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Letter to the Editor

Age, gender and efflux transporter activity influence imatinib efficacy in chronic myeloid leukemia patients

Chronic myeloid leukemia (CML) is a malignant hematopoietic disease caused by constitutively activated BCR-ABL tyrosine kinase, product of the Philadelphia (Ph) chromosome. In the last years, imatinib mesylate has been the treatment of choice for CML. Although imatinib response has been demonstrated in many reports, some patients present resistance due to a variety of factors including *BCR-ABL* mutation [1]. Imatinib resistance related to multidrug resistance (MDR) related to P-glycoprotein (Pgp), an efflux transporter protein has been investigated by our group in CML patient samples [2–5].

The purpose of the present study was to evaluate the relevance of gender and age with regard to clinical response to imatinib. Therefore, we analyzed the relationship of MDR profile, age and gender with molecular response to imatinib in CML patients. The study was run in a cohort of 402 CML patients at diagnosis enrolled at National Cancer Institute (INCA), Southeast Brazil. After diagnosis, patients were treated with imatinib 400 mg/day. We used analysis of variance (ANOVA) to compare the level of Pgp expression and/or efflux transporter activity into three or more groups, using test *F* correction, and Student *t* test when comparing two groups. To compare the distribution of patients into the groups we used the Chi-square test.

The Sokal score was available in 293 of 402 patients at CML diagnosis. Major molecular response (MMR) was analyzed in samples from 235 CML patients. Molecular analysis was done in accordance to European Leukemia Net guideline [6]. We used *ABL1* as control gene and all results were expressed in International Scale. We found MMR in 72 of 93 (77.4%) female samples, and 78 out of 142 (54.9%) male samples.

As shown in Table 1 female patients had better response to imatinib than male patients at 12 months after starting treatment ($p = 0.0005$). The best response in women was age dependent. Women in the 21–40 and > 60 years age groups had better response to imatinib than male patients ($p = 0.01$ and $p = 0.01$, respectively; Table 1). MMR was not influenced by Sokal score or interferon (IFN) previous treatment. Most patients were classified as low Sokal risk (143 out of 218; 65.6%) and achieved MMR at 12 month time-point ($p = 0.0005$). Female (13 of 72 or 18.05%) and male (21 of 78 or 26.92%) patients that achieved MMR had received IFN prior to imatinib, hence demonstrating that the best response to imatinib in women, observed in the present study, was not related to previous IFN-treatment.

Pgp expression and efflux transporter activity (MDR profile) were analyzed by flow cytometry using monoclonal antibody and Rhodamine-123 assay, respectively [2]. CML cell lines were used as controls for MDR profile (Fig. 1A–D). Samples were classified as positive (RFI $\geq$ 1.1) and negative (RFI < 1.1) based on CML cell line results (Fig. 1C and D) and, also analyzed regarding the level (without cutoff) of efflux transporter activity or Pgp expression. The Rhodamine-123 assay was positive in 84 out of 126 (66.7%) samples. Interestingly, the number of positive samples was lower in female (33 out of 60) than in male (51 out of 66) patient samples ($p = 0.013$). Pgp expression was found in 107 out of 121 (88.4%) patient samples. There was no difference in Pgp expression between male and female patient samples ($p = 0.5709$). Additionally, there was no correlation between Rhodamine-123 assay and Pgp expression suggesting the presence of efflux transporter other than Pgp.

The age impact on MDR profile was analyzed. The number of positive samples for Rhodamine-123 and Pgp expression assays was similar for all age groups ($p = 0.99$ and $p = 0.925$, respectively). The analysis of the level of Pgp expression revealed no statistical difference among the age groups ($p = 0.43$). However, the level of efflux transporter activity was significantly different ($p = < 0.0001$). The samples were stratified into age groups, gender and MDR profile. Women presented higher efflux transporter activity level compared to men ($p = 0.04$) in the under 20 years age group (Fig. 1). An opposite scenario was observed in the 21–40 age group where women had lower efflux transporter activity than men ($p < 0.0001$; Fig. 1E–G) even when outlier samples were removed from the analysis ($p = 0.0034$). It is important to notice that women with better molecular response were in the same age group of women with lower efflux transporter activity (21–40 age group). Although the women in the > 60 years age group had better molecular response than men ($p = 0.010$; Table 1) the efflux activity and molecular response were not influenced by gender in this group ($p = 0.37$; Fig. 1G). It could have been influenced by the fewer CML samples (7 male and 8 female) in that age group.

There is growing evidence in literature demonstrating that women have better response to imatinib than men [7,8]. Although the clinical significance of CML gender differences was inconclusive, our data suggest differences in efflux transporter activity in both, age categories and gender. The efflux transporter activity analyzed by Rhodamine-123

Table 1
Major molecular response (MMR) to imatinib according to gender and age in patients with chronic myeloid leukemia.

Age	Female		Male		*p*-value
	Total	MMR (%)	Total	MMR (%)	
≤ 20 years	5	4 (80.0)	9	6 (66.7)	0.597
21-40 years	31	26 (83.9)	35	19 (54.3)	0.010
41-60 years	32	22 (68.8)	62	36 (58.1)	0.312
> 60 years	25	20 (80.0)	36	17 (52.8)	0.010
Total	93	72 (77.4)	142	78 (54.9)	0.0005

MMR was evaluated after 12-month starting imatinib.

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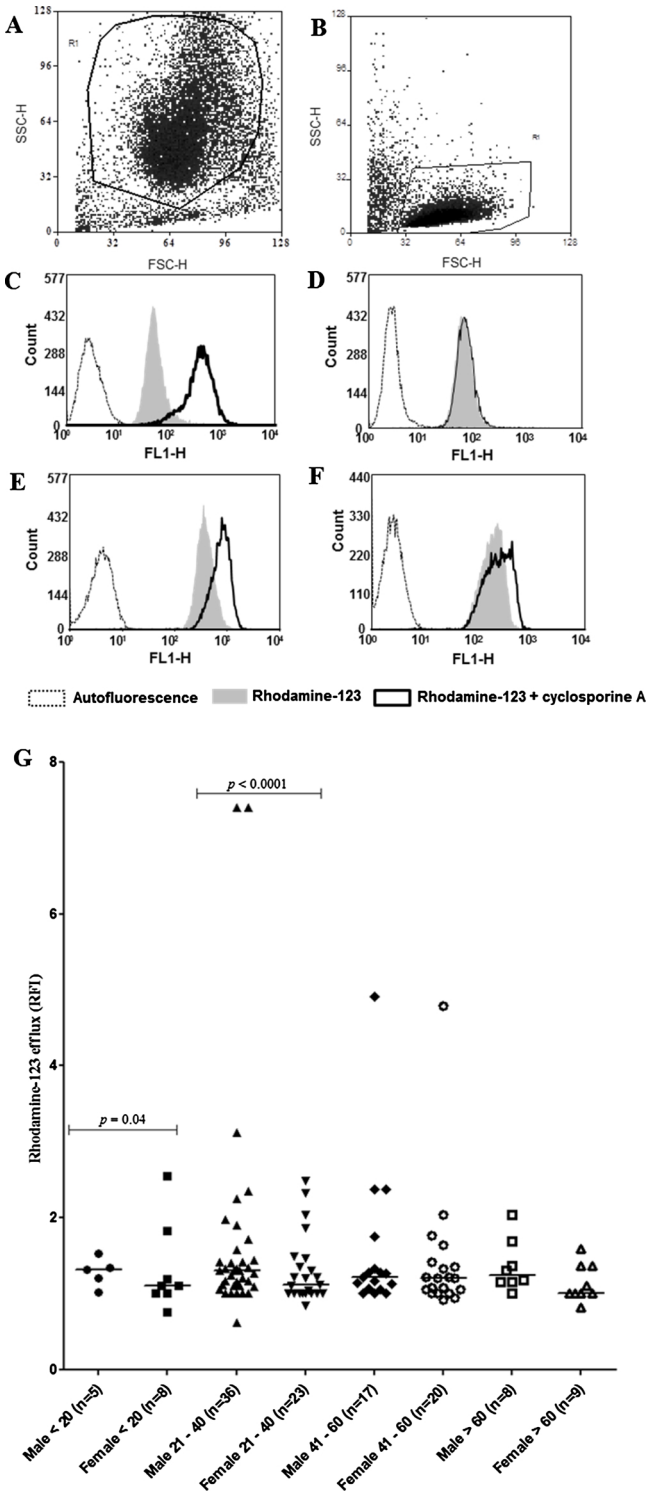

Fig. 1. Analysis of the efflux transporter activity in chronic myeloid leukemia cells.
Rhodamine-123 assay results were analyzed using flow cytometer and expressed as ratio of mean fluorescence intensity (RFI). The mean fluorescence intensity (MFI) of cells incubated with the fluorochrome Rhodamine-123 and cyclosporine A (modulator agent) were divided by MFI of cells with fluorochrome only. Representative gating strategy applied to (A) samples from CML patient at chronic phase and (B) cell lines was based on relative size (FSC) and granularity (SSC). (C) Chronic myeloid leukemia (CML) K562-Lucena Pgp positive (Pgp^+), and (D) K562 Pgp negative (Pgp^-) cell lines were used to establish the positivity cutoff in which RFI $\geq$ 1.1 was considered positive. Patient samples of the 21–40 age group were chosen to represent the differences in efflux transporter activity between (E) male and (F) female patient samples. (G) CML patient samples analyzed without cutoff. Data were expressed as raw data (RFI values). Variance analysis was used to compare the levels of Rhodamine-123 efflux between male and female samples according to age. $p < 0.05$ was considered significant. "n" means the number of samples in each group.

assay was not related to Pgp. As demonstrated before [2,9] a plausible explanation could be the existence of other efflux transporters, such as MRP1 and ABCG2/BCRP. In the present study, young female exhibited low efflux transporter activity levels. In this age range, women have physiological differences in contrast to men, such as menstrual cycle and fertility window. Besides that, the age range observed in our study gives insights to the possible influence of progesterone hormone, which is mainly secreted by *the corpus luteum* in the ovary during the second half of the menstrual cycle [10]. Yang et al [11] indicated that progesterone, an endogenous hydrophobic steroid, was able to inhibit the efflux of vinblastine, a chemotherapeutic drug substrate for Pgp [12]. Also, Fröhlich et al [13] observed potent Pgp inhibition by several synthetic progestins *in vitro* and *ex vivo*.

Additionally, imatinib is a substrate for efflux transporter proteins that could be reducing drug bioavailability, intracellular drug concentration and consequently contributing for resistance. However, progesterone could be acting as endogenous efflux transporter modulator reducing the imatinib efflux and contributing for a better response to imatinib in female than in male CML cells. Further studies should be focused on illuminating hormonal influence on these findings.

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Authors' disclosures of potential conflicts of interest

No potential conflicts of interest relevant to this article were reported.

Ethical approval

This study was approved by the institutional review board (CAAE 70945317.4.0000.5274) and is in accordance with the Declaration of Helsinki.

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