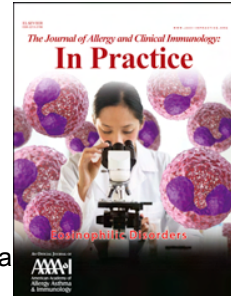


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Immunophenotype anomalies predict the development of autoimmune cytopenia in 22q11.2 Deletion Syndrome

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4 *memory B cells are predictive of development of autoimmune cytopenia in 22q11.2*  
5 *Deletion Syndrome.*

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**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<sup>+</sup> 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 naïve B cells (83,5% vs 71,4%,  $p=0,02$ ); increased CD16<sup>+</sup>/56<sup>+</sup> 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  $\leq 30\%$  (OR 14.0  $p=0.002$ ) and for SMB  $\leq 2\%$  (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.

**92 Highlights box:****93 1. What is already known about this topic?**

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

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

- 98 • 22q11.2 DS patients with hematologic autoimmunity have peculiar B and T  
99 immunophenotype anomalies, the anomalies precede the onset of  
100 autoimmunity and may be used for risk stratification.

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

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

105

106 **Key words:** autoimmune cytopenia, thrombocytopenic purpura, hemolytic anemia,  
107 22q11.2 Deletion Syndrome, DiGeorge Syndrome, B immunophenotype, T  
108 immunophenotype, NK cells, CD4 naïve cells, switched memory B cells

**109 Abbreviations:**

110 22q11DS: 22q11.2 deletion syndrome

111 ITP: Idiopathic thrombocytopenic purpura

112 AIHA: hemolytic anemia

113 PID: primary immunodeficiencies

114 HA: hematological autoimmunity

115 IPINet: Italian Primary Immunodeficiency Network

116 cTFH: follicular helper T cells

- 117 Treg: regulatory T cells
- 118 RTE: Recent Thymic Emigrants
- 119 SMB: switched memory B cells
- 120 CVID: common variable immunodeficiency
- 121 MA: multivariate analysis
- 122

## 123 **Introduction**

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

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

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



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

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

## 157 **Methods**

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

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

169 22q11DS patients without hematologic autoimmunity (non-HA group), randomly  
170 selected.

171 All del22q11 patients of the Italian registry are assigned a specific anonymous  
172 alphanumeric code when their data are entered in the DB. Using the alphanumeric code  
173 we extracted through a random electronic generator ([www.random.org](http://www.random.org)) 45 patient in  
174 the whole non-HA patients of the DB. The random assignment was made by a  
175 blinded examiner. Afterwards the alphanumeric code was used to detect the  
176 anagraphical data and caring physician of each patient.

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

182 Lymphocyte specific population were defined as follows. Regarding T cells:  
183 CD4<sup>+</sup>CD45RA<sup>+</sup> naïve helper T cells, CD4<sup>+</sup>CD45R0<sup>+</sup> activated/memory helper T cells,  
184 CD4<sup>+</sup>CD45R0<sup>+</sup>CXCR5<sup>+</sup> circulating follicular helper T cells (cTFHs),  
185 CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>low</sup>FOXP3<sup>+</sup> regulatory T cells (Treg) (all expressed as percentage  
186 of CD4<sup>+</sup> T cells); CD4<sup>+</sup>CD45RA<sup>+</sup>CD31<sup>+</sup> Recent Thymic Emigrants (RTEs)  
187 (expressed as percentage of naïve CD4<sup>+</sup> T cells); and CD8<sup>+</sup>CD27<sup>+</sup>CD28<sup>+</sup> naïve  
188 cytotoxic T cells (expressed as percentage of CD8<sup>+</sup> T cells). B cells subsets were  
189 subdivided as CD38<sup>++</sup>IgM<sup>++</sup> transitional B cells, CD27<sup>-</sup>IgM<sup>+</sup>IgD<sup>+</sup> naïve B cells,  
190 CD27<sup>+</sup>IgM<sup>+</sup>IgD<sup>+</sup> IgM memory B cells, CD27<sup>+</sup>IgM<sup>-</sup>IgD<sup>-</sup> switched memory B (SMB)  
191 cells, and CD21<sup>low</sup>CD38<sup>low</sup> B cells; all B cell subpopulations are expressed as  
192 percentage of total CD19<sup>+</sup> B cells.

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

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

204 Finally Kaplan-Meier curves were calculated for all the candidate predictors using the  
205 same cut-offs<sup>17,18</sup>.

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

## 212 **Results**

### 213 *HA and non-HA patients have similar clinical features*

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

217 collected demographic, clinical and laboratory data of 23 HA and 45 non-HA  
218 patients; among HA patients, 16 had ITP and 2 AIHA; 5 patients were affected by  
219 both (Evans Syndrome). Table 1 shows clinical and laboratory features of enrolled  
220 patients. There was no statistical difference in median age at the time of 22q11DS  
221 diagnosis between HA and non-HA. In the case group the mean delay between  
222 22q11DS diagnosis and HA development was 7.9 years.

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

### 231 *HA patients have specific immunophenotypic alterations*

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

233 No difference in CD4<sup>+</sup> total cell count was found between HA and non-HA groups.  
234 HA patients had significantly decreased percentage of naïve CD4<sup>+</sup> cells, (26.8% vs  
235 43.2%,  $p=0.003$ ) and RTEs (48.6% vs 80.5%,  $p=0.046$ ), with increased memory  
236 CD4<sup>+</sup> cells (74.0% vs 55.5%,  $p=0.001$ ). No difference between HA and non-HA  
237 patients was found in the frequencies of naïve cytotoxic T cells (41.4% vs 49.6%),  
238 Treg (3.9% vs 5.9%), and cTFHs (16.0% vs 13.5%). (Figure1A)

239 Furthermore, HA patients had decreased class-switched memory B cells (SMB)  
240 (2.0% vs 5.9%  $p=0.037$ ), increased naive B cells (83.5% vs 71.4%,  $p=0,017$ ) and

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

246 *Naïve CD4<sup>+</sup> cells and SMB cells are predictive of development of HA*

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

249 HA patients had significantly decreased percentage of naïve CD4<sup>+</sup> cells (29.0% vs  
250 51.0%,  $p=0.021$ ) and decreased SMB (1.7% vs 4.3%,  $p=0.015$ ); at the time of  
251 diagnosis NK number and percentage did not differ between HA and non-HA groups.

252 We therefore estimated the OR of immune cytopenia development based on the  
253 following cut-offs:  $\leq 30\%$  for CD4 naïve and  $\leq 2\%$  for SMB. Cut-offs were identified by  
254 comparison with previous work on the stratification of patients with common variable  
255 immunodeficiency (CVID) <sup>17,18</sup>. The OR was 14.0 (2.6-74.6;  $p=0.002$ ) for CD4 naïve  
256  $\leq 30\%$  group, while for SMB  $\leq 2\%$  group the OR was 44.0 (2.2-98.3;  $p=0.010$ ).

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

260 Survival curves were estimated for both subpopulations using the same cut-offs. All  
261 curves reached statistical significance respectively of  $p=0.0001$  for CD4<sup>+</sup> naïve  
262  $\leq 30\%$  and of  $p=0.0018$  for SMB  $\leq 2\%$ . (Figure2)

263 **Discussion**

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

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

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

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

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

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

308 Reduction of both NK number and percentage is an intriguing issue. NK are mostly  
309 innate immunity, extra-thymic derived cells, whereby alteration in its number or  
310 function cannot be a direct consequence of an inadequacy of thymic environment.

311 The role of NK cells in autoimmune disease has been evaluated in animal models,  
312 but only a few studies, mainly descriptive, have demonstrated NK alterations in  
313 human diseases, with conflicting results<sup>25</sup>. It is interesting to note that some

314 22q11.2DS patients display a functional defect of NK direct cytolytic and antibody-  
315 dependent cell-mediated cytotoxicity due to haploinsufficiency of CRKL gene,  
316 included in the typical deleted region <sup>15</sup>. These findings, however, have not been  
317 related to development of autoimmunity. The association of reduced NK numbers  
318 and HA suggests the involvement of a pathway independent from thymic function,  
319 maybe related to an intrinsic lymphocyte defect.

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

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



339 In conclusion our study highlights that, among 22q11.2DS patients, those with HA  
340 have characteristic anomalies regarding T, B and NK cells; these anomalies appear  
341 considerably before HA onset, therefore systematic immunophenotyping of  
342 22q11.2DS patients at diagnosis is advisable for early identification of patients at risk  
343 for this severe complication. In view of the evident immunological features  
344 characterizing this subgroup of patients, it is conceivable that further pathogenetic  
345 mechanisms might be involved with respect to the remaining patients with  
346 22q11.2DS. We are therefore conducting an in-depth genetic investigation in this  
347 specific group of patients with peculiar features to identify possible new genetic  
348 determinants of immune impairment in 22q11.2DS.

349

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427 **Table and figures**

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

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

438 **Figure1:** Comparison of lymphocyte subpopulations between patients with  
439 hematological autoimmunity (grey box, HA) and patients without hematological  
440 autoimmunity (white box, non-HA). **A.** CD4 naïve % of CD4<sup>+</sup>CD3<sup>+</sup> cells, RTE % of  
441 CD4 naïve cells and cTFH % of CD4 memory cells. **B.** Naïve B cells, Switched  
442 Memory B cells and CD21<sup>Low</sup> cells % of CD19<sup>+</sup> cells. **C.** Peripheral % of total  
443 lymphocytes and absolute number of CD3<sup>-</sup>CD16<sup>+</sup>CD56<sup>+</sup> NK cells. \*P < 0.05 and \*\*P  
444 < 0.01.

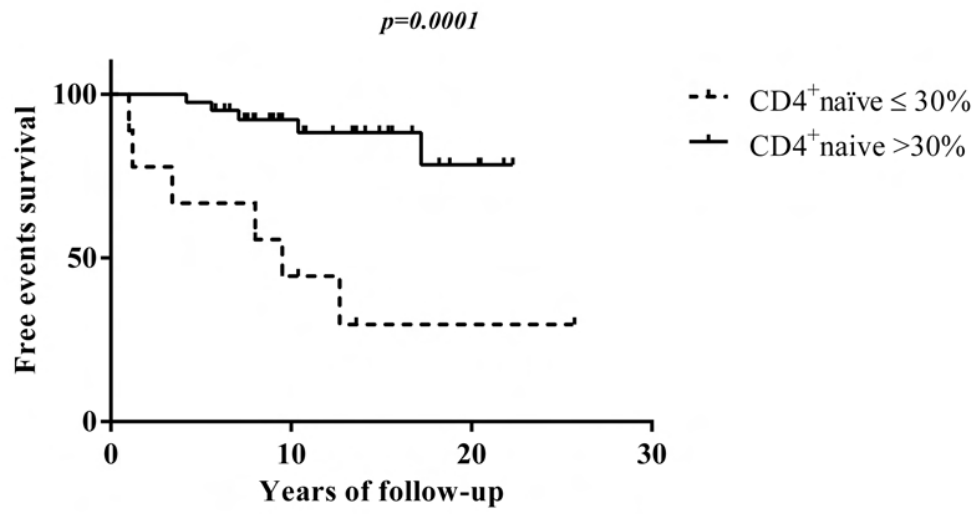
445 **Figure2:** Probability of free survival event as a function of the CD4 naïve T helper  
446 cells levels (A) and of switched memory B cells (B). The event was defined as the  
447 onset of hematologic autoimmunity either thrombocytopenic purpura (ITP) or  
448 hemolytic anemia (AIHA)

Patient	Sex	Age	Hypocalcemia	Cardiopathy	Cardiosurgery	ENT Alterations	CAKUT	Epilepsy	JIA/RA	Thyroiditis	Psoriasis	HA	Age of Onset of HA	Ongoing Immunosuppressive treatment*	Previous RTX therapy	Leucocytes cell/mm <sup>3</sup>	Neutrophils %	Neutrophils cell/mm <sup>3</sup>	Lymphocytes %	Lymphocytes cell/mm <sup>3</sup>	CD3 %	CD4 %	CD8 <sup>+</sup> %	CD19 <sup>+</sup> %	CD16 <sup>+</sup> /56 <sup>+</sup> %	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	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	<b>0.02</b>
Abnormal Thymus (Hypoplastic/Absent)	69.7 %	70.0 %	1.00
Persistent Hypocalcemia	45.4 %	17.8 %	<b>0.02</b>
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



ACCEPTED MANUSCRIPT

**A****B**