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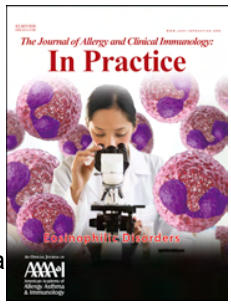
Accepted Manuscript



Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 Deletion Syndrome

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PII: S2213-2198(19)30278-8

DOI: https://doi.org/10.1016/j.jaip.2019.03.014

Reference: JAIP 2163

To appear in: *The Journal of Allergy and Clinical Immunology: In Practice*

Received Date: 4 December 2018

Revised Date: 6 March 2019

Accepted Date: 6 March 2019

Please cite this article as: Montin D, Marolda A, Licciardi F, Robasto F, Di Cesare S, Ricotti E, Ferro F, Scaioli G, Giancotta C, Amodio D, Conti F, Giardino G, Leonardi L, Ricci S, Volpi S, Baselli LA, Azzari C, Bossi G, Consolini R, Dellepiane RM, Duse M, Gattorno M, Martire B, Caterina Putti M, Soresina A, Plebani A, Ramenghi U, Martino S, Pignata C, Cancrini C, Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 Deletion Syndrome, *The Journal of Allergy and Clinical Immunology: In Practice* (2019), doi: https://doi.org/10.1016/j.jaip.2019.03.014.

Title (12 words): Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 Deletion Syndrome

Original proposed title (21 words)*: CD4^{+} naïve helper T cells and class-switched memory B cells are predictive of development of autoimmune cytopenia in 22q11.2 Deletion Syndrome.*

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Funding source: No external funding for this manuscript

Conflict of interest: The authors have indicated they have no potential conflicts of interest to disclose.

Abstract word count: 248

Manuscript word count: 2451

Abstract

Background: patients with 22q11.2DS may develop severe thrombocytopenic purpura (ITP) and hemolytic anemia (AIHA). There are no reliable predictors for the development of hematologic autoimmunity (HA) in these patients.

Objective: describe the peculiar B and T subpopulations defects in 22q11DS patients that have developed HA and test if these defects precede the development of HA.

Methods: We performed a case-control multicenter study. Patients with HA were compared with a control population of 22q11.2DS without hematologic autoimmunity (non-HA). A complete immunological evaluation was performed at diagnosis and at last follow-up including extensive T and B phenotype.

Results: Immunophenotype at last follow-up was available in 23 HA and 45 non-HA patients. HA patients had significantly decreased percentage of naïve $CD4^+$ cells, (26,8% vs 43,2%, *p=0,003)* and recent thymic emigrants (48,6% vs 80,5%, *p=0,046*)*;* decreased class-switched B cells (2,0% vs 5,9% *p=0,04)* and increased naive B cells (83,5% vs 71,4%, *p=0,02);* increased $CD16^+/56^+$ both in absolute number (312 vs 199, *p=0,009*) and percentage (20,0% vs 13,0%, *p=0,03*).

Immunophenotype was performed in 36 patients (11 HA and 25 non-HA) at diagnosis. Odds ratio (OR) of immune cytopenia were estimated for both CD4 naïve ≤30% (OR 14.0 *p=0.002*) and for SMB ≤2% (OR 44.0 *p=0.01*). The estimated survival curves reached statistical significance respectively *p=0.0001* and *p=0.002.*

Conclusion: Among 22q11.2DS patients those with HA have characteristic lymphocytes anomalies that appear considerably before HA onset. Systematic immunophenotyping of 22q11.2DS patients at diagnosis is advisable for early identification of patients at risk for this severe complication.

Highlights box:

1. *What is already known about this topic?*

- Some patients with 22q11.2DS may develop severe hematologic autoimmunity, it is impossible to predict which patients will develop this severe complication.

2. *What does this article add to our knowledge?*

- 22q11.2 DS patients with hematologic autoimmunity have peculiar B and T immunophenotype anomalies, the anomalies precede the onset of autoimmunity and may be used for risk stratification.

3*. How does this study impact current management guidelines?*

- Extensive B and T immunophenotyping is helpful in all 22q11.2 DS patients at diagnosis. Patients with CD4 naïve ≤30% or Switched Memory B cells ≤2% are at risk of developing severe hematologic autoimmunity.

Key words: autoimmune cytopenia**,** thrombocytopenic purpura, hemolytic anemia, 22q11.2 Deletion Syndrome, DiGeorge Syndrome, B immunophenotype, T immunophenotype, NK cells, CD4 naïve cells, switched memory B cells

Abbreviations:

22q11DS: 22q11.2 deletion syndrome

ITP: Idiopathic thrombocytopenic purpura

AIHA: hemolytic anemia

PID: primary immunodeficiencies

HA: hematological autoimmunity

IPINet: Italian Primary Immunodeficiency Network

cTFH: follicular helper T cells

Treg: regulatory T cells

RTE: Recent Thymic Emigrants

SMB: switched memory B cells

CVID: common variable immunodeficiency

MA: multivariate analysis

Introduction

Chromosome 22q11.2 deletion syndrome (22q11DS) is the most common microdeletion disease in humans, with a prevalence of 1:4000 to 1:6000 [1-2]; the deletion is most frequently associated to Di-George syndrome (Online Mendelian Inheritance in Man [OMIM] number, 188400) and velocardiofacial syndrome (OMIM number, 192430). Usually, the 22q11.2DS is caused by a de novo heterozygous deletion of approximately 2.5 Mb in length between low-copy repeats (LCR22) A and D. Less frequently, the syndrome is the result of deletions between LCR22 A and B, between B and D, or between C and D[3].

While the majority (90%) of patients share the same deletion the phenotypic expression of 22q11DS is widely variable, with over 190 features reported, among which the most frequent are congenital heart disease, velopharyngeal insufficiency and cleft palate, immune disorders, feeding difficulties, and hypocalcemia secondary to hypoparathyroidism[4].

The spectrum of immune deficiency ranges from nearly normal immune function to T-negative severe combined immunodeficiency (SCID). The majority of patients displays an intermediate form of immune disorder with T-cell lymphopenia more evident in the early age[5,6], decreased naïve and increased memory CD4+ cells, reduced T-cell receptor (TCR) repertoire[6,7] and impaired T-cell function[8]. Furthermore, patients with 22q11.2DS may exhibit hypogammaglobulinemia with defective response to pneumococcal polysaccharide[9,10], decreased CD27+ memory B cells[11,12], increased CXCR4+ circulating follicular helper T cells[12,13], low natural regulatory T cells [7,14], and deficient NK cytotoxic activity[15] .

Another hallmark of 22q11DS patients is the increased risk of autoimmune diseases such as thrombocytopenic purpura (ITP), hemolytic anemia (AIHA), thyroiditis and arthritis[4]. The development of autoimmunity is possibly a consequence of immune dysregulation as it has been proven in patients with other primary immunodeficiencies (PID). In particular T-cell PIDs are especially prone to develop hematological autoimmunity (HA)[16].

In this paper we have compared the immunophenotype of 22q11DS patients with HA (ITP and/or AIHA) with other 22q11DS patients without HA in order to understand if the former group has a distinctive immunological hallmark. Then, we tested the hypothesis that these immunological hallmarks may precede HA and therefore may be used as reliable predictors for this serious complication.

Methods

Italian Primary Immunodeficiency Network (IPINet) 22q11DS National Registry is a web-based application for the collection of clinical and laboratory data of 22q11DS patients[4]. Launched in May 2005, it consists in a secure database, compliant to International Conference on Harmonisation for Good Clinical Practice guidelines and European regulations. From 2006 to 2018, 16 Italian centers have registered retrospective and prospective data of 22q11DS patients. Clinical diagnosis was confirmed by fluorescence in situ hybridization 22 or molecular methods (multiplex ligation-dependent probe amplification 22 or comparative genomic hybridization microarray for 22q11.2 microdeletion).

In the context of the IPINet 22q11DS National Registry we selected all the patients with AIHA and/or ITP (HA group) and we compared them to a control population of

22q11DS patients without hematologic autoimmunity (non-HA group), randomly selected.

All del22q11 patients of the Italian registry are assigned a specific anonimous alfanumeric code when their data are entered in the DB. Using the alfanumeric code we extracted through a random electronic generator (www.random.org) 45 patient in the whole non-HA patients of the DB. The random assignment was made by a blinded examinator. Afterwards the alfanumeric code was used to detect the anagraphical data and caring physician of each patient.

Clinical and immunologic data sets (complete blood count, serum immunoglobulin levels, lymphocyte subsets) were extrapolated from the registry. A specific case report form was elaborate to confirm the registry data and to collect details about lymphocyte immunophenotype. All the patients provided written informed consent. The study was approved by the local ethics committees.

Lymphocyte specific population were defined as follows. Regarding T cells: $CD4^+CD45RA^+$ naïve helper T cells, $CD4^+CD45R0^+$ activated/memory helper T cells, $CD4^+CD45R0^+CXCR5^+$ circulating follicular helper T cells (cTFHs), $CD4^+CD25^+CD127^{low}FOXP3^+$ regulatory T cells (Treg) (all expressed as percentage of $CD4^+$ T cells); $CD4^+CD45RA^+CD31^+$ Recent Thymic Emigrants (RTEs) (expressed as percentage of naïve $CD4^+$ T cells); and $CD8^+CD27^+CD28^+$ naïve cytotoxic T cells (expressed as percentage of $CD8^+$ T cells). B cells subsets were subdivided as $CD38^{++}IgM^{++}$ transitional B cells, $CD27^-IgM^+IgD^+$ naïve B cells, $CD27^+IgM^+IgD^+$ IgM memory B cells, $CD27^+IgM^-IgD^-$ switched memory B (SMB) cells, and $CD21^{low}CD38^{low}$ B cells; all B cell subpopulations are expressed as percentage of total $CD19^+$ B cells.

The presence of peculiar lymphocyte subpopulation anomalies in the HA group, in the respect of the non-HA group, was tested firstly considering the immunophenotype at last follow-up and after that considering the immunophenotype at 22q11DS diagnosis. Any subpopulation reaching statistical significance in the comparison between HA and non-HA group at diagnosis was considered as a possible predictor of HA development in 22q11DS patients. Using cut-offs defined according to previous work on the stratification of patients with common variable immunodeficiency (CVID)[17,18] we estimated the odds ratio (OR) of HA development.

In order to define if the candidate predictors were independently associated with HA development a multivariate analysis (MA) was performed, in the MA analysis age and gender were included as well.

Finally Kaplan-Meier curves were calculated for all the candidate predictors using the same cut-offs[17,18].

Statistical analysis was performed using IBM SPSS Statistics 20.0 and GraphPad Prism 6.0. The differences between groups were analyzed using Mann-Whitney *U* test for continuous data, and Fisher's exact test for categorical data. All tests were two tailed and the significance was set at $P \leq 0.05$. Survival curves were estimated with Kaplan-Meier model and compared with Mantel-Cox test, significance for survival curves was set at $P \leq 0.05$.

Results

HA and non-HA patients have similar clinical features

At January 2018 a total of 358 patients were registered on IPINet 22q11DS National Registry, with follow-up data available for 294. Global prevalence of autoimmunity was 24% (72/294), while hematological autoimmunity was 8% (23/294). We

collected demographic, clinical and laboratory data of 23 HA and 45 non-HA patients; among HA patients, 16 had ITP and 2 AIHA; 5 patients were affected by both (Evans Syndrome). Table 1 shows clinical and laboratory features of enrolled patients. There was no statistical difference in median age at the time of 22q11DS diagnosis between HA and non-HA. In the case group the mean delay between 22q11DS diagnosis and HA development was 7.9 years.

Table2 provides a comparison of demographic and clinical features of HA and non-HA patients. At follow-up HA patients were significantly older (18.0 vs 14.0 years, *p=0.015*) and frequently had persistent hypocalcemia (45.4% vs 17.8%, *p=0.016*). None of the other clinical features examined (renal, otorhinolaryngological-ENT, epilepsy, orthopedic, gastroenterological, cardiac, thymic anatomy or thymectomy related to cardiac surgery) differed between HA and non-HA groups. The rate of respiratory recurrent infections, severe infections and the frequency of non-hematological autoimmunity (thyroiditis, arthritis, psoriasis) showed no difference.

HA patients have specific immunophenotypic alterations

Immunophenotype at last follow-up was available in all 68 patients.

No difference in $CD4^+$ total cell count was found between HA and non-HA groups. HA patients had significantly decreased percentage of naïve $CD4^+$ cells, (26.8% vs 43.2%, *p=0.003*) and RTEs (48.6% vs 80.5%, *p=0.046*), with increased memory $CD4^+$ cells (74.0% vs 55.5%, *p=0.001*). No difference between HA and non-HA patients was found in the frequencies of naïve cytotoxic T cells (41.4% vs 49.6%), Treg (3.9% vs 5.9%), and cTFHs (16.0% vs 13.5%). (Figure1A)

Furthermore, HA patients had decreased class-switched memory B cells (SMB) (2.0% vs 5.9% *p=0.037*), increased naive B cells (83.5% vs 71.4%, p=0,017) and

CD21^{low} (9.9% vs 2.4%, *p=0.018*) (Figure1B). Finally, non-HA patients had increased CD3^{-}CD16^{+}/56^{+} NK cells as absolute number (312 vs 199 cells per microliter, *p=0.009*) and percentage (20.0% vs 13.0%, *p=0.029*) (Figure1C). No difference has been found between HA and non-HA in transitional B cells (7.6% vs 9.4%) and in IgM Memory B cells (9.8% vs 8.3%).

Naïve CD4^{+} cells and SMB cells are predictive of development of HA

Immunophenotype at diagnosis was available in 36 patients (11 HA and 25 non-HA). The age at immunophenotype was not different between groups.

HA patients had significantly decreased percentage of naïve CD4^{+} cells (29.0% vs 51.0%, *p=0.021*) and decreased SMB (1.7% vs 4.3%, *p=0.015*); at the time of diagnosis NK number and percentage did not differ between HA and non-HA groups.

We therefore estimated the OR of immune cytopenia development based on the following cut-offs: ≤30% for CD4 naïve and ≤2% for SMB. Cut-offs were identified by comparison with previous work on the stratification of patients with common variable immunodeficiency (CVID) [17,18]. The OR was 14.0 (2.6-74.6; *p=0.002*) for CD4 naïve ≤30% group, while for SMB ≤2% group the OR was 44,0 (2.2-98.3; *p=0.010*).

In the MA both predictors confirmed their predictivity with a *p=0.008* for CD4 naïve (OR 1.8-55.9) and *p=0.022* for SMB (OR 1.4-88.7), sex and age at diagnosis were not associated with HA development.

Survival curves were estimated for both subpopulations using the same cut-offs. All curves reached statistical significance respectively of *p=0.0001* for CD4+ naïve ≤30% and of *p=0.0018* for SMB ≤2%. (Figure2)

Discussion

In this study we report the immunological findings in 23 HA 22q11DS patients, the largest cohort described so far in literature. Our data, based upon the Italian registry, show that HA affects 8% of patients with 22q11.2DS, the appearance of autoimmune disease is usually 8 years after the 22q11DS diagnosis. HA patients have an almost complete demographical and clinical overlap compared to non HA patients, making almost impossible for the physician to predict the development of this complication.

Nevertheless, our follow-up data shows that HA patients have specific immunophenotypic alterations. The absolute count and percentage value of $CD4^+$ T cells, which is known to be generally reduced in 22q11.2DS patients compared with healthy subject [7], do not differ within HA and non-HA subjects. Conversely, we find a significantly decreased percentage of $CD45RA^+$ naïve and $CD31^+$ RTE T helper cells, confirming some previous observations [19,20].

Overall our findings suggest a defective thymic output, which is known to be associated to autoimmunity in several PIDs. The mechanisms that link both phenomena are still unclear, but certain key pathways have been described in various PID: a reduced TCR repertoire diversity, a homeostatic IL-7 driven proliferation of T lymphocytes, and a lack of naturally occurring regulatory T cells (nTregs)[21]. Indeed, an intra-thymic defect has also been suggested as a possible explanation of autoimmunity in 22q11.2DS through incomplete negative selection or compromised AIRE expression[22].

It should be underlined that in our cohort HA patients have a greater incidence of persistent hypocalcemia suggesting, as another previous study[23], the association between hypoparathyroidism and defective T cell immunity, and linking these alterations to a defective common organogenesis; indeed an association between persistent and recurrent hypocalcemia and thymus defects was also reported[24].

However, in our cohort the two groups do not differ for thymic anatomy nor for thymectomy related to cardiac surgery, suggesting that this association could not be explained solely by a bare anatomic defect.

In effect, immunophenotypic alterations in HA patients are not confined to T cells. In particular, we observed a significant reduction of class-switched memory B cells (SMB) and NK cells. SMB are generated in the germinal centers of lymph-nodes by interaction with their cognate IL-21 expressing TFH cells. Reduced capacity of generating effective SMB cells is a hallmark of several PIDs, like common variable immunodeficiency (CVID) spectrum disorders, reflecting in some cases an intrinsic alteration in THF function[17]. Previous works underlined that 22q11.2DS adult patients exhibit a reduction of SMB cells and a decrease rate of somatic hypermutation, while circulating T follicular helper (cTFH) cells are present at higher percentages at all ages and display a more activated phenotype [12,13]. Indeed, in our cohort both HA and non-HA patients have increased percentages of cTFH, and the subjects with higher cTFH values have more severe autoimmune manifestations (ITP+AIHA) (data not shown). Nevertheless, the overall difference between cTFH in HA and non-HA do not reach the statistical significance. Definitely, our data are in lines with an aberrant germinal center function, although it is impossible to establish whether this defect is ascribable primarily to cTFH cells or instead intrinsic to B cells.

Reduction of both NK number and percentage is an intriguing issue. NK are mostly innate immunity, extra-thymic derived cells, whereby alteration in its number or function cannot be a direct consequence of an inadequacy of thymic environment. The role of NK cells in autoimmune disease has been evaluated in animal models, but only a few studies, mainly descriptive, have demonstrated NK alterations in human diseases, with conflicting results[25]. It is interesting to note that some

22q11.2DS patients display a functional defect of NK direct cytolytic and antibody-dependent cell-mediated cytotoxicity due to haploinsufficiency of CRKL gene, included in the typical deleted region [15]. These findings, however, have not been related to development of autoimmunity. The association of reduced NK numbers and HA suggests the involvement of a pathway independent from thymic function, maybe related to an intrinsic lymphocyte defect.

Overall our data at follow-up shows that HA patients exhibit a distinctive immunophenotypic hallmark. To exclude that the mentioned anomalies were due to the difference of age between HA and non-HA patients or to possible ongoing treatment we compared the immunophenotype at 22q11DS diagnosis. Interestingly some peculiar anomalies of HA immunophenotype were present even at diagnosis, suggesting that HA should be considered a peculiar complication of 22q11DS patients with a more severe immunological phenotype rather than a cause of it.

The most prominent result of this study is the prognostic value of these immunophenotypic alterations. Analyzing prospectively the data from a long follow-up period, we demonstrated that reduced levels of naïve $CD4^+$ and SMB cells are already present at diagnosis and are strong predictor of HA development. This finding may represent a critical point in the clinical management of 22q11.2DS patients. We suggest to clinicians to use lymphocyte immunophenotype to stratify patient at diagnosis, in order to offer a more personalized follow-up and to early diagnose potentially severe complications such AIHA and PTI. In particular, we recommend to utilize $CD4^+CD45RA^+$ naïve helper T cells percentage, which is a simple and reliable test and is an extremely good marker of HA development. When B lymphocytes subtyping is available SMB analysis increases the predictivity and may be very useful especially in older patients

In conclusion our study highlights that, among 22q11.2DS patients, those with HA have characteristic anomalies regarding T, B and NK cells; these anomalies appear considerably before HA onset, therefore systematic immunophenotyping of 22q11.2DS patients at diagnosis is advisable for early identification of patients at risk for this severe complication. In view of the evident immunological features characterizing this subgroup of patients, it is conceivable that further pathogenetic mechanisms might be involved with respect to the remaining patients with 22q11.2DS. We are therefore conducting an in-depth genetic investigation in this specific group of patients with peculiar features to identify possible new genetic determinants of immune impairment in 22q11.2DS.

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Table and figures

Table1: clinical and laboratory features of enrolled HA patients. ND: Not Defined; Hypocalcemia: A absent, T transitory, P persistent; ENT: Ears Nose and Throat; CAKUT: Congenital Anomalies of the Kidneys and of the Urinary Tract; JIA: Juvenile idiopathic arthritis; HA: Hematological Autoimmunity; ITP: Idiopathic Thrombocytopenic Purpura; AIHA: Autoimmune Hemolytic Anemia; CS: corticosteroids; IVIG: intravenous immunoglobulin; RTX: Rituximab; MMF: mycophenolate. *None of the patient was splenectomized

Table2: comparison of demographic and clinical features of HA and non-HA patients. ENT: Ears Nose and Throat; CAKUT: Congenital Anomalies of the Kidneys and of the Urinary Tract; JIA/RA: Juvenile idiopathic arthritis/Rheumatoid Arthritis.

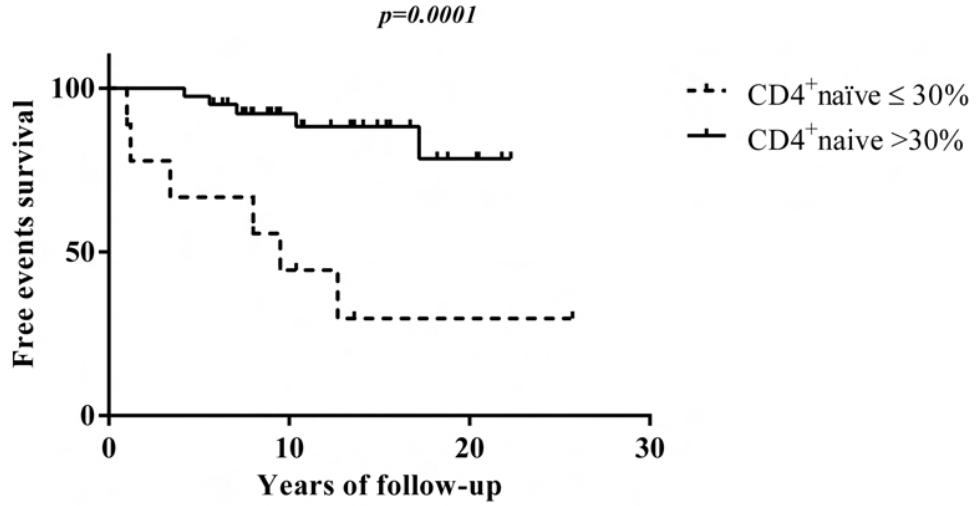
Figure1: Comparison of lymphocyte subpopulations between patients with hematological autoimmunity (grey box, HA) and patients without hematological autoimmunity (white box, non-HA). **A.** CD4 naïve % of $CD4^+CD3^+$ cells, RTE % of CD4 naïve cells and cTFH % of CD4 memory cells. **B.** Naïve B cells, Switched Memory B cells and CD21Low cells % of $CD19^+$ cells. **C**. Peripheral % of total lymphocytes and absolute number of $CD3^-CD16^+CD56^+$ NK cells. *$P < 0.05$ and **$P < 0.01$.

Figure2: Probability of free survival event as a function of the CD4 naïve T helper cells levels (A) and of switched memory B cells (B). The event was defined as the onset of hematologic autoimmunity either thrombocytopenic purpura (ITP) or hemolytic anemia (AIHA)

	Sex	Age	Hypocalcemia	Cardiopathy	Cardiosurgery	ENT Alterations	CAKUT	Epilepsy	JIA/RA	Thyroiditis	Psoriasis	HA	Age of onset of HA	Ongoing Immunosoppressive treatment*	Previous RTX therapy	Leucocytes cell/mm^3	Neutrophils %	Neutrophils cell/ mm^3	Lymphocytes %	Lymphocytes cell/ mm^3	CD3$^+$%	CD4$^+$ %	CD8$^+$ %	CD19$^+$ %	CD16$^+$/56$^+$ %	IgG mg/dl	IgM mg/dl	IgA mg/dl
Patient 1	F	29	P	•								ITP	18	CS		8920	ND	ND	33,0	2943	64,0	23,0	30,0	33,0	ND	1800	249	580
Patient 2	M	4	P	•	•							ITP	1			9830	53,5	5250	26,7	2620	39,7	20,2	14,3	28,2	28,5	842	79	112
Patient 3	M	12	A									ITP	4			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Patient 4	M	19	P			•						AIHA	8			4200	ND	ND	30	1260	57	26,0	29,0	18	17	909	36	180
Patient 5	M	41	A									ITP	ND			6330	57,0	3600	27,8	1760	65,8	35,8	25,2	11,8	18,4	1160	124	309
Patient 6	M	48	P					•				ITP	ND			5300	ND	ND	24,3	1290	65,5	26,4	36,7	29	5	1190	120	6
Patient 7	F	23	P	•	•	•				•		ITP	15			5220	66,9	3492	15,1	788	60,0	30,0	24,0	17,0	21,0	1640	25	117
Patient 8	M	19	P			•		•				ITP+AIHA	7	CS		4280	53,6	2294	36,8	1575	60,0	32,0	25,0	23,0	17,0	864	20	351
Patient 9	F	19	ND						•			ITP	4			8210	79,1	6500	15,4	1260	65,0	23,2	40,6	25,4	8,6	213	166	4
Patient 10	M	16	A	•	•	•						ITP	12			3760	ND	ND	38,0	1460	50,0	22,0	18,0	6,0	41,0	1390	22	133
Patient 11	F	17	T			•				•		ITP+AIHA	12	CS + IVIG		5470	57,0	3130	30,0	1640	68,0	38,0	27,0	18,0	12,0	1088	306	207
Patient 12	F	18	A				•	•				ITP	15			8970	40,0	4090	38,4	3450	76,0	57,5	15,6	11,0	10,0	1350	115	323
Patient 13	F	23	T	•	•	•				•		ITP	15			6450	ND	ND	26,3	1700	64,0	29,0	31,0	20,0	2,0	1480	102	218
Patient 14	M	9	A	•	•	•	•			•		ITP	3			5520	49,6	2740	28,6	1580	41,2	22,0	14,9	23,9	33,5	598	72	126
Patient 15	M	23	A	•		•						ITP	17			6410	62,4	4000	22,7	1460	75,4	34,7	33,9	9,5	13,6	1455	64	300
Patient 16	M	12	P	•								ITP	8	CS	•	2560	54,7	1400	28,4	727	87,9	60,0	15,6	0,0	12,0	1292	55	38
Patient 17	F	25	A	•	•							ITP+AIHA	ND	PDN		3050	51,7	1580	35,2	51,7	79,0	52,2	25,6	18,3	2,6	474	34	25
Patient 18	M	18	P	•	•	•		•		•		ITP+AIHA	9	CS + Sirolimus		5620	62,5	3510	24,8	1390	61,0	41,0	20,0	22,0	16,0	1760	154	231
Patient 19	M	14	P	•		•						ITP+AIHA	1	Sirolimus		3850	57,9	2230	23,4	900	67,0	27,0	30,0	24,0	8,0	559	41	23
Patient 20	M	34	T				•	•			•	ITP	22			10500	49,5	5220	37,5	3940	40,7	29,7	10,6	45,4	12,4	159	19	9
Patient 21	F	18	A	•	•				•			ITP	5			5040	ND	ND	25,0	1260	57,9	44,3	11,8	28,1	11,7	1493	58	144
Patient 22	F	9	T	•	•							ITP	7	CS + IVIG		4340	56,9	2470	31,1	1350	63	46	15	20	14	629	107	89
Patient 23	M	12	P	•	•	•						AIHA	9	MMF	•	5030	71,4	3590	11,8	590	85,0	33,0	48,0	0,0	13,0	621	4	57

	HA	non-HA	p-value
Female	39 %	51 %	0.44
Median age (years)	18.0	14.0	**0.02**
Abnormal Thymus (Hypoplastic/Absent)	69.7 %	70.0 %	1.00
Persistent Hypocalcemia	45.4 %	17.8 %	**0.02**
ENT Anomalies	36.4 %	57.7 %	0.10
Cardiopathy	60.7 %	64.4 %	0.80
Cardiosurgery	43.5 %	46.7 %	1.00
CAKUT	14.3 %	6.7 %	0.32
Epilepsy	22.7 %	17.8 %	0.63
Gastrointestinal Anomalies	30.4 %	37.8 %	0.55
JIA/RA	8.6 %	2.2 %	0.22
Thyroiditis	21.7 %	22.2 %	0.96
Psoriasis	4.3 %	6.7 %	0.70
Severe Infections	27.3 %	37.2 %	0.42

A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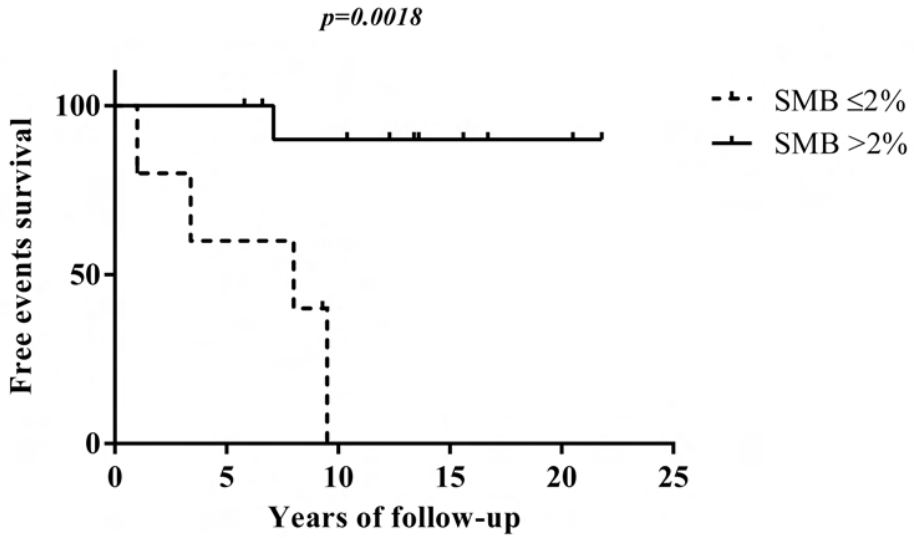
Free events survival
100
50
0
0
10
20
30
Years of follow-up
CD4+naïve ≤ 30%
CD4+naive >30%

B


p=0.0018
Free events survival
100
50
0
0
5
10
15
20
25
Years of follow-up
SMB ≤2%
SMB >2%