Development of hypogammaglobulinemia in patients treated with imatinib for chronic myeloid leukemia or gastrointestinal stromal tumor

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ABSTRACT

Imatinib mesylate is a tyrosine kinase inhibitor used as first line treatment in chronic myeloid leukemia and gastrointestinal stromal tumorpatients. Although several in vitro and animal studies demonstrated that imatinib affects immune response, few immune alterations are described in humans. We retrospectively studied hematologic and immunological parameters in 72 chronic myeloid leukemia and 15 gastrointestinal stromal tumor patients treated with imatinib at standard dosage and in 20 chronic myeloid leukemia patients treated before the introduction of imatinib in clinical practice. Both chronic myeloid leukemia and gastrointestinal stromal tumor patients developed a significant reduction of gammaglobulin and immunoglobulin serum levels. No significant hypogammaglobulinemia was observed in chronic myeloid leukemia patients in the pre-imatinib era. These data demonstrate that imatinib treatment induces hypogammaglobulinemia that can reach a severe entity in 10% of cases, both in chronic myeloid leukemia and in gastrointestinal stromal tumor patients. Prospective studies are needed to evaluate immune humoral alterations and to define the real incidence of infectious events, including viral reactivations.

Key words: chronic myeloid leukemia, imatinib, hypogammaglobulinemia.

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Introduction

Imatinib mesylate (Gleevec; Novartis Pharmaceuticals, East Hanover, NJ, USA) is a selective tyrosine kinase inhibitor effective in the treatment of malignancies characterized by constitutive tyrosine kinases activation such as chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST).

Imatinib has been rationally developed as a selective inhibitor of target proteins involved in cellular transformation including BCR-ABL, ABL, c-KIT, ARG and PDGFR-α and β. However, tyrosine kinases are involved in various intracellular signaling pathways and several *in vitro* experimental studies demonstrated that imatinib affects immune response. Differentiation, cytokine production and ability to elicit T cell responses of dendritic cells were impaired by imatinib treatment *in vitro* and in animal models.[1-3] Moreover, imatinib reduces T-cell proliferation by arresting the cells in G0/G1 and inhibits T-cell effector functions affecting T-cell receptor signal transduction.[4-6] The inhibitory effect of imatinib on memory cytotoxic T-cell expansion, B-cell proliferation as well as IgM production in response to lipopolysaccharide (LPS) stimulation may be therapeutically useful in treatment of autoimmune diseases.[7] Physiologically, tyrosine kinases play a prominent role in both T-cell and B-cell receptor signal transduction: c-ABL and ARG tyrosine kinases are necessary for TCR dependent transcriptional activation. Primary T-cells lacking functional ABL showed decreased interleukin-2 production and cell proliferation in response to TCR stimulation.[8] Moreover, ABL phosphorylates the B-cell receptor (BCR) co-receptor CD19, suggesting a role for ABL also in regulation of B-cell proliferation.[9] According to this observation, ABL knocked-out mice display several defects in T- and B-cell development.[10]

In spite of *in vitro* experimental evidence of imatinib-related immunity impairment, several years after its introduction in clinical practice no significant major incidence of infection has been reported. In the present study, we report a noteworthy reduction of immunoglobulin (Ig) levels in 72 CML patients and in 15 GIST patients treated with imatinib at standard dosage suggesting the direct role of imatinib in a significant alteration in humoral immunity.

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Design and Methods

Patient population

Seventy-two CML patients and 15 GIST patients treated with imatinib mesylate at standard dosage were enrolled in this retrospective study. All patients were seen in the Department of Haematology and Oncology of Modena University Hospital and of the Piacenza Civil Hospital (Italy). This study was approved by the Ethics Committee of Modena and informed consent was obtained according to the Helsinki declaration. CML patients at the time of data collection were on average 61 years old (range 26-86), 39 were males and 33 females. Forty-three CML patients received imatinib as first line treatment, whereas 29 CML patients received a prior cytoreductive therapy. In particular, 18 CML patients received interferon-α (IFN-α), 4 patients hydroxyurea (HU), 5 patients both IFN-α and HU, and 2 cytosine arabinoside (Ara-C). The daily median dosage of imatinib was 385 mg/d (range 300-600 mg/d), for a median of 32 months of therapy (range 6-64) with a median cumulative dosage of 374 gr (range 72-937). Average age of GIST patients (n=15) was 64 years (range 33-79). They were treated with imatinib as first line therapy after surgery. All GIST patients were treated at the dosage of 400 mg/d for a median time of treatment of 30 months (range 5-48) with a median cumulative dose of 360 gr (range 48-840). In addition, we collected data on gammaglobulin serum levels of 20 CML patients followed before imatinib became available for CML treatment.

Disease monitoring

For CML patients, minimal residual disease was monitored by cytogenetic, FISH and RT-PCR analyses. These evaluations were performed on bone marrow and peripheral blood samples at diagnosis and every three months until complete cytogenetic response, subsequently every four months on peripheral blood, and yearly on blood marrow samples. Cytogenetic studies were carried out on bone marrow samples by standard G-banding technique on 20 metaphases. FISH studies were performed on 300 cells using the Poseidon BCR/ABL t(9;22) Dual-color Dual-fusion translocation probe (Kreatech Biotechnology B.V., Amsterdam, ND). Moreover, RT-PCR was performed by standardized quantitative method and β_2-microglobulin was used as control gene.[11,12]

Hematologic and immunological evaluation

Data on blood cell counts, serum biochemistry, serum protein electrophoresis, serum levels of IgG, IgA and IgM were collected at diagnosis and after imatinib treatment. In 15 patients the same evaluation was also available at diagnosis and after 18 and 36 months of therapy. In 23 CML patients, we obtained percentages and absolute counts of lymphocyte subpopulations after imatinib treatment.

Statistical methods

Data were analyzed using SPSS for Windows Version 14.0 (SPSS Inc, Chicago, IL, USA). Data were presented as median and range for continuous variables and as count and percentage for categorical variables. Comparisons of distributions of continuous variables were made by two-tailed *t*-test. The Pearson coefficient for estimating correlations between quantitative parameters was used. Analyses of variance of repeated measures were used to study the temporal evolution of quantitative variables. An effect was considered statistically significant at $p \leq 0.05$.

Results and Discussion

Sixty-nine out of the 72 CML patients treated with the imatinib regimen reached a complete cytogenetic response, 2 patients reached a major cytogenetic response and only one did not reach a partial cytogenetic response. A minor and a major molecular response was reached respectively in 38 and 33 patients, according to the response criteria proposed by Baccarani *et al*.[13]

The median gammaglobulin dosage in all 72 CML patients was 0.92 g/dL (range 0.7-2.1) at baseline and only 5 patients presented a dosage slightly inferior to the normal range (between 0.7 and 0.8 g/dL). After a median treatment time of 32 months, almost all 72 CML patients experienced a significant reduction of serum gammaglobulin with a median dosage after treatment of 0.81 g/dL (0.26-1.63) ($p<0.0001$) (Table 1). In particular, only 3 patients maintained a stable gammaglobulin dosage respectively after 61, 56 and 9 months of treatment, whereas 30 patients (42%) developed hypogammaglobulinemia, 7 of whom (10%) of severe entity (gammaglobulin <0.5 g/dL). Interestingly, both CML patients treated with imatinib as first line therapy and patients pre-treated with HU, Ara-C and INF-α developed a similar reduction in gammaglobulin level, being the median dosage 0.90 and 0.94 g/dL at baseline compared to 0.81 and 0.82 after treatment respectively ($p<0.001$) (Figure 1).

A similar reduction of G, A and M immunoglobulin dosage was documented. Immunoglobulin dosages were 961, 201 and 96 mg/dL at baseline compared to 832, 174 and 59 mg/dL after treatment for IgG, IgA and IgM respectively (percentage reduction 23.2±13.2% for IgG, 25.0±14.1% for IgA and 35.4±16.5% for IgM). After treatment, 19 patients had IgG and IgM dosage

Table 1. Comparison of gammaglobulin and immunoglobulin dosage in 72 chronic myeloid leukemia patients before and after imatinib treatment.

	Pre-imatinib treatment Median (range)	*Post-imatinib treatment Median (range)*	*p value*
Gammaglobulin	0.92 (0.7-2.1)	0.81 (0.3-1.6)	$p<0.0001$
IgG	961 (560-1500)	832 (259-1568)	$p<0.0001$
IgA	201 (44-541)	174 (4-455)	$p=0.001$
IgM	96 (26-233)	59 (4-187)	$p<0.0001$

Normal range: gammaglobulin:0.8-1.35 g/dL; IgG: 800-1700 mg/dL; IgA: 100-490 mg/dL; IgM: 50-320 mg/dL.

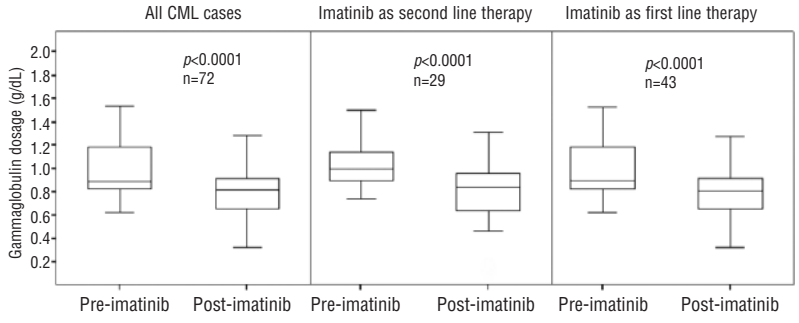

Figure 1. Box-plot representation of gammaglobulin dosage in chronic myeloid leukemia patients before and after imatinib treatment. Note the reduction of gammaglobulin after treatment in subset of patients treated with Imatinib as first line therapy (n=43) as well as in subset of patients pre-treated with interferon-α or hydroxyurea (n=29) ($p<0.0001$, *t*-test). The horizontal line inside boxes indicates the median value.

inferior to normal laboratory range (800 and 50 mg/dL respectively) and 8 patients presented a reduction of IgA dosage (<100 mg/dL). Both CML patients treated with imatinib as first line therapy and those pre-treated with other cytoreductive therapy displayed a similar decrease in immunoglobulin levels. We did not observe significant differences in reduction of gammaglobulin levels compared to CML response to treatment. In particular, a median decrease in gammaglobulin equal to 27.1±19% and 27.6±24% was identified in patients with a major and minor molecular response. Moreover, in patients with available gammaglobulin and IgG, IgA and IgM serum levels dosage at baseline and after 18 and 36 months of treatment, a progressive reduction of both has been documented. In this cohort of patients the median gammaglobulin dosage was 0.89 mg/dL (range 0.74-1.41 mg/dL) at baseline, 0.83 mg/dL (range 0.28-1.20 mg/dL) after 18 months and 0.78 mg/dL (range 0.27-1.35 mg/dL) after 36 months ($p<0.001$). Although a clear linear correlation between the cumulative dosage of imatinib and the gammaglobulin reduction was not evident, patients who had assumed less than a cumulative dosage of 200 gr of imatinib had a median gammaglobulin reduction of 7.4% whereas patients who had assumed more than 200 gr had a median reduction of 22.4% ($p=0.013$).

In order to define if the decrease in serum antibody level could be a consequence of imatinib treatment or disease related, gammaglobulin serum levels were also evaluated in 20 CML patients treated before the introduction of imatinib in clinical practice and in 15 GIST patients. Among CML patients treated in the pre-imatinib era, no significant reduction in gammaglobulin dosage developed during treatment. A slight hypogammaglobulinemia developed only in 4 young patients (average age, 45 years old) during accelerated or blastic phase, probably as a consequence of the aggressive chemotherapy regimens established. Significantly, GIST patients showed a median gammaglobulin dosage of 0.83 g/dL after therapy compared to 0.98 g/dL at baseline ($p=0.02$) with a percentage reduction of 21.9±14.6%. Hypogammaglobulinemia developed in 7 out of 15 GIST patients (46%), and reached a severe grade (<0.5 g/dL) in 2 GIST patients after imatinib treatment (Figure 2).

In both CML and GIST patients no statistically significant reduction of the lymphocyte count developed during imatinib therapy. The analysis of lymphocyte subpopulations was available in 23 CML patients after treatment: a circulating $CD19^+$ lymphocyte count less than 50 cell/m^3 was observed in 5 patients, 3 of whom with severe hypogammaglobulinemia; a clear alteration of the $CD4^+/CD8^+$ ratio was observed in only 3 patients. One serious infectious event (pneumonia) occurred in a CML patient in whom a severe hypogammaglobulinemia was documented (gammaglobulin dosage, 0.38 g/dL).

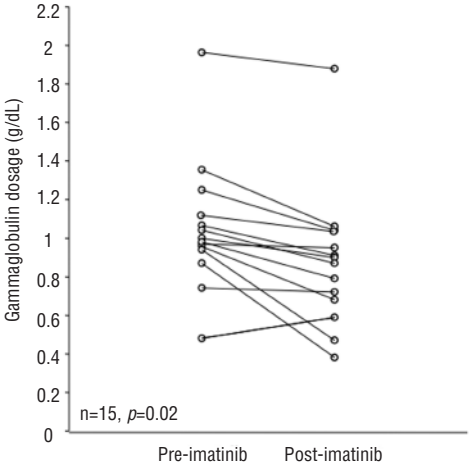

Figure 2. Representation of gammaglobulin dosage measured both before and after imatinib treatment in 15 GIST patients. We observed a statistically significant gammaglobulin reduction with percentage of decrement equal to 21.9±14.6% ($p=0.02$, *t*-test).

A single previous study describes only a slight reduction of the Ig levels in imatinib treated CML patients who became resistant or intolerant to interferon-α.[14] Authors cannot rule out the possibility that the previous treatments favor the development of hypogammaglobulinemia in this subset of patients. They also assume that hypogammaglobulinemia development may be related to an imatinib-mediated impairment of Philadelphia-positive B-lymphocytes or, more likely, to the inhibition of physiological ABL tyrosine kinase activity.

The data reported here confirm and expand this initial observation. Indeed, the Ig reduction was also observed in CML patients treated with imatinib as first line ther-

apy and therefore it is certainly not ascribed to previous treatments. Moreover, a significant reduction of gammaglobulin equally arose also in the imatinib treated GIST patients in whom no baseline alteration of lymphopoiesis was expected. No significant hypogammaglobulinemia was observed in CML patients followed in the pre-imatinib era. These data demonstrated that the Ig reduction observed must be considered the consequence of the imatinib treatment.

Several years after the introduction of imatinib in clinical practice no significant major incidence of infection has been reported but much experimental evidence demonstrates that imatinib can impair many of the different cellular functions involved in the immune response. On the basis of this experimental evidence, it is not surprising that imatinib could determine *in vivo* a reduction of antibody production and an impairment of humoral immunity. We noted only one severe infective event out of 87 patients, however we are aware that some infectious episodes may be underestimated in retrospective studies. Therefore, we suggest a periodical monitoring of gammaglobulin dosage and special clinical attention to infectious events in patients who develop a severe hypogammaglobulinemia. Prospective studies are needed to evaluate modification of T- and B-cell immunity of these patients during the treatment and to define the real incidence of infectious events, including viral reactivations.

Authorship and Disclosures

RS, RMaf and RMar conceived the study, analyzed and interpreted the data and wrote the paper; FP, GLo and GLu collected and interpretated data about GIST patients, SM performed and interpretated quantitative PCRs; AA, TE, PA, AF, GLe, DV collected and interpreted data about CML patients, GT interpreted data and gave financial support. All authors have critically revised the paper and gave their approval to the final version.

The authors reported no potential conflicts of interest.

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