Original Article

The Immune Phenotype of Patients with CHARGE Syndrome

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What is already known about this topic? Several case reports indicate that, like 22q11.2 deletion syndrome, severe combined immune deficiency (SCID) can occur in CHARGE (Coloboma, Heart disease, choanal Atresia, growth/mental Retardation, Genitourinary malformations, Ear abnormalities) syndrome. However, whether more subtle forms of immune deficiency such as antibody deficiency are also frequent in CHARGE syndrome is unknown.

What does this article add to our knowledge? The results of this study suggest that CHARGE syndrome is associated with lymphopenia and hypocalcemia early on; however, significant immune deficiencies are uncommon by the time these children reach school age.

How does this study impact current management guidelines? In the absence of recurrent infections or SCID presentations, routine immunological investigations are not indicated in children with CHARGE syndrome.

BACKGROUND: Recurrent sinopulmonary infections are common in children with CHARGE (Coloboma, Heart disease, choanal Atresia, growth/mental Retardation, Genitourinary malformations, Ear abnormalities) syndrome, but no prospective studies on immune function have been conducted.
OBJECTIVE: This study aims to examine and compare the immune phenotype of patients with CHARGE syndrome to those with 22q11.2 deletion and healthy controls.
METHODS: A total of 21 patients attended a multidisciplinary CHARGE clinic. All patients had *CHD7* mutational analysis performed. Patients with CHARGE syndrome had lymphocyte subsets, immunoglobulins (IgG, A, M), functional protein, and polysaccharide vaccine responses measured at initial evaluation. A total of 55 healthy controls were prospectively recruited, whereas 40 patients with 22q11.2 deletion were retrospectively identified through medical records. A separate analysis compared serial lymphocyte counts and ionized calcium levels between patients with CHARGE syndrome and those with 22q11.2 deletion in the first 72 months of life.
RESULTS: Despite recurrent childhood ear and chest infections, only 2 children with CHARGE syndrome had an identifiable immune defect (reduced serum IgA). In contrast, T-cell lymphopenia, low immunoglobulin levels, and specific antibody deficiency were noted in patients with 22q11.2 deletion. A greater proportion of patients with 22q11.2 deletion had persistent lymphopenia (57% vs 30%) and hypocalcemia (60% vs 37.5%) compared with patients with CHARGE syndrome in the first 72 months of life.
CONCLUSIONS: Although phenotypic overlap exists between CHARGE and 22q11.2 deletion syndromes, no significant immune defects were detected in this cohort of patients with CHARGE syndrome at the time of testing. Lymphopenia and hypocalcemia occur in both conditions early in life, but is more pronounced in patients with 22q11.2 deletion. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;■:■-■)

Key words: *CHARGE syndrome; 22q11.2 deletion syndrome; Lymphopenia; Specific antibody deficiency*

The acronym "CHARGE" (Coloboma, Heart disease, choanal Atresia, growth/mental Retardation, Genitourinary malformations, Ear abnormalities) encapsulates the multisystemic and complex nature of CHARGE syndrome. CHARGE syndrome remains a clinical diagnosis, based on major and minor criteria as defined by Verloes.[1] However, up to 90% of patients with

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Abbreviations used
CHARGE- Coloboma, Heart disease, choanal Atresia, growth/mental Retardation, Genitourinary malformations, Ear abnormalities
CHD7- Chromodomain Helicase DNA-binding protein 7
22qDS- 22q11.2 deletion syndrome
SAD- Specific antibody deficiency

typical clinical features of CHARGE syndrome have a heterozygous mutation or deletion involving *CHD7*, encoding Chromodomain Helicase DNA-binding protein 7,[2] which is thought to be important in neural crest formation.[3]

22q11.2 deletion syndrome (22qDS) or velocardiofacial syndrome is a genetic disorder caused by a submicroscopic deletion at 22q11.2.[4] There are phenotypic overlaps between 22qDS and CHARGE syndrome, such as cardiac defects, cleft lip/palate, and growth retardation. Very rarely severe combined immune deficiency (SCID) due to DiGeorge anomaly (athymia, hypocalcemia, congenital heart disease) can occur in both disorders.[5] Notable differences also exist, with the presence of ocular coloboma, cranial nerve deficits, and the absence of semicircular canals that occur much more frequently in CHARGE syndrome.[6]

Immune deficiency is a well-studied and recognized feature of 22qDS. Patients with 22qDS often have asymptomatic lymphopenia[7] and specific antibody deficiency (SAD),[8] defined as the inability to mount antibody responses to polysaccharide antigens.[9] Little is known regarding immune defects in CHARGE syndrome. Although CHARGE syndrome with SCID has been described in several case reports,[10-12] this likely represents a reporting bias of severe cases. In the largest retrospective case series of 25 patients, only 9 had immune phenotyping, of whom 2 had SCID, 3 had lymphopenia, and 2 had IgA deficiency.[13]

There have been no prospective studies that evaluated the immune function of patients with CHARGE syndrome. We aimed to prospectively recruit children with CHARGE syndrome and systematically assess and compare their immune phenotype with those with 22qDS and healthy controls.

METHODS

Multidisciplinary CHARGE clinic

A multidisciplinary CHARGE clinic at the Children's Hospital at Westmead (CHW), Sydney, was established in 2012. Patients with CHARGE syndrome at CHW were identified via medical records by the genetics department. An invitation letter was sent out to patients with a likely clinical diagnosis of CHARGE syndrome (n = 23). Six clinics were run once every 3 months from January 2012 to January 2014, and a total of 21 patients attended the clinic; 2 patients did not respond to invitation. At each clinic, patients were assessed by a consultant pediatric geneticist, endocrinologist, immunologist, and a general pediatrician. Any specific medical issues were managed and/or followed up in appropriate subspecialty clinics.

The immunological assessment included completion of a standard proforma by the treating immunologist, which encompassed clinical symptoms, examination findings, and investigation results. Recurrent otitis media (OM) was defined as more than 2 doctor diagnosed OM per year. Suppurative infections were defined as any one of x-ray diagnosed pneumonia, at least 1 episode of purulent ear discharge, and/or chronic productive cough or purulent nasal discharge (cough or discharge for > 90 days). Bloods for immunophenotyping were taken just before or on the day of the clinic, and included full blood count, lymphocyte subsets, immunoglobulins (IgG, A, M), IgG subclasses, and IgG antibody levels to tetanus, diphtheria, Haemophilus influenzae type B, and pneumococcus (14 serotypes). All patients more than 18 months of age were offered Pneumovax (unconjugated 23-valent) vaccination (bioCSL, Victoria, Australia) with subsequent assessment of functional antibodies.

Healthy controls, patients with 22q11.2 deletion, and comparison with CHARGE syndrome

Patients with a genetic diagnosis of 22qDS diagnosed between 2005 and 2014 (n = 63) were identified by the genetics department via the departmental database. Fluorescence *in situ* hybridization was used for detection of 22q11.2 deletion in all patients. A total of 40 patients who had lymphocyte subsets and/or immunoglobulins were included in the analysis (median, 2 years; range, 10 days to 12 years). The latest lymphocyte subset and/or immunoglobulin results were recorded. Of the 40 patients with 22qDS, 23 patients had formal testing for functional antibody responses.

Controls consisted of healthy children (median, 4 years; range, 1 month to 14 years) who underwent elective surgery. Children with autoimmune diseases or primary immune deficiencies were excluded. A total of 55 healthy controls were recruited, and bloods were collected for lymphocyte subset analysis. Lymphocyte subset counts were compared between healthy controls, CHARGE, and 22qDS. Immunoglobulin levels were compared between patients with CHARGE syndrome and those with 22qDS (antibody levels were not measured in healthy controls).

To account for the older age of our cohort of patients with CHARGE syndrome, all available lymphocyte counts and ionized calcium levels from the first 72 months of life were also retrospectively collected from the same patients with CHARGE syndrome and 22qDS for comparison. Results were analyzed for persistent lymphopenia, defined as at least 2 lymphocyte counts $< 2 \times 10^9$/L 2 months apart. An ionized calcium level of < 1.2 mmol/L was considered low. All tests were performed at the Children's Hospital at Westmead.

Flow cytometry

One hundred microliters of whole blood per tube was incubated with fluorescent conjugated antibodies against CD45, CD3, CD4, CD8, CD16, CD56, and CD19 for 15 minutes at room temperature. Red cells were then lysed using 2 mL of Easylyse Erythrocyte Lysing solution (Dako, Sydney, Australia) per tube. Samples were acquired through a BD FACS Canto flow cytometer and analyzed and gated using FACS Diva(BD biosciences, Sydney, Australia).

Measurement of immunoglobulins

IgG, IgA, IgM, and IgG subclass levels were measured using immunonephelometry using the BN (Dade Behring, Marburg) system according to the manufacturer's protocols (Dade Behring). The deficiency of antibody levels was determined based on established laboratory reference ranges. Specific IgG to tetanus, diphtheria, Haemophilus influenzae type B, and 14 serotypes of *Streptococcus pneumonia* were measured by a multiplexed fluorescent bead-based assay according to the manufacturer's instructions (xMAP Pneumococcal Immunity Assay from Luminex, Sydney, Australia). A protective level of antibody to tetanus, diphtheria, and Haemophilus influenzae type B was defined as >0.16 IU/mL, >0.1 IU/mL, and >1 μg/mL, respectively.[14,15] An adequate response to