abnormalities in a cohort of 32 children with CHH followed up for over 330 patient-years. To our knowledge, no previous studies with high numbers of pediatric participants and long follow-up have addressed the longitudinal changes in cell counts, or the association of clinical features and laboratory indices with subsequent disease course. While the decision to perform HSCT in individuals with CHH fulfilling SCID definitions is straightforward, the optimal management of children with less profound T-cell lymphopenia has been unclear. Our data provide several novel clinical observations that can assist clinicians caring for such patients with CHH. Most importantly, we demonstrated that several clinical and laboratory immunologic parameters correlated with the development of infections. Traditional statistical analyses combined with machine learning showed that shorter birth length, low naive T cells, and abnormal lymphocyte proliferation responses contributed most to the infection risk.

We also provide novel longitudinal data on lymphocyte subclass counts during childhood, demonstrating that the counts remain mostly stable after the first year of life. We have previously reported on progressive clinical features of immunodeficiency in CHH.[9] Also, we have described late-onset clinical manifestations of immunodeficiency in patients with metaphyseal dysplasia without hypotrichosis, which had been previously been considered a mild form of CHH with skeletal manifestations only.[26] Thus, clinical features of CHH are not always predictable by lymphocyte count kinetics—which, once again, calls for regular follow-up of all patients with CHH, regardless of laboratory manifestations.

Our observations can guide the frequency of blood sampling during follow-up, which is of particular importance in short-statured children with CHH, when obtaining a sufficient amount of blood is problematic. Importantly, 1 of our patients developed hypogammaglobulinemia and simultaneous decline in B-cell counts at the age of 4 while remaining free of respiratory infections. This patient already had profound T-cell abnormalities and then continued to develop the most severe clinical immunodeficiency in our cohort. This calls for regular laboratory follow-up of all children with CHH and low T cells, including those asymptomatic in terms of infections.

All participants with absent TRECs, but none with TREC copies over 1, developed severe infections. This probably relates directly to the differences in the severity of T-cell laboratory abnormalities between these 2 groups, consistent with a previous report of SCID diagnoses in CHH children with absent, but not in those with decreased, TRECs.[10] Although TRECs were measured in only 7 children in our study, RTEs, the next closest approximate of T-cell production, were assessed in the majority of participants. We have previously shown that low RTEs associated with more severe clinical manifestations in 56 children and adults with CHH,[5] and we confirmed this finding in the present study that included extended follow-up data on 10 children from our previous report. In our participants with TREC counts over 1, naive $CD4^+$ cell counts were decreased, although not to the level of

Leaky-SCID, and they did not develop significant infections. Importantly, TREC counts were unavailable in 3 children who could have been reclassified to Leaky-SCID patients in cases when their TREC counts were low. Systematic assessment of TREC counts in children with CHH would help to validate our preliminary findings and may guide counseling and management decisions.

Abnormal lymphocyte proliferation responses associated with the development of severe infections in our cohort, particularly in combination with other T-cell abnormalities. Although previous studies have reported contradictory results,[3,25] our methods of measuring proliferation responses have changed over time. Recent study has demonstrated good discriminating ability of flow-cytometric assay for specific cell-mediated immune response in activated whole blood for detecting patients with more severe immunodeficiency.[27] Among our patients with normal proliferation responses and low T cells, only 1 (a patient with absent TREC) developed severe infections. Our findings suggest that low T-cell counts alone should not guide management decisions in patients with CHH; rather, a combination of evidence for poor T-cell production and functioning should be considered.

Testing for T-cell receptor repertoire is not routinely available in Finland, and we could not assess its relevance in predicting the clinical course in our patients. Previous studies have shown restricted T-cell receptor repertoire in the majority of tested individuals with CHH[6,28,29] and can help guide management.[30] Therefore, it would be of interest in the future to evaluate the prognostic potential of this immunologic parameter.

The severity of short stature has been previously reported to be associated with increased mortality in CHH.[9] In our study, shorter birth length clearly correlated with lower T-cell counts and was identified by machine learning as the clinical feature contributing to the development of infections. Therefore, the severity of short stature can reflect the degree of generalized cell proliferation impairment in *RMRP* deficiency, translating into poor clinical outcome. The lower prevalence of severe short stature in our older CHH cohort[9] can thus be explained by failure to recruit severely short-statured patients due to their early mortality. These data suggest that severe short stature at birth should be considered as a risk factor for adverse outcome in CHH, in combination with laboratory immunologic parameters.

We identified certain differences between the analyses by using traditional statistical methods and machine learning. Both short birth length (continuous variable) and low naive T-cell counts (numerical) contributed most to the risk of severe infections with random forest analysis; however, the association was not obvious with traditional methods (where both variables were transformed into categorical variables). In contrast, while abnormal proliferation responses were most significantly correlated to infection risk with traditional statistics, their contribution with machine learning was modest. Random forest analysis compensates for small sample size and therefore brings out associations that may be obscured with traditional statistical methods. When using bootstrapping and a higher cutoff for birth length, we did get significant correlation of shorter birth length and the development of severe infections with traditional statistical methods. Therefore, we believe that the 2 approaches complement each other.

Surprisingly, children with Hirschsprung disease in our cohort did not develop respiratory infections. Also, only 1 patient with Hirschsprung disease had severe clinical course, contrary to previous observations of poor prognosis of Hirschsprung disease in CHH.[9,31] We did not collect data on day care attendance in our patients, which may have been delayed (resulting in decreased pathogen exposure) in children with Hirschsprung disease and concomitant short bowel syndrome, the presence of bowel stomas, or multiple hospitalizations due to intestinal issues. Additionally, enterocolitis is common in CHH-related Hirschsprung disease[32] and is often treated with repeated and prolonged antibiotic courses. Both increased receipt of antimicrobials and alterations in microbiota can affect susceptibility to respiratory infections. The higher prevalence of Hirschsprung disease in our cohort can arise from undiagnosed and early fatal CHH cases with Hirschsprung disease earlier in the 20th century, which were not included in previously described cohorts.

While anemia and/or macrocytosis is a well-recognized feature of CHH, reported in as many as 86% of children,[33] severe anemia necessitating repeated red blood cell transfusions is less frequent (6%).[22] Severe anemia was unexpectedly common in our patients (31%) and did not correlate with the degree of T-cell deficiency or with clinical course. The increased prevalence of severe anemia can be explained by differences in management of CHH children in the 20th and 21st centuries. Anemia in CHH is often transient, and some of the children born in the 20th century may have never been tested for anemia. The pathogenesis of anemia in CHH remains unclear, but recent study suggests immune dysregulation as a possible explanation.[34] Autoimmunity was uncommon in our cohort, represented by 1 child who developed autoimmune hemolytic anemia.

Despite describing the largest pediatric CHH cohort with long-term follow-up, our study has limitations of small sample size, which is inevitable in country-level rare disease reports. In addition, a minority of children in our cohort had *RMRP* variants other than n.71A>G, which is known to associate with milder disease course.[4] Indeed, our patients with variants other than homozygous n.71A>G more often developed infections, although this trend did not reach statistical significance, probably because of the high number of homozygous individuals.

In conclusion, we suggest that a combination of clinical (shorter birth length) and laboratory (abnormal T-cell production and function) features should guide management decisions in infants with CHH not fulfilling classical SCID criteria.

DISCLOSURE STATEMENT

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