Accuracy of leukocyte alkaline phosphatase score to predict *JAK2* V617F mutation

Granulocyte activation parameters have been described in patients with myeloproliferative disorders (MPD). We have evaluated the accuracy of leukocyte alkaline phosphatase (LAP) score to predict *JAK2* V617F mutation. LAP score was obtained using a cytochemical reaction in granulocytes of patients' peripheral blood with MPD.

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Patients with chronic myeloproliferative disorders (MPD) show granulocyte activation parameters such as increased values for leukocyte alkaline phosphatase (LAP) expression.[1] In 1955, Kaplow described a cytochemical technique for assessing LAP activity, and later, a score greater than 100 in granulocytes of peripheral blood was included in the Polycythemia Vera Study Group (PVSG) diagnostic criteria.[2,3] Recently, studies described a unique gain-of-function mutation of *JAK2* (V617F) in almost all the patients with polycythemia vera (PV), and in about half of the patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF).[4,5] Flow cytometry showed patients with the *JAK2* V617F mutation had significantly higher neutrophil counts, total white blood cell counts, and LAP levels.[6,7] A significant relationship between percentage of *JAK2* mutant alleles and LAP expression was also noted.[6] We aimed to study the accuracy of LAP scores based on a cytochemical reaction to detect *JAK2* V617F MPD.

Between March and September 2006, we studied 44 consecutive patients with diagnosis of MPD according to WHO criteria, at the Hospital Privado de Córdoba: 21 PV, 20 ET and 3 CIMF.[8] We also included 6 patients that did not fulfill the WHO criteria for PV. LAP score from peripheral blood smears of all 50 patients had been obtained at diagnosis. At that time, no fever or inflammation were noted and none of the patients had received previous cytoreductive treatment. Ten healthy controls were also studied. Sodium α napthyl acid was used as substrate as previously described and a TRIS solution was used as buffer.[2] All LAP slides were revised by two independent biochemists and a blind mutation test was carried out. *JAK2* V617F mutation was performed at diagnosis in 4 patients and during the follow up in 46 patients. We used allele-specific polymerase chain reaction (PCR) and PCR by restriction enzyme BsaXI on granulocytes DNA of peripheral blood, as previously published.[4] The study was approved by the local ethics committee and all patients signed a written informed consent. Data were analyzed using StatsDirect statistical software, version 2.5.6.

JAK2 V617F mutation was identified in 19 patients with PV (90.5%), 14 patients with ET (70%) and 1 patient with CIMF (33.3%). No mutation was detected in patients with other variants of polycythemia or in healthy controls. Patient characteristics at diagnosis are shown in Table 1. Distribution of LAP score according to different patient categories and normal controls is shown in Figure 1. A LAP score above 100 had a sensitivity of 88.2% (30/34 *JAK2* positive patients) and a specificity of 87.5% (2/16 *JAK2* negative patients). LAP score identified patients carrying *JAK2* mutation (area under ROC curve=0.89). Highest LAP levels were found only in patients carrying *JAK2* V617F mutation and a cut off point ≥245 was found in 7/34 *JAK2* positive (6 PV and 1 ET) and in 0/16 *JAK2* negative (specificity 100%; sensitivity 20.6%). The highest LAP score in patients with PV may be due to a greater prevalence of homozygosity for *JAK2* V617F mutation in PV.[9] Two patients with ET and PV who were negative for the mutation presented LAP score of 190 and 243 respectively (less than 245). Indeed, this finding may be explained by a previous report that patients with MPD and fully wild-type *JAK2* alleles showed higher LAP expression than controls, however lower than *JAK2* V617F MPD.[6] A higher transcription of wild-allele *JAK2* in patients without the mutation may be the reason for this.[10] Four patients had a LAP score <100 but they were positive for *JAK2* mutation. Interaction between the expression of other genes or a very early disease could explain the low score in a small group of patients. Although the mean of white blood cell count was higher in *JAK2* V617F positive patients (Table 1), we found a weak direct correlation between LAP score and white blood cell count for all patients (r= 0.34; p=0.0153). This

Table 1. Patients' characteristics at diagnosis (n= 50).

Variable	*V617F JAK2 positive (n= 34)*	*V617F JAK2 negative (n= 16)*	*p value**
Age (years)	60.5±14.6	52.1±14.9	NS
Sex			
Male	15	9	NS
Female	19	7	
Disease duration (months)	31.8 (0.9-298.2)	41.1 (0.4-179.2)	NS
LAP score, all patients	190.4±68.5	72.9±59.6	<0.0001
White cell count (×10⁹/L)	12.4 ±4.1	9 ±4.3	0.011
Hemoglobin (g/dL)	16.6 ±3.1	15.4 ±3.6	NS
Platelet count (×10⁹/L)	717.7 ±323.3	722.1 ±773.4	NS

*Data are mean ±standard deviation, number for sex and median (range) for disease duration. NS: not significant; LAP: leukocyte alkaline phosphatase. *All p were two sided; unpaired T test or Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables were used.*

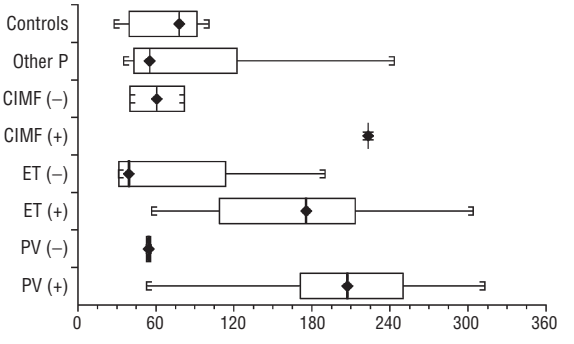

Figure 1. Leukocyte alkaline phosphatase (LAP) score in granulocytes of peripheral blood. LAP values obtained in different categories of patients and controls are shown in a box plot where the diamond indicates median. Differences between the groups studied are statistically significant (Kruskal-Wallis test; p<0.0001). PV: polycythemia vera; ET: essential thrombocythemia; CIMF: chronic idiopathic myelofibrosis; (+) or (–) are JAK2 V617F positive or negative respectively.

however was not confirmed for *JAK2* positive (r= 0.18; *p*=0.2863) or *JAK2* negative patients (r= 0.07; *p*=0.7792). Our results are consistent with the previously reported association between *JAK2* mutation and LAP levels detected by flow cytometry.

We have confirmed the validity of the technique described some 50 years ago (LAP score) that predicts the novel *JAK2* V617F mutation in patients with MPD.

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