Original article

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POPULATIONS OF PERIPHERAL BLOOD LYMPHOCYTES IN PEDIATRIC PATIENTS WITH CONOTRUNCAL CARDIAC ANOMALIES

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Objective - To determine the abundance of principal lymphocyte subpopulations in the peripheral blood of patients with conotruncal cardiac anomalies. Conotruncal cardiac anomalies arise from a developmental defect of the conotruncal septum and often occur as part of 22q11.2 deletion syndrome, also characterized by hypoparathyroidism, growth retardation, characteristic facial dysmorphia and an immune system impairment due to thymic hypoplasia.

Patients and Methods - Deletion of 22q11.2 is detected by fluorescent in situ hybridization in over 90% of patients showing the characteristic phenotype. In these patients, a broad spectrum of immunological defects has been described. Most typical of these is a mild to moderate decrease of T cell number and function. In this study, we determined the numbers of lymphocyte subpopulations (CD3+, CD19+, CD3-CD16/CD56+, CD3+CD4+, CD3+CD8+, CD3+HLA-DR+) in patients treated for conotruncal anomalies (8 patients with 22q11.2 deletion, 7 without 22q11.2 deletion and 4 in whom deletion was not sought) by immunofluorescence/flow cytometry.

Results - The number of patients whose CD3+ cells were decreased in number, as compared to the age-specific reference range, was 4/8, 3/7 and 2/4 in the three groups, respectively (total: 9/19 patients). The remaining lymphocyte subpopulations investigated demonstrated wide variability in all three groups of patients.

Conclusion - This finding is in concurrence with published data and, overall, supports the significance of determining the numbers of lymphocyte subpopulations in all patients with conotruncal anomalies, whether carrying 22q11.2 deletion or not.

Key words: Conotruncal anomalies ▪ Deletion 22q11.2 ▪ Lymphocytes

Introduction

Conotruncal anomalies are characterized by defects of the conotruncal septum and include ventricular septal defect with „riding" aorta, tetralogy of Fallot, syndrome of absent pulmonary artery valves, double outlet right ventricle (DORV), transposition or malposition of great vessels and truncus arteriosus (Figure 1). Syndrome of 22q11.2 deletion (formerly Di George syndrome) consists of conotruncal cardiac anomalies, hypoparathyroidism, growth retardation, characteristic facial dysmorphia (micrognathia, short philtrum, hypertelorism, epicanthus, antimongoloid eye position, earlobe deformities), as well as immune system defects brought about by thymic hypoplasia/aplasia (Figure 2). It is a maldevelopment of structures origina-

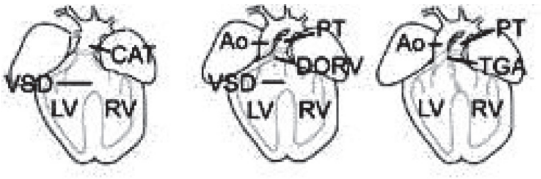

Figure 1 *Conotruncal defects: Truncus arteriosus (left), double outlet right ventricle (DORV, middle), transposition of great arteries (TGA, right). VSD, ventricular septal defect; RV, right ventricle; LV, left ventricle; Ao, aorta; PT, pulmonary trunk (Source: Kelly RG. An introduction to outflow tract development. European Society of Cardiology 2009; web presentation)*

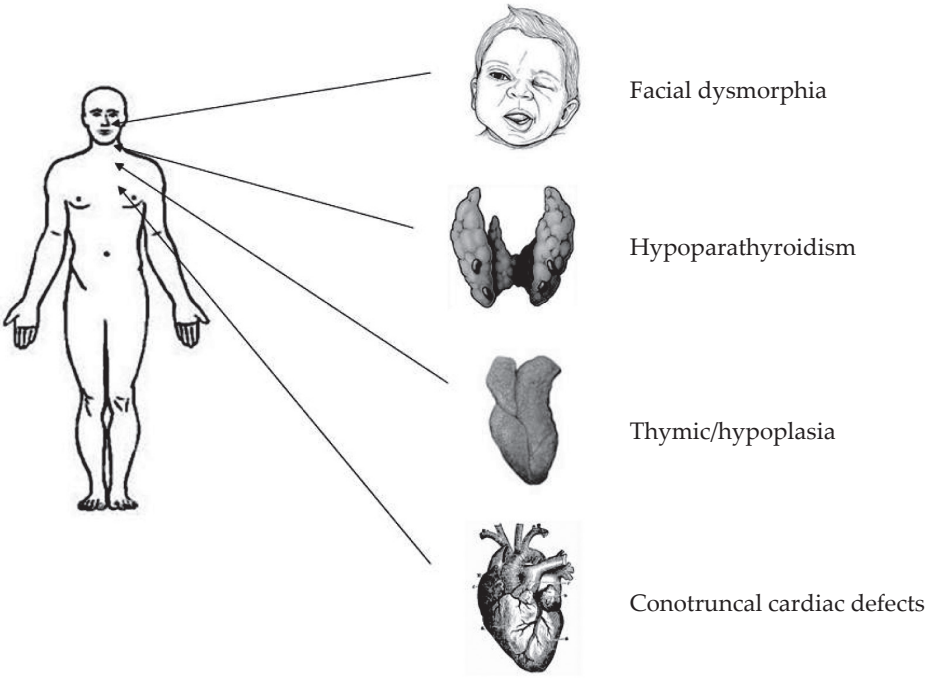

Figure 2 *Most important developmental defects in 22q11.2 deletion syndrome*

ting in the 3rd and 4th branchial arches. The estimated incidence of the 22q11.2 deletion syndrome is about 1 in 3000 live births (1). Deletion 22q11.2 is most often (>95%) a de novo occurrence, but it may also be inherited in autosomal dominant fashion (2). The great phenotypic diversity of patients affected by this syndrome does not correlate with the size of the deleted segment (3). In over 90% of patients exhibiting the characteristic phenotype, 22q11.2 deletion is readily detected by fluorescent in situ hybridization (2). In the remaining ≥10% of patients, 22q11.2 deletion is not detected, and the syndrome is assumed to be the result of other genetic anomalies and/or exposure in utero to alcohol or isotretinoin (4, 5, 6).

A broad spectrum of immune system defects has been described in patients with 22q11.2 deletion syndrome (7, 8). The characteristic defect is a mild to moderate decrease in T lymphocyte number, resulting from thymic hypoplasia (9). Patients that completely lack a thymus show a severe T cell immunodeficiency, but they comprise no more than 1% of all patients, since most of those without a macroscopically visible thymus do possess microscopic islets of thymic epitelial cells in the neck and/or mediastinum, allowing a certain number of T cells to mature (9). B cell numbers are normal in most patients, although defects in immunoglobulin production are encountered (in as many as 43% of patients in one study) (10), probably as a result of the reduced number and/or function of T helper lymphocytes, necessary for mounting the humoral immune response directed at T-dependent antigens. Similar to the anatomical and functional changes, susceptibility to infection in patients with 22q11.2 deletion syndrome is highly variable and does not always correlate with numbers of lymphocytes and their subpopulations. This is partly due to the fact that many of these patients have functional T cell defects, dictating the necessity of assessment of T cell proliferation in response to mitogen or antigen stimulation, in order to fully appreciate the status of the immune system. Immune system defects in 22q11.2 deletion syndrome usually tend to be mitigated with the passage of time. In addition to infection susceptibility, these patients have a somewhat higher incidence of autoimmune disorders compared to the general population, indicating possible defects in the regulatory components of the immune system (9). The objective of this work was to examine the abundance of principal lymphocyte subpopulations in the peripheral blood of patients with conotruncal cardiac anomalies.

Patients and Methods

Patients

A total of 19 patients (8 boys and 11 girls), aged from 2 months to 16 years, treated for conotruncal cardiac anomalies in the University Children's Hospital, were recruited in the study at the time of the appointed control examination in the period 2008-2009. Seven of 19 patients showed the characteristic facial dysmorphia, two had non-specific changes of the facies, while in ten patients no facial dysmorphia could be observed. In three patients, signs of hypoparathyroidism were present. Two patients had a family history of 22q11.2 deletion. In two children, immunodeficiency was manifested by severe and/or recurrent bacterial or fungal infections.

By fluorescent in situ hybridization (FISH), 22q11.2 deletion was found in 8 patients (including the two patients with clinically manifest immunodeficiency), while in 7 patients the deletion was shown to be absent. In the remaining 4 patients, the analysis was not performed.

Immunofluorescence/flow cytometry

A quantity of 2 ml of peripheral blood

was drawn from each patient in a tube with ethylenediaminetetraacetate (EDTA) as anticoagulant. After the determination of total leucocyte number and lymphocyte percentage by an automated blood counter, the blood was diluted so that 100 μL of the suspension contained 1-10 x109/L leucocytes. The blood was then incubated with appropriate volumes of commercial solutions of monoclonal antibodies anti-CD45, anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD16, anti-CD56, anti-HLA-DR, conjugated with fluorescein-isothiocyanate, phycoerythrin or peridinin-chlorophyll-protein (Beckton-Dickinson, USA), according to the manufacturer's instructions. After incubation, red blood cells were lysed by ammonium-chloride solution, after which the cells were washed in physiological saline solution and centrifuged at 500 g. The samples were subsequently analyzed by flow cytometer (BD FACSCalibur, Beckton-Dickinson, USA) in order to determine the abundance of cell subpopulations, defined by the examined molecular markers within the lymphocyte population, determined by the side scatter of laser light beam and expression intensity of CD45 (CD45highSSClow). Absolute numbers of lymphocyte subpopulations were calculated based on the known total leucocyte number and relative number of lymphocytes.

Cell numbers belonging to the respective subpopulations were compared to the reference range determined by values found in healthy persons of appropriate age (average ± 2 SD) (12).

Results

1. Absolute lymphocyte numbers

In the group of patients with detected 22q11.2 deletion (N=8), lymphopenia was found in three patients, in four patients the lymphocyte number was within the reference range, while one patient had lymphocytosis. A similar distribution was noted in the group where 22q11.2 deletion was not detected (N=7), with two patients having lymphopenia, four showing normal lymphocyte numbers and one patient exhibiting lymphocytosis. Lymphopenia was also found in two of four patients in whom the presence of 22q11.2 deletion was not examined, while one patient had normal lymphocyte number and lymphocytosis, respectively (Table 1).

2. Numbers of T Lymphocytes (CD3+)

Of patients with the documented presence of 22q11.2 deletion, one half (4/8) had T lymphocyte numbers below the lower margin of age-respective reference range, including one of the two patients with clinically manifest immunodeficiency, while the other half (4/8) had T lymphocyte numbers within the reference range. In the group without 22q11.2 deletion, 3/7 patients had a reduced number of T lymphocytes, 3/7 had a number within the reference range, while 1/7 had an elevated number of T lymphocytes. Of the two patients in whom the presence of 22q11.2 deletion was not sought (N=4), one had elevated and one reduced T lymphocyte numbers, respectively (Table 1).

3. CD4/CD8 Ratio

The ratio of T helper/regulatory cells to cytotoxic T cells (CD4/CD8) is one of the most sensitive parameters of altered funcional state of the immune system. Five of eight patients in the group with 22q11.2 deletion had CD4/CD8 ratio within the reference range, while 1/8 had a reduced, and 2/8 an elevated ratio. Similarly, in the group without 22q11.2 deletion, 5/7 patients had a normal and 2/7 an increased CD4/CD8 ratio. Among the patients in whom the presence of the deletion was not examined, 2/4 had a normal, and 1/4 reduced and elevated ratio, respectively (Table 1).

4. Number of activated T lymphocytes (CD3+HLA-DR+)

T lymphocytes that express on their cell surface the molecules of the class II of the major histocompatibility complex (MHC-II; HLA-DR) are activated T lymphocytes, since T lymphocytes do not constitutively express MHC II molecules. The number of CD3+HLA-DR+ lymphocytes was, in the group of patients with 22q11.2 deletion, within the reference range in 6/8 patients, while one patient had a reduced and one an elevated number, respectively. Likewise, in patients without 22q11.2 deletion, 5/7 had a normal and 2/7 a reduced number of CD3+HLA-DR+ lymphocytes (Table 1).

5. Number of B lymphocytes (CD19+)

In the group of patients with 22q11.2 deletion, 6/8 had B lymphocyte numbers within the reference range, while 2/8 had a reduced number of B lymphocytes compared to the age-respective reference range. In the group without 22q11.2 deletion, 5/7 patients had a number of B lymphocytes within the reference range, while 1/7 had a reduced and one an elevated number, respectively. In patients where the presence of 22q11.2 deletion was not sought, 3/4 had a normal, and 1/4 an elevated number of B lymphocytes (Table 1).

6. Number of NK cells (CD3-CD16/CD56+)

Seven of eight patients with confirmed 22q11.2 deletion had a NK cell number within the age-respective reference range, while one had an elevated number. In the group without 22q11.2 deletion, 4/7 patients had NK cell numbers within the reference range, 2/7 had a reduced, and 1/7 an elevated number of NK cells. Of the patients in whom 22q11.2 deletion was not examined, 1/4 had a reduction, 1/4 a normal number, and 2/4 an increase in NK cells (Table 1).

Discussion

The great variability of T lymphocyte numbers in our series of patients with conotruncal anomalies is in keeping with previously found data (2), as is the fact that in approximately half the patients (9/19) the T lymphocyte number was below the lower limit of the age-respective reference range. Interestingly, the proportion of patients with reduced T lymphocyte numbers was quite similar in patients in whom 22q11.2 could not be

Table 1 Populations of peripheral blood lymphocytes in pediatric patients with conotruncal anomalies

Del 22q11 (FISH)	Patients (n)	Relation to healthy population	Number of patients					
			Lymphocytes n	$CD3^+$	CD4/CD8	$CD3^+$ HLA-DR^+	$CD19^+$	$CD3^-$ CD16/$CD56^+$
+	8	Decrease	3	4	1	1	2	0
		Reference range	4	4	5	6	6	7
		Increase	1	0	2	1	0	1
-	7	Decrease	2	3	0	2	1	2
		Reference range	4	3	5	5	5	4
		Increase	1	1	2	0	1	1
?	4	Decrease	2	2	1	2	0	1
		Reference range	1	0	2	2	3	1
		Increase	1	2	1	0	1	2

demonstrated by FISH, although the number of patients was far from sufficient for parametric tests of statistical significance. It is also of note that none of our patients had T lymphocyte numbers lower than 3 SD below the average value for the appropriate age group, which is in accordance with literature data, indicating that this has been found in less than 1% of patients (9, 13).

The increased number of T lymphocytes that was found in one patient in the 22q11.2 deletion group, one patient without deletion 22q11.2, and two in whom deletion was not examined (overall 4/19 patients) might be a compensatory phenomenon resulting from impaired T lymphocyte function and/or narrowed repertoire of specificity. The reason may lie in the inadequate course of positive and negative selection processes taking place in the hypoplastic thymus or scattered islets of thymic tissue. An increased or decreased number of activated T cells expressing on their surface the molecules of class II of the major histocompatibility complex could also be a compensatory phenomenon (14). Variations in the CD4/CD8 ratio are difficult to interpret in the context of immunodeficiency in 22q11.2 syndrome, since this parameter is also very sensitive to the action of a multitude of factors that ephemerally influence the functional state of the immune system, such as infection by various microorganisms or concomitant chronic disorders with an inflammatory component (15, 16).

The increase in B lymphocyte numbers in two of the nineteen patients might be yet another compensatory phenomenon; on the other hand, since increase in B lymphocyte number was not found in any of the patients harboring 22q11.2 deletion, in those two patients with conotruncal anomalies and an elevated number of B lymphocytes, some other biological factors, innate or acquired, may be in play, leading to the observed increase in B cell numbers. Similar holds true for patients with an increased or decreased number of NK cells. A decrease in B lymphocyte numbers, found in two patients with 22q11.2 deletion, may arise from the inadequate function of helper T lymphocytes, and consequent impairment of cognate B-T cell interactions, necessary for mounting immune responses toward thymus-dependent antigens. The maturation of B cells may also be somewhat hampered due to less than adequate quantities of stimulating cytokines produced by the Th2 subpopulation of CD4+ lymphocytes (17). It is worth noting that simple counting of the lymphocytes belonging to principal subpopulations (T and B lymphocytes and NK cells) is not sufficient for a complete insight into the state of the immune system, and this data should be complemented by lymphocyte functional investigations (11).

Finally, it should also be noted that both our patients with clinically manifest immunodeficiency had 22q11.2 deletion detected. Of these two, one patient died of sepsis that did not respond to any antibiotic therapy, while the other patient suffered from frequent and severe respiratory infections. This patient had a reduced number of CD3+ lymphocytes, while the number of CD3+ cells in the deceased child was within the age-specific reference range

Conclusion

Taken as a whole, the obtained data indicate that in about one half of the patients with conotruncal anomalies, T lymphocyte numbers were significantly lower than those found in the healthy population. The data also indicate the great diversity of abundance of principal lymphocyte subpopulations in patients with conotruncal anomalies. Bearing in mind the importance of determining the exact functional state of the immune system in order to optimally follow these patients up and, upon necessity, undertake appropriate

measures of prevention and/or treatment, the enumeration of lymphocyte subpopulations is indicated in all patients with conotruncal anomalies, whether carrying 22q11.2 deletion or not.

Conflict of Interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

Literatura

1. Sullivan KE, McDonald-McGinn D, Driscoll DA, Emanuel BS, Zackai EH, Jawad AF. Longitudinal analysis of lymphocyte function and numbers in the first year of life in chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). Clin Diagnostic Lab Immunology. 1999;6(6):906-11.

2. Le Deist F, Fischer A. Primary T cell immunodeficiencies. In: Rich RR, Fleisher TA, Shearer WT, Schroeder Jr HW, Frew AJ, Weyland CM, editors. Clinical immunology: principles and practice. 3rd edition. Philadelphia: Elsevier Limited. 2008. p.531-51.

3. Carlson C, Sirotkin H, Pandita R, Goldberg R, McKie J, Wadey R et al. Molecular definition of 22q11 deletions in 151 velocardio-facial syndrome patients. Am J Hum Genet. 1997;61:620-9.

4. Fukushima Y, Ohashi H, Wakui K, Nishida T, Nakamura Y, Hoshino K et al. DiGeorge syndrome with del(4) (q21.3q25): possibility of the fourth chromosome region responsible for DiGeorge syndrome. Am J Hum Genet. 1992;51:A80.

5. Greenberg F, Elder FF, Haffner P, Northrup H, Ledbetter DH. Cytogenetic findings in a prospective series of patients with DiGeorge anomaly. Am J Hum Genet. 1988;43:605-11.

6. Monaco G, Pignata C, Rossi E, Mascellaro O, Cocozza S, Ciccimarra F. DiGeorge anomaly associated with 10p deletion. Am J Med Genet. 1991;39:215-6.

7. Barrett DJ, Ammann AJ, Wara DW, Cowan MJ, Fisher TJ, and Stiehm ER. Clinical and immunologic spectrum of the DiGeorge syndrome. J Clin Lab Immunol. 1981;6:1-6.

8. Bastian J, Law S, Vogler L, Lawton A, Herrod H, Anderson S et al. Prediction of persistent immunodeficiency in the DiGeorge anomaly. J Pediatr. 1989;115:391-6.

9. McLean-Tooke A, Barge D, Spickett GP, Gennery AR. Immunologic defects in 22q11.2 deletion syndrome. J Allergy Clin Immunol. 2008;122(2):362-7e4.

10. Finocchi A, Di Cesare S, Romiti ML, Capponi C, Rossi P, Carsetti R et al. Humoral immune responses and CD271 B cells in children with DiGeorge syndrome (22q11.2 deletion syndrome). Pediatr Allergy Immunol. 2006;17:382-8.

11. Lima K, Abrahamsen TG, Foelling I, Natvig S, Ryder LP, Olaussen RW. Low thymic output in the 22q11.2 deletion syndrome measured by CCR9+CD45RA+ T cell counts and T cell receptor rearrangement excision circles. Clin Experimental Immunology. 2010;161:98-107.

12. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997;130(3):388-93.

13. Ryan AK, Goodship JA, Wilson DI, Philip N, Levy A, Seidel H et al. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet 1997; 34:798–804.

14. Ko HS, Fu SM, Winchester RJ, Yu DT, Kunkel HG. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells. J Exp Med. 1979;150:246-55.

15. Chen ZG, Li M, Ji JZ, Chen H, Chen YF, Chen FH. Significance of changes of T lymphocytes subsets in children with infectious mononucleosis and the effects of different interventions. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2009;23(2):118-20.

16. Liu DY, Zhao HM, Zhao N, Lu C, Lu AP. Effect of Bawei Xilei powder on CD3, CD4, CD8 T-lymphocytes of rats with ulcerative colitis. Zhongguo Zhong Yao Za Zhi. 2008;33(11):1301-4.

17. Abbas AK, Lichtman AH. Cellular and molecular immunology. 5th edition. Philadelphia: Saunders; 2003.