

Long-term assessment of T-cell populations in DiGeorge syndrome

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Background: Patients with DiGeorge syndrome present with a broad range of T-cell deficiency. Partial DiGeorge syndrome (pDGS) is a preferred designation for patients with detectable T-cell function. Among immunology experts, there is no uniform opinion on the necessity of T-cell precautions for pDGS patients. Few studies have addressed the natural course of their immune function over time.

Objective: The objective of this study was to describe the natural history of immune parameters in pDGS.

Methods: We reviewed the medical records of 45 pDGS patients. Peripheral blood T-cell subsets counts and percentages were recorded at 1, 6, 12, 18, 24, 30, 48, 60, 72, 96, and 120 months of age, and the rates of change of T-cell measurements over the follow-up period (slopes) were calculated for each individual. Humoral immunity was evaluated by quantification of immunoglobulins and by testing antibody titers to recall antigens.

Results: T-cell subsets counts from pDGS patients were generally lower than those of age-matched normal populations but were not severely depressed (ie, CD4⁺ T-cell percentage less than 15%). The median of the slopes for CD3⁺, CD4⁺, and CD8⁺ T-cell percentages were -0.7%, -0.8%, and -0.1%/month, respectively, in the first year of age and 0.1%/month for each subpopulation from 12 to 120 months of age. Lymphoproliferative responses to phytohemagglutinin were adequate at all ages. Immunoglobulin deficiencies or inadequate production of specific antibodies were not detected. **Conclusions:** In our pDGS patient cohort, a significant deterioration of T-cell number or function did not occur over time. Clinical implications of this finding include the possibility of discontinuing T-cell deficiency precautions and frequency of reevaluations of pDGS patients with stable and adequate immune function. (*J Allergy Clin Immunol* 2003;111:573-9.)

Key words: Immunodeficiency, DiGeorge syndrome, 22q11 deletion, velocardiofacial syndrome, T-cell counts

DiGeorge syndrome (DGS) is a common primary immunodeficiency, with an estimated frequency of 1 to 4000 live births. The clinical diagnosis of DGS relies on the presence of conotruncal cardiac defects, hypocalcemia, cleft palate, characteristic facies, and hypoplastic

Abbreviations used

ConA:	Concanavalin A
DGS:	DiGeorge syndrome
FISH:	Fluorescent in situ hybridization
pDGS:	partial DiGeorge syndrome
PTH:	Parathyroid hormone
PWM:	Pokeweed mitogen
SI:	Stimulation index
URI:	Upper respiratory infection

thymus.^{1,2} The molecular defect has not been identified yet; however, more than 90% of DGS cases are associated with a deletion in the chromosome 22q11.2 region, detected by fluorescent in situ hybridization (FISH).³ Recently, it has been shown that mice deficient of the *tbox1* gene, contained within this critical region, reproduced the cardiac anomalies seen in this syndrome.⁴

DGS patients present with diverse degrees of T-cell deficiency, ranging from an absence of T cells to almost normal T-cell counts and percentages. The presentation of DGS with a very low T-cell percentage (less than 1% to 2%) is commonly referred to as complete DGS; in contrast, patients with partial DiGeorge syndrome (pDGS) have greater T-cell percentages and a broad range of lymphoproliferative responses. The CD4⁺ and CD8⁺ T-cell receptor V repertoires are apparently conserved in blood and thymic lymphocytes from pDGS patients.^{5,6} Complete DGS is uncommon and usually presents with severe infections caused by opportunistic pathogens. Bone marrow transplant or thymus transplant has been shown to restore the immune function and therefore decrease the risk of fatal infections.^{7,8} Early studies on the immune function of pDGS reported an unpredictable course, supporting the recommendation for regular immunologic evaluation of these patients for an indefinite period of time. These studies had small numbers of patients and were not regularly followed after 1 year of age.^{9,10} More recently, Sullivan et al¹¹ reported on T-cell counts and function in 19 patients with a chromosome 22q11 deletion, followed from birth to 1 year of age. Lymphoproliferative responses to PHA were largely preserved. The averages of T-cell counts at the newborn period were lower than those of normal control subjects but were not significantly different at 1 year of age. They also showed that children with cardiac anomalies but without DGS did not have low T-cell counts. Subsequently, a study of 195 patients with chromosome 22q11 deletion analyzed lymphocyte counts in 4 age brackets (0

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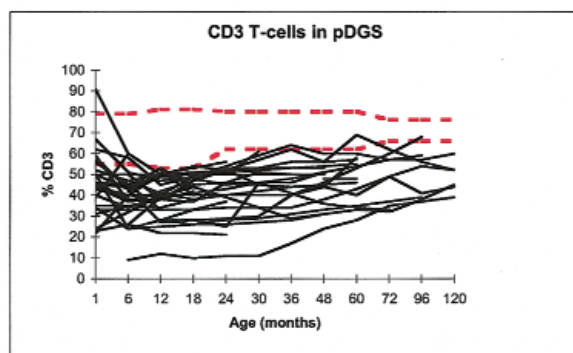
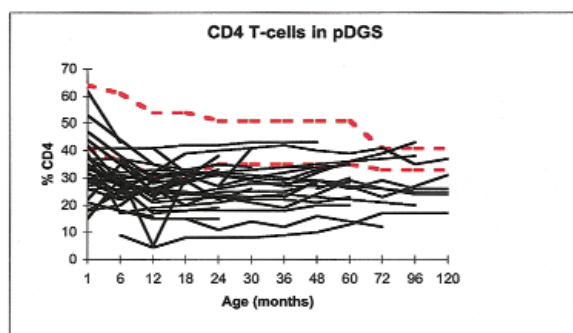
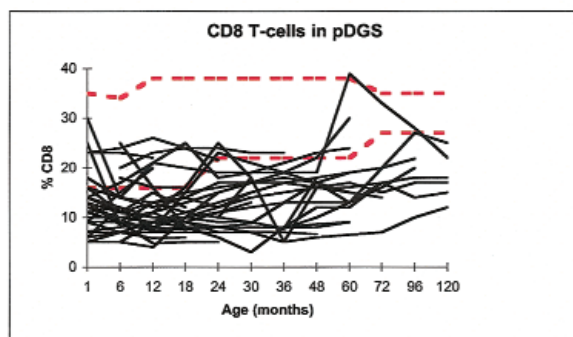
a**b****c**

FIG 1. T-cell percentages of pDGS patients followed over time. Each line describes 1 patient. Broken lines represent upper and lower limits for normal values (from Denny et al¹⁴). **a**, CD3⁺ T cell; **b**, CD4⁺ T cell; **c**, CD8⁺ T cell.

to 6 months, 6 to 38 months, 38 to 109 months, and more than 109 months of age). Although mean T-cell counts in each group were lower than values of normal control children, there was a slower decline over time.¹² There are few published data addressing lymphocyte counts and function in children with DGS. This information is necessary to define immuno-deficiency precautions (ie, live vaccine administration) and follow-up studies for pDGS patients. We reviewed the medical records of 45 patients with diagnosis of pDGS to define the natural history of their immune function.

TABLE I. Characteristics of pDGS patients at Texas Children's Hospital, 1991-2000

	n = 45 (%)
Gender	
Male	20 (44.4)
Female	25 (55.5)
Ethnicity	
Hispanic	18 (40.0)
White	9 (20.0)
Black	5 (11.1)
Asian/Indian	1 (2.2)
No data	12 (26.7)
Selected clinical features	
22q11.2 deletion	30* (96.7)
Cardiac defect	45 (100.0)
Calcium levels	
Hypocalcemia	14 (31.1)
Neonatal hypocalcemia	21 (46.7)
No hypocalcemia	10 (22.2)
Recurrent URI	11 (24.4)

*31 patients were tested.

METHODS

Patients

We obtained the medical records of 45 children with diagnosis of DGS followed from 1991 to 2000 by the Service of Allergy and Immunology, Texas Children's Hospital, Houston, Texas. None of the patients presented with complete DGS. We recorded the presence of conotruncal cardiac defects, hypocalcemia, and recurrent infections. Recurrent upper respiratory infection (URI) was defined as 6 or more episodes of sinusitis or otitis media in 1 year. We analyzed peripheral blood T-cell subsets counts and percentages that were available at 1, 6, 12, 18, 24, 30, 48, 60, 72, 96, and 120 months of age. We included the evaluations done between 1 month before or after each determined time point.

Laboratory tests

The FISH studies for chromosome 22q11.2 deletion were performed by the Kleberg Genetic Clinic at Baylor College of Medicine.¹³ Immunophenotyping and T-cell proliferation tests were done by the Clinical Immunology Laboratory at Texas Children's Hospital. Immunophenotyping was performed by flow cytometry (Coulter Epics XL; Coulter Corporation, Hialeah, Fla). Age-matched normal values were obtained from Denny et al.¹⁴ Lymphocyte proliferation tests were measured in response to the following mitogens: PHA, pokeweed mitogen (PWM), and concanavalin A (Con A). Immunoglobulin levels and specific antibody titers to diphtheria, tetanus toxin, *Haemophilus influenza*, and pneumococcal serotypes 3, 7, 9, and 14 were performed at the Specialty Laboratories Inc (Santa Monica, Calif).

Statistical analysis

T-cell subsets counts and percentages available for each patient were recorded in a worksheet (Excel 5.0; Microsoft Inc, Redmond, Wash), and graphs for each T-cell subset were generated. We divided the data regarding age before and after 12 months, considering the variability previously reported in infants with pDGS.¹¹ The medians of cell counts and percentages were calculated for each time interval. To demonstrate whether changes occur over time, differences of cell counts and percentages over time (slopes) were calculated for each patient who had more than 1 immunologic evaluation by using all the available time points. Wilcoxon rank sum test was used to evaluate whether the slope samples were different from zero.

TABLE II. T-cell subsets medians from 1 to 120 months of age in patients with pDGS*

Age in mo	CD3 ⁺ T cells		CD4 ⁺ T cells		CD8 ⁺ T cells	
	(%) (range) Normal values	(cell/mm ³) (range) Normal values	(%) (range) Normal values	(cell/mm ³) (range) Normal values	(%) (range) Normal values	(cell/mm ³) (range) Normal values
1 (n = 32)	45 (23-91)	1485 (651-5964)	33 (15-62)	1178 (286-4109)	12 (3-30)	338 (72-2019)
6 (n = 26)	55-78 40 (9-60)	2070-6540 1566 (440-4186)	41-64 28 (9-44)	1460-5116 1094 (440-2735)	16-35 11 (5-24)	650-2450 436 (24-1240)
12 (n = 25)	45-79 39 (12-52)	2280-6450 1660 (279-2573)	36-61 26 (5-41)	1690-4600 974 (105-1788)	16-34 11 (4-26)	720-2490 392 (189-1273)
18 (n = 16)	53-81 43 (10-54)	1460-5440 1228 (497-3900)	31-54 29 (8-33)	1020-3060 785 (368-3000)	16-38 11 (7-25)	570-2230 376 (48-1082)
24 (n = 15)	53-81 50 (21-56)	1460-5440 1738 (560-3018)	31-54 27 (11-38)	1020-3060 949 (332-2266)	16-38 11 (5-25)	570-2230 500 (213-1305)
30 (n = 6)	62-80 45 (11-61)	1610-4230 1037 (245-1600)	35-51 27 (8-41)	900-2860 662 (164-1076)	22-38 16 (3-18)	630-1910 332 (63-485)
36 (n = 13)	62-80 44 (28-64)	1610-4230 1349 (119-2678)	35-51 24 (12-34)	900-2860 707 (93-1721)	22-38 14 (5-23)	630-1910 489 (29-1441)
48 (n = 13)	62-80 47 (24-60)	1610-4230 1013 (423-2928)	35-51 29 (10-43)	900-2860 631 (179-1570)	22-38 17 (6-19)	630-1910 335 (89-1425)
60 (n = 12)	62-80 47 (28-69)	1610-4230 857 (445-3092)	35-51 27 (13-39)	900-2860 559 (176-1206)	22-38 14 (9-39)	630-1910 227 (155-1612)
72 (n = 5)	62-80 35 (32-57)	1610-4230 847 (448-2132)	35-51 23 (12-41)	900-2860 505 (179-1536)	22-38 15 (7-17)	630-1910 241 (203-548)
96 (n = 7)	66-76 55 (41-68)	1400-2000 939 (656-2142)	33-41 26 (20-43)	700-1100 513 (419-1443)	27-35 20 (14-28)	600-900 466 (221-725)
120 (n = 6)	66-76 48 (39-60)	1400-2000 691 (432-1180)	33-41 25 (17-31)	700-1100 389 (185-676)	27-35 17 (12-25)	600-900 202 (160-314)

*n denotes the number of available data at each time point. Normal values are taken from Denny et al.¹⁴

RESULTS

Thirty-one patients had FISH test results for the chromosome 22q11.2 deletion, and 30 patients had positive results for the deletion. The rest of the patients were diagnosed clinically, on the basis of the presence of at least 3 of the following 4 features: conotruncal heart defect, characteristic facies, hypocalcemia, and decreased T-cell counts. More of the patients were female (25 of 45, 55%) and of Hispanic ethnicity (18 of 45, 40%). All the patients presented with a conotruncal cardiac defect (45 of 45), and 77% of the patients had at least 1 episode of hypocalcemia. Eleven patients had a history of recurrent URIs including sinusitis and otitis media. One patient presented with vitiligo (Table I).

Median cell counts and percentages of T-cell subsets at different ages are shown in Table II. The median values of CD3⁺ T-cell percentages of all patients were below normal range, although we did not find a significant decrease over time (Fig 1, A). Median CD3⁺ T-cell counts decreased from 1485 cells/mm³ at 1 month of age to 691 cells/mm³ at 10 years of age (Table II). Most patients maintained their CD4⁺ T-cell percentages between 15% and 35% (Fig 1, B). Only 6 patients had their CD4⁺ T-cell counts at normal levels (not shown). One patient persisted with CD4⁺ T-cell percentage around 15% and CD4⁺ T-cell count below 200 cells/mm³ (not shown). CD8⁺ T-cell percentages ranged between 5% and 25% at most time points (Fig 1, C). We analyzed

TABLE III. Changes (slope) in T-cell subsets over time in pDGS patients

Changes in	Age: 1-12 months old			Age: 12-120 months old		
	Slope median (n = 31)	Slope range	P value	Slope median (n = 31)	Slope range	P value
CD3 T cells						
Percentage/mo	−0.7	(−6.2 to 1.0)	.01*	0.1	(−2.0 to 1.5)	.001*
(cell/mm ³)/mo	9.0	(−786 to 384)	>.1	−4.2	(−212 to 156)	>.1
CD4 T cells						
Percentage/mo	−0.8	(−4.4 to 1.2)	.000*	0.1	(−1.5 to 4.1)	.002*
(cell/mm ³)/mo	−7.6	(−528 to 244)	>.1	−3.1	(−222 to 125)	.001*
CD8 T cells						
Percentage/mo	−0.1	(−4.1 to 1.2)	.03*	0.1	(−0.5 to 0.9)	.005*
(cell/mm ³)/mo	−2.6	(−304 to 91)	>.1	−0.2	(−88 to 71)	>.1

*Wilcoxon test, statistically different from zero.

the rates of change of the T-cell subsets before and after 12 months of age, because pDGS patients may have an initial period of stabilization up to this age, as suggested previously.¹¹ Thirty-one patients who had more than one T-cell phenotyping study before 12 months of age and 31 patients who had the same between 12 and 120 months of age were studied. The rate of change of CD3⁺, CD4⁺, and CD8⁺ T-cell percentages over time was −0.7%, −0.8%, and −0.1%/month, respectively, for the first year of age and 0.1% in each T-cell subset for 12 to 120 months of age (Table III). The difference of the rates of change of CD3⁺, CD4⁺, and CD8⁺ T-cell counts from zero did not reach statistical significance, except for CD4⁺ T-cell counts for the age period of 12 to 120 months, which was −3.1 cells/mm³/mo (Table III).

Adequate lymphoproliferative responses to mitogens (greater than 79,000 cpm) were found in 41 of 45 patients. The medians and ranges of the responses to PHA are shown in Table IV. Patients 25 and 43 had initial low proliferative response that increased to normal levels in subsequent follow-up evaluations. Patients 31 and 35 had lymphoproliferative responses below normal range, and their CD3⁺ T-cell percentages were 57% and 34%, respectively. Unfortunately, these patients were evaluated only in the newborn period and then were lost to follow-up.

Thirty-six patients had a determination of immunoglobulin levels at least once, and all had normal levels of IgG, IgA, IgM, except for 1 patient who had low IgA during the neonatal period and another patient with low IgM (Table IV). We were unable to follow these patients to determine whether the deficiency was transient or permanent. Twenty-five of 45 patients were tested and had adequate protective antibody levels against diphtheria, tetanus toxoid, and at least 1 pneumococcal serotype (Table IV).

Six of the 11 patients who presented with recurrent respiratory infections had low CD3⁺ T-cell counts. Two of these 11 patients had normal CD3⁺ T-cell counts, and 3 had initial low T-cell counts that became normal in the follow-up evaluation. We were unable to find any statistical association between presence of recurrent infections and decreased T-cell counts (data not shown).

DISCUSSION

We reviewed the immunologic evaluations of 45 pDGS patients to define the long-term natural history of their T-cell deficiency. They were predominantly of Hispanic origin, reflecting the ethnic distribution of our referral population.¹⁵ None of our patients had a diagnosis of complete DGS, in agreement with the reported rarity of this severe presentation in other published DGS patient series.^{9-11,16} All tested patients except one had the chromosome 22q11.2 deletion. It is possible that some of the patients who were not tested are negative for this chromosomal defect. Kornfeld et al¹⁶ analyzed the initial immunologic evaluation of 16 patients with clinical diagnosis of pDGS in regard to the presence of the 22q11.2 deletion. No clinical or immunologic differences were found between the 2 groups.

Almost 47% of our patients presented with hypocalcemia only during the neonatal period and did not require calcium supplements later. The pathogenesis of the hypocalcemia in DGS is explained by hypoparathyroidism as a result of hypoplasia of the parathyroid glands. These patients may later develop transient or permanent hypocalcemia if they are in stress situations, such as surgical procedures or severe infections.¹⁷

Previously, Barrett et al¹⁰ described 13 patients with pDGS and followed 5 of them longitudinally. Three of these 5 patients had low peripheral blood T-cell counts and percentages in the newborn period that improved in the following follow-up evaluations. The T-cell counts of the fourth patient declined from 3900 cells/mm³ at 3 months of age to 1000 cells/mm³ at 11 months of age, with decreasing lymphoproliferative responses to PHA. The fifth patient had poor lymphoproliferative response to PHA, although his T-cell counts increased from 798 cells/mm³ at 4 months of age to 1215 cells/mm³ at 7 months of age. The medians of the CD3⁺ T-cell counts and percentages from our pDGS patients were lower than those of normal age-matched populations. A decreasing trend across the time points were not observed in the percentage values; in contrast, median CD3⁺ T-cell counts decreased from 1660 cells/mm³ at 12 months of age to

TABLE IV. Mitogenic responses and immunoglobulin evaluations in pDGS*

Patient	PHA ($\times 1000$ cpm)	n (range)	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Specific antibody response
1	176	2 (87-264)	859	59	72	Present
2	175	1 (175)	554	71	55	Present
3	203	1 (203)	427	8	11	NA
4	88	6 (40-154)	1083	85	62	Present
5	180	5 (113-338)	833	16	88	Present
6	106	6 (78-197)	566	55	62	Present
7	126	5 (99-102)	NA	NA	NA	NA
8	166	2 (127-204)	937	56	116	Present
9	106	4 (99-112)	2026	368	228	Present
10	190	6 (139-228)	NA	NA	NA	NA
11	216	3 (112-250)	1059	116	77	Present
12	240	4 (204-265)	NA	NA	NA	NA
13	90	4 (86-109)	NA	NA	NA	NA
14	170	5 (110-256)	1097	96	75	Present
15	160	3 (98-200)	NA	NA	NA	NA
16	144	6 (89-273)	821	111	81	Present
17	137	3 (133-224)	701	86	112	NA
18	79	1 (79)	757	69	48	Present
19	133	5 (63-264)	1672	123	123	Present
20	123	7 (80-181)	1060	129	188	Present
21	161	3 (59-167)	799	232	164	Present
22	164	5 (123-233)	1058	143	125	Present
23	179	1 (179)	NA	NA	NA	NA
24	157	2 (148-165)	NA	NA	NA	NA
25	73	6 (23-95)	1022	215	88	Present
26	181	4 (117-284)	1290	329	105	Present
27	202	7 (119-246)	1349	110	126	Present
28	158	4 (111-310)	1279	160	131	Present
29	143	2 (87-198)	871	131	38	Present
30	142	2 (138-146)	651	169	141	NA
31	41	1 (41)	458	28	52	NA
32	140	4 (49-231)	1530	208	309	NA
33	170	3 (114-188)	1060	101	185	Present
34	176	4 (128-225)	1700	212	257	Present
35	65	1 (65)	NA	NA	NA	NA
36	181	3 (111-233)	518	27	90	NA
37	107	4 (27-142)	1212	165	72	Present
38	177	2 (166-187)	980	131	95	Present
39	136	3 (83-223)	678	54	45	NA
40	127	1 (127)	NA	NA	NA	NA
41	117	1 (117)	340	70	88	NA
42	89	3 (70-148)	1179	137	143	Present
43	76	2 (62-89)	643	62	76	NA
44	126	2 (114-138)	760	40	70	NA
45	142	5 (111-351)	1718	275	99	NA

NA, Not available.

*Median lymphoproliferative response to PHA, cpm, normal range 79-179; n = number of time points available. Shown immunoglobulin levels correspond to the last measurement available for each patient. Patient 3 immunoglobulin values were taken in the newborn period; they are within normal range for age.

691 cells/mm³ at 120 months of age. This finding was not surprising, because T-cell counts from normal children present a similar decrease.¹⁴ Medians of CD4⁺ T-cell counts and percentages were not decreased to immunodeficiency levels, defined as per guidelines for HIV infection (0 to 1 year of age, less than 1500 cells/mm³; 1 to 5 years of age, less than 750 cells/mm³; older than 5 years of age, less than 500 cells/mm³; less than 15% at all ages).¹⁸ Patients with lower T-cell counts

or with recurrent respiratory infections were evaluated more frequently and may be responsible in part for the lower median values; however, it should not affect the analysis of changes over time.

The median of the calculated slopes for the rate of change of T-cell subsets over time showed modest values, which may not be clinically significant, even though some of the values were statistically different from zero. The negative median of the slopes calculated for T-cell

percentages from 1 to 12 months may be explained by the decrease that occurs in normal children.¹⁴ The analysis done for time points from 12 to 120 months of age showed medians of 0.1% increase per month for the 3 subsets. The analysis of T-cell subsets counts slopes did not reach statistical significance, except for CD4⁺ T-cell counts from 12 to 120 months of age, with a median of -3.1 cells/mm³/mo. This small decline of T-cell absolute numbers may be clinically significant, and longer clinical and immunologic follow-up would be necessary to answer this question. These results support the hypothesis of long-term stability of the T-cell subsets and confirm the results from a previous study by Jawad et al,¹² which found no severe immunodeficiency and a slow decline of T-cell counts in patients with 22q11 deletion grouped in 4 age brackets up to 109 months of age.¹²

The proliferative responses to mitogens were adequate for all patients, which is consistent with the absence of an intrinsic defect in the T cells of pDGS patients. The maintenance of normal proliferative responses of T cells in pDGS contrasts with that of patients with infection (eg, HIV), in whom both T-cell numbers and T-cell function are decreased. Several reports have suggested the possibility of alterations in the humoral immune response of DGS patients. One study reported hypergammaglobulinemia in 10 of 13 DGS patients.¹⁹ Hypogammaglobulinemia in DGS has been described in several case reports, including specific polysaccharide deficiency in 3 members of a family affected with DGS.¹⁹⁻²² In addition, prevalence of IgA deficiency was reported to be increased in DGS.²³ Junker and Driscoll²⁴ evaluated 13 patients with DGS and reported normal immunoglobulin levels, except in 2 patients who had transient borderline low IgG level. These patients generated good antibody responses to different pathogens, including measles, rubella, and varicella. Kornfeld et al¹⁶ also analyzed immunoglobulin levels, finding mild increases or decreases in 5 of 13 patients tested, plus 1 patient with IgA deficiency. We found only 1 case of low IgA level, which was during the neonatal period, possibly transient. However, 11 of our patients presented with recurrent respiratory infections, which are commonly found in patients with antibody disorders. We are currently evaluating the possibility of a decreased polysaccharide antibody response in these patients. The association of DGS with autoimmune disorders is controversial. Jawad et al¹² described 4 patients with concomitant juvenile rheumatoid arthritis and 8 patients with idiopathic thrombocytopenic purpura in a cohort of 195 patients with chromosome 22q11 deletion. In addition, the association of DGS with Graves' disease has been reported in 2 patients.^{25,26} In our cohort, vitiligo in 1 patient was the only condition of probable autoimmune etiology.

In conclusion, our data show a cohort of children with pDGS who have maintained a relative stability of the cellular immunity, although the CD4⁺ T-cell data at 120 months may be predictive of further decline with age. Larger collaborative and prospective studies are needed to better define the natural history of the immune function in

pDGS patients, which would also help to establish more cogent recommendations concerning T-cell deficiency precautions and the frequency of immunologic evaluations currently required for the care of these patients.

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REFERENCES

1. Lischner HW, Huff DS. T-cell deficiency in DiGeorge syndrome. *Birth Defects: Original Article Series* 1975;11:16-21.
2. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. *Clin Immunol* 1999;93:190-7.
3. Driscoll DA, Budarf ML, Emanuel BS. A genetic etiology for DiGeorge Syndrome: consistent deletions and microdeletions of 22q11. *Am J Hum Genet* 1992;50:924-33.
4. Lindsay EA, Vitelli F, Su H, Morishika M, Huynh T, Pramparo T, et al. Tbx1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* 2001;410:97-101.
5. Pierdominici M, Marziani M, Giovannetti A, Oliva A, Rosso R, Marino B, et al. T cell receptor repertoire and function in patients with DiGeorge syndrome and velocardiofacial syndrome. *Clin Exp Immunol* 2000;121:127-32.
6. Conley ME, Beckwith JB, Mancer JFK, Tenckhoff L. The spectrum of the DiGeorge syndrome. *J Pediatr* 1979;94:883-90.
7. Markert ML, Boeck A, Hale LP, Kloster AL, McLaughlin TM, Batchvarova MN, et al. Transplantation of thymus in complete DiGeorge Syndrome. *N Engl J Med* 1999;341:1180-9.
8. Markert ML, Hummel DS, Rosenblatt HM, Schiff SE, Harville TO, Williams LW, et al. Complete DiGeorge syndrome: persistence of profound immunodeficiency. *J Pediatr* 1998;132:15-23.
9. 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.
10. Barrett DJ, Ammann AJ, Wara DW, Cowan MJ, Fisher TJ, Stiehm ER. Clinical and immunological spectrum of DiGeorge syndrome. *J Clin Lab Immunol* 1981;6:1-6.
11. 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 Diagn Lab Immunol* 1999;6:906-11.
12. Jawad FA, McDonald-McGinn DM, Zackai E, Sullivan KE. Immunologic features of chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *J Pediatr* 2001;139:715-23.
13. Berend SA, Spikes AS, Kashork CD, Wu JM, Daw SC, Scambler PJ, et al. Dual-Probe fluorescence in situ hybridization assay for detecting deletions associated with VCFS/DiGeorge Syndrome I and DiGeorge Syndrome II loci. *Am J Med Genet* 2000;91:313-7.
14. Denny T, Yoge R, Gelman R, Skuza C, Oleske J, Chadwick E, et al. Lymphocyte subsets in healthy children during the first five years of life. *JAMA* 1992;267:1484-8.
15. US Census Bureau. The Hispanic population: Census 2000 brief. Washington: US Census Bureau; 2001. p. 7.
16. Kornfeld SJ, Zeffren B, Christodoulou CS, Day NK, Cawkwell G, Good RA. DiGeorge anomaly: a comparative study of the clinical and immunologic characteristics of patients positive and negative by fluorescence in situ hybridization. *J Allergy Clin Immunol* 2000;105:983-7.
17. Greig F, Paul E, DiMartino-Nardi J, Saenger P. Transient congenital hypoparathyroidism: resolution and recurrence in chromosome 22q11 deletion. *J Pediatr* 1996;128:563-7.
18. Centers for Disease Control and Prevention. 1994 revised classification system for children with human immunodeficiency virus infection in children less than 13 years. *MMWR* 1994;43:1-19.
19. Muller W, Peter HH, Kallfelz HC, Franz A, Rieger CHL. The DiGeorge sequence. II. Immunological findings in partial and complete forms of the disorder. *Eur J Pediatr* 1989;19:96-103.
20. Etzioni A, Pollack S. Hypogammaglobulinemia in DiGeorge sequence. *Eur J Pediatr* 1989;150:144-5.
21. Mayumi M, Kimata H, Suehiro Y, Hosoi S, Ito S, Kuge Y, et al. DiGeorge

- syndrome with hypogammaglobulinemia: a patient with excess suppressor T cell activity treated with fetal thymus transplantation. *Eur J Pediatr* 1989;148:512-22.
22. Schubert MS, Moss RB. Selective polysaccharide antibody deficiency in familial DiGeorge syndrome. *Ann Allergy* 1992;69:231-8.
23. Smith CA, Driscoll DA, Emanuel BS, McDonald-McGinn DM, Zackai EH, Sullivan KE. Increased prevalence of immunoglobulin A deficiency in patients with the chromosome 22q11.2 deletion syndrome. *Clin Immunol Immunopathol* 1998;86:141-6.
24. Junker AK, Driscoll DA. Humoral immunity in DiGeorge syndrome. *J Pediatr* 1995;127:231-7.
25. Ham Pong AJ, Cavallo A, Holman GH, Goldman AS. DiGeorge syndrome: long-term survival complicated with Graves disease. *J Pediatr* 1985;106:619-20.
26. Kawamura T, Nomura I, Hanafusa M, Fujikawa R, Okubo M, Egusa G, et al. DiGeorge syndrome with Graves disease: a case report. *Endocr J* 2000;47:91-5.