Table 1. Clinical data regarding the 13 hips with AVN.

Patient #.	Hip R	Hip L	Age (yr)	Sex	Genotype	Ht (%)	Stage *(b) x-ray	Stage *(b) MRI	Treatment	Stage *(a) x-ray	Stage *(a) MRI	Follow-up
1.	+		11	F	SS	25	4	–	**S	2	–	Improved
		+	11				4	–	S	2	–	Improved
2.	+		7	M	$S\beta^0$	26	3	–	S	2	2	Improved
3.	+		12	F	$S\beta^+$	29	5	–	S	3	3	Improved
		+	12				2	–	**C	3	3	Deteriorated
4.	+		7	M	$S\beta^0$	27	4	–	S	2	–	Improved
		+	7				4	–	S	2	–	Improved
5.	+		18	M	Sβ+	28	5	–	S	4	4	Stable
		+	18				3	–	S	2	2	Improved
6.	+		13	M	$S\beta^+$	30	2	2	S	2	2	Improved
		+	18				normal	1	C	normal	1	Stable
7.	+		10	F	$S\beta^+$	28	2	2	C	2	2	Improved
		+	10				normal	1	C	normal	1	Stable

**b = before treatment, *a = after treatment, **S = surgical treatment, **C = conservative treatment.*

prompt identification of the disease and initiation of earlier treatment regimens, in order to achieve a better outcome.

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HLA-DRB1*15 and pediatric aplastic anemia

We report a positive association between HLA-DRB1*15 (p = 0.0002) in Turkish patients with pediatric severe aplastic anemia (SAA) and a paradoxically favorable influence of the susceptibility marker on the clinical response to immunosuppressive therapy. These findings point to an immune mechanism mediated by DRB1*15 in SAA which confers responsiveness to treatment.

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Severe aplastic anemia (SAA) is a hematologic disorder characterized by peripheral blood cytopenias and reduced bone marrow cellularity in the absence of an underlying myeloproliferative disorder or malignancy. Most cases are described as idiopathic. Immune mechanisms have been considered in the pathogenesis of SAA and an HLA-DR-restricted immune reaction against hematopoietic cells has been reported.[1] Immunogenetic studies in different populations have identified an association between SAA and the serologically detected HLA-DR2 antigen or its molecular correspondents, DRB1*15/ *1501.[2-4] DRB1*1501 has also been reported to be closely associated with a favorable response to cyclosporin-A (CSA) therapy in patients with SAA.[5]

We examined the HLA-DRB1 locus in 33 Turkish pediatric SAA patients diagnosed and treated in Istanbul between 1992 and 2000. All patients with SAA had the following laboratory findings: hemoglobin < 100 g/L, hematocrit ≤ 30%, platelet count ≤ 50×10^9/L, white blood cell count ≤ 3.5×10^9/L and granulocytes ≤ 1.5×10^9/L. Bone marrow biopsy and aspirations showed decreased cellularity and the absence of significant fibrosis or neoplastic infiltration in all cases. Seventeen patients who did not have an HLA-matched donor received high dose methyl-

Table 1. Number of subjects possessing each HLA-DRB1 allele and HLA-DRB1 marker frequencies (%) in patients and controls.

HLA-DRB1	*Patients (n=33)*	*p*	*Controls (n=97)*
01	1 (3.03%)		14 (14.43%)
15 (02)*	20 (60.61%)	$p = 0.0002^{\#}$	22 (22.68%)
03	7 (21.21%)		14 (14.43%)
04	10 (30.30%)		35 (36.08%)
11 (05)	13 (39.39%)		38 (39.18%)
13 (06)	1 (3.03%)	$p = 0.042$	17 (17.53%)
14 (06)	1 (3.03%)		11 (11.34%)
07	6 (18.18%)		14 (14.43%)
Others° (08/09/10/12/16)	4 (12.12%)		12 (12.37%)

**Numbers in brackets in the HLA-DRB1 column denote the broad groups of DRB1 specificities; °relatively rare HLA-DRB1 alleles were pooled together for this analysis and there was no difference between the groups in the pooled frequencies; #The comparisons were made by Fisher's exact test. All p values are two-tailed. The global distribution of HLA-DRB1 markers between patients and controls was significantly different by the G-test (p = 0.007).*

prednisolone (HDMP) + antithymocyte globulin (ATG) ± CSA treatment. The controls were 97 healthy unrelated bone marrow donors. Molecular HLA-DRB1 typing was performed by low-resolution sequence specific primers (PCR-SSP) using 24 primer mixes (Olerup SSP primers).

Partial response (PR) and complete response (CR) were considered as responses for the evaluation of the success of immunosuppressive therapy. Complete response was defined as a return to normal counts in response to treatment. Partial response was defined as the absence of infections and transfusion dependency and sustained increase in all cell counts (reticulocyte count = 20×10^9/L; platelet count = 20×10^9/L: absolute neutrophil count = 0.5×10^9/L).

As shown in Table 1, there was a significant deviation in the distribution of HLA-DRB1 marker frequencies between patients and controls ($p = 0.007$; corrected G = 20.99). Two alleles were primarily responsible for this overall difference. The frequency of HLA-DRB1*15 was higher in patients ($p = 0.0002$; OR = 5.25, 95% CI = 2.25 to 12.21), and HLA-DRB1*13 appeared to be protective ($p = 0.042$; OR = 0.51, 95% CI = 0.02 to 1.15). In 17 patients who underwent immunosuppressive treatment, the overall response rates in patients positive and negative for HLA-DRB1*15 were 83.3% (9 CR [and 1 PR]; n=12) and 20.0% (1 CR; n=5), respectively ($p = 0.028$). Since the first report on HLA-DR antigens in SAA by Chapius *et al*., who found an association with the serological specificity HLA-DR2,[3] further studies have confirmed the DRB1*15 association in adult SAA patients.[2-5] We have now extended this observation to pediatric SAA. DRB1*1501 was previously shown to be closely associated with good response to CSA therapy in patients with aplastic anemia.[5-7] In the present study we could not analyze CSA and ATG treated patients separately because most patients received both. Clinical response to immunosuppressive treatment in general was much better in DRB1*15 bearing patients than in DRB1*15-negative patients. This finding was paradoxical in that a genetic susceptibility factor is usually also associated with more aggressive disease and unresponsiveness to treatment.

The results suggest that HLA-DRB1*15 confers susceptibility to SAA in a proportion of patients who are more likely to respond to immunosuppressive treatment. This results in the paradoxical finding that the susceptibility marker predicts a favorable clinical response to treatment in the HLA-DRB1*15-positive subgroup. This can be explained by the involvement of HLA-DRB1*15 in an autoimmune response resulting in aplastic anemia rather than increased susceptibility to an undetermined viral agent (as suspected in childhood acute lymphoblastic leukemia). Consequently, HLA-DRB1*15 would also predict responsiveness to immunosuppressive treatment as noted in our and others' studies. This aspect of the association also shows consistency in different populations. Nakao *et al*.[5] noted the same HLA-DRB1*1501-associated susceptibility to CSA-dependent aplastic anemia in Japan. We do not have high-resolution typing results on our patients and thus cannot confirm the restriction of the association to the HLA-DRB1*1501 allele.

SAA is one of the rare diseases that show a very consistent HLA association in different populations with almost no exception. All these data point towards a strong immunologic background in so-called idiopathic SAA. As happened to what is now called immune thrombocytopenic purpura, it may be time to transform the name of *idiopathic SAA* to *immune SAA*.

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Elevated serum vascular endothelial growth factor levels in patients with polycythemia vera and thrombotic complications

Vascular endothelial growth factor (VEGF) induces platelet activation in a thrombin-dependent manner. We tested the serum VEGF levels in patients with polycythemia vera (PV) and found a significant correlation between increased VEGF and thrombosis. These findings suggest that high VEGF levels might contribute to the occurrence of thrombosis in this hematologic malignancy.

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The thrombotic risk remains difficult to predict in patients with polycythemia vera (PV). Bellucci *et al.*[1] studied β-TG and PF4 levels in patients with normal platelet counts receiving antiaggregant and cytoreductive therapy. These authors failed to establish a clear correlation between increased β-TG and PF4 and thrombosis.[1] On this basis, we evaluated platelet function and number in a series of patients with polycythemia and tried to correlate the results with a history of thrombosis. Increased bone marrow angiogenesis has been demonstrated in patients with PV.[2] Recent studies indicate that vascular endothelial growth factor (VEGF), the major stimulus of angiogenesis, promotes platelet adhesion and activation in a thrombin-dependent manner and that on activation the platelets release VEGF.[3] Thus, we also measured VEGF levels.

The study group comprised 19 patients (13 men, 6 women; mean age 63.1 years [range 20-81]) suffering from PV, as defined by the Polycythemia Vera Study Group criteria.[4] Their mean duration of disease was 5 years (range 2-12). A group of 10 healthy subjects (3 men, 7 women; mean age 55.3 years [range 35-85]) acted as controls. Sixteen out of nineteen patients received hydroxyurea and antiaggregant agents, either aspirin or ticlopidine, indobufen and dipirydamole. The remaining three were managed with phlebotomy alone. Thrombotic complications had occurred in 8 patients (5 men, 3 women; mean age: 65.38 years) and included two episodes of deep vein thrombosis, two of transient ischemic attacks, two of myocardial infarction and two of microvascular thrombosis of extremities (erythromelalgia) (symptomatic group). The other eleven patients (7 men, 4 women; mean age: 60.55 years) had not experienced thrombosis (asymptomatic group). We excluded the presence of both acquired thrombotic risk factors (hypertension, smoking, obesity, hyperlipidemia, antiphospholipid syndrome) and inherited ones (antithrombin III, protein C and protein S deficiency, factor V Leiden, prothrombin G20210A and MTHFR C677T mutations). Serum VEGF and plasma β-TG and PF4 were measured by ELISA (Quantikine Human VEGF Immunoassay, R&D Systems, Minneapolis, MN, USA; Diagnostica Stago Boehringer Mannheim, Germany). To be sure that platelets did not release VEGF differently during the preparatory steps, depending on whether they came from a patient with a history of thrombosis or not, in 19 patients VEGF measurement was subsequently repeated in another sample collected after 1 or 2 months. These measurements showed concordance for VEGF concentration. The time elapsed between venipuncture and centrifugation for VEGF was 30 min. Platelet counts were determined by a Sysmex SF-3000 analyzer (Dasit, Milan, Italy). The results are summarized in Tables 1 and 2. All patients had significantly higher values of β-TG and PF4 than did the controls ($p<0.001$) and normal platelet counts. Of these 19 patients, 8 exhibited significantly higher VEGF levels than the normal subjects ($p<0.0001$) and had experienced thrombosis, whereas 11 showed normal VEGF levels and had not had thrombosis.

A significant difference was observed in VEGF levels between symptomatic and asymptomatic patients ($p<0.0001$, Student's t-test). A highly significant correlation was found between VEGF levels and thrombotic events estimated as Spearman's coefficient ($p<.0001$). No correlation was found between increased VEGF and platelets. In agreement with Bellucci *et al.*,[1] we found no correlation between elevated β-TG and PF4 and thrombosis.

Table 1. Bioclinical data of 19 patients with PV.

Patient No.	*Age/Sex*	*βTG 10-40 IU/mL**	*PF4 0-5 IU /mL**	*Platelets 150-450 ×10⁹/L**	*VEGF 62-707 pg/mL**	*Thrombotic events*	*Concomitant therapy*
1	78/F	180	85	305	621	No	Hu+IND+DYP
2	47/M	220	90	262	228	No	Phlebotomy
3	37/M	350	245	227	556	No	Phlebotomy
4	69/M	210	140	547	1119	Yes/TIA	Hu+IND+DYP
5	65/F	240	235	310	1354	Yes/DVT	Hu+ASA
6	53/M	260	245	522	1506	Yes/E	Hu+ASA
7	60/M	150	90	301	225	No	Hu+ASA
8	70/M	246	97	245	1366	Yes/MI	Hu+Ind
9	71/F	243	106	292	392	No	Hu+ASA
10	67/F	200	95	320	1845	Yes/DVT	Hu+ASA
11	79/M	250	104	230	444	No	Hu+ASA
12	81/M	230	120	299	791	Yes/E	Hu+ASA
13	71/F	240	95	564	1510	Yes/TIA	Hu+TIC
14	20/M	220	198	223	575	No	Phlebotomy
15	69/M	245	480	357	671	No	Hu+IND+DYP
16	57/M	210	180	230	848	Yes/MI	Hu+ASA
17	74/M	200	200	217	357	No	Hu+ASA
18	59/M	240	118	374	638	No	Hu+ASA
19	81/F	246	106	423	591	No	Hu+ASA
Mean°	63.11 ±15.69	230.531 ±39.86	59.42 ±96.17	328.84± 110.82	823± 482		
Controls°	55.3± 18.81	26.0± 8.59	2.68± 1.14	244.4± 66.86	194± 180		

**In brackets normal range; °Values shown are means±SD. TIA: transient ischemic attack; DVT: deep vein thrombosis; E: erythromelalgia; MI: myocardial infarction.*

Table 2. Statistics of 19 patients with PV.

	Patients with thrombosis (n=8)	*Patients without thrombosis (n=11)*	*p*
VEGF	1292±355	481±161	<.0001
VEGF/Thrombosis			<.0001
VEGF/Platelets			NS