Chapter 6

Hypocalcemia and hypophosphatemia are no predictive biomarkers of imatinib efficacy in patients with gastrointestinal stromal tumors

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Abstract

Gastrointestinal stromal tumors (GIST) are rare neoplasm of the gastrointestinal tract. First line treatment of metastatic or irresectable disease consists of imatinib, a tyrosine kinase inhibitor. A minority of the patients has primary resistance to this therapy. Recognition of these primary resistant patients with reliable upfront or early predictive biomarkers would enable early switch to effective treatment. In patients with chronic myelogenous leukemia treated with imatinib, development of hypophosphatemia was associated with response. We therefore evaluated whether hypophosphatemia or hypocalcaemia are predictive biomarkers for GIST patients treated with imatinib.

GIST patients treated with imatinib between 2004 and 2007 at the University Medical Center Groningen formed the test cohort. We longitudinally measured calcium, phosphate and parathyroid hormone levels before and during imatinib treatment. The efficacy of imatinib was defined as time to progression with response assessment by computed tomography. Results were validated in an independent patient cohort treated at the Erasmus University Medical Center between 2002 and 2007. All patients gave written informed consent.

In the test cohort (n = 35 patients) imatinib initiation led to a significant decrease in the serum concentration of calcium corrected for albumin and phosphate, while parathyroid hormone concentrations increased. Patients who developed hypophosphatemia had a shorter time to progression (median 8.4 months) than patients who did not develop hypophosphatemia (median 25.3 months), p = 0.016. In the validation cohort (n = 31) a similar decrease in plasma calcium and phosphate was found, but a difference in time to progression between patients with and patients without hypophosphatemia could not be confirmed.

Although calcium and phosphate concentrations in serum decrease during imatinib treatment, these changes do not predict treatment efficacy.

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract with an annual age-adjusted incidence rate of 0.68 per 100,000 capita [1]. The majority of GISTs harbors a gain-of-function mutation in the proto-oncogene *c-kit* [2], which encodes KIT, or in the gene encoding PDGFRα [3, 4]. The prognosis of patients with GIST has changed significantly since imatinib which inhibits both KIT and platelet derived growth factor receptor α (PDGFRα) became available. An observed off-target phenomenon of imatinib treatment is the development of secondary hyperparathyroidism with hypocalcaemia and hypophosphatemia [5, 6]. In a study of 36 patients with chronic myelogenous leukemia (CML) treated with imatinib, phosphate and calcium concentration in serum decreased significantly dur-

ing treatment compared to baseline [7]. This decrease became evident after 3 months of therapy and lasted until the final analysis after one year of treatment. Parathyroid hormone was increased after 9–12 months of treatment in 19 out of 33 evaluable patients. In the group of patients who actually developed hypophosphatemia, all patients had hyperparathyroidism. An interesting observation was that reduction of phosphate levels was significantly higher in patients with a major genetic response of their CML. The reduction of phosphate level after 3 months was 23% in this group, while it was only 11% in patients without a major genetic response.

Although the majority of patients with GIST benefit from imatinib, a subgroup of about 15% experience disease progression within 3 months [8, 9]. A D842V mutation in exon 18 of the *PDGFRA* gene in GIST is a known predictive biomarker for unresponsiveness to imatinib [10]. However, that mutation is present in less than 5% of GISTs. Recognition of primary resistance with reliable upfront or early predictive biomarkers would enable an early switch to another tyrosine kinase inhibitor like sunitinib [11], safe costs and protect patients from unnecessary side effects. Preferably such a biomarker should be easy to measure and interpret. The objective of our study is to analyze whether early changes in serum phosphate and calcium levels predict imatinib efficacy in GIST patients, and to validate findings in an independent patient cohort.

Patients and methods

Patients

Thirty-five patients with a histologically confirmed locally advanced or metastatic GIST who started treatment with imatinib between 2001–2007 at the University Medical Center Groningen were included in the test cohort. For validation 31 GIST patients treated with imatinib starting between 2002–2007 from the Erasmus University Medical Center in Rotterdam were included. Patients who used calcium or vitamin D supplements were not eligible. All patients gave written informed consent. For the test cohort serum samples and for the validation cohort plasma samples were available for biochemistry analyses. Samples were taken at baseline, after 1 week, 4 weeks, 3 months and 6 months of imatinib treatment.

Electrolyte and hormone measurement

After collection of blood, tubes were placed on ice immediately. Blood samples were centrifuged at 4°C within 30 minutes of collection and serum (test cohort) or plasma (validation cohort) was separated. The samples were stored in a freezer at -20°C until analysis.
Calcium, phosphate and albumin were measured on a Roche Modular P automated analyzer (Roche Diagnostics, Mannheim, Germany). Normal value for calcium ranges from

2.20–2.60 mmol/L, for phosphate from 0.70–1.50 mmol/L and for albumin from 35–50 g/L. We corrected calcium for albumin concentration, with the following formula: calcium(corr) = calcium (mmol/L) + 0.02 · (40 - albumin (g/L)). Hypophosphatemia and hypocalcaemia were defined as a phosphate level < 0.70 mmol/L and calcium(corr) level < 2.20 mmol/L, respectively. In the test cohort, also parathyroid hormone and parathyroid hormone-related peptide (PTHrp) were measured. Serum parathyroid hormone was analyzed using a two-site chemiluminescent enzyme-labeled immunometric assay (Immulite 2500, Siemens, The Hague, the Netherlands). The normal value is < 8.7 pmol/L. PTHrp was measured with an immunoradiometric assay (Diagnostic System Laboratories, Beckman Coulter). The normal value is < 2.0 pmol/L. Hyperparathyroidism was defined as parathyroid hormone levels > 8.7 pmol/L.

Response assessment

Response to imatinib treatment was evaluated with computed tomography (CT) scans every 3 months during the first year of treatment. Thereafter the interval was at the discretion of the treating physician. Response was assessed according to RECIST 1.1 [12]. The primary endpoint was time to progression (TTP), defined as time from start of imatinib therapy until the date of the first CT scan demonstrating progressive disease. Patients were censored on the last date of follow-up when no progressive disease occurred.

Statistics

Statistical analysis has been performed with Predictive Analytics SoftWare (PASW) v18.03. Changes in serum or plasma concentrations were evaluated with the paired samples T-test. For the comparison of electrolyte levels between the cohorts the Mann-Whitney U test was used. The log rank test was applied to analyze the correlation between biochemical changes and TTP. Time to progression was plotted in a Kaplan-Meier curve. Correlations were described by the Pearson's correlation coefficient (r). Significance statements refer to p-values of two-tailed tests < 0.05.

Results

In het test cohort 35 patients (23 male, 12 female) with a median age of 62 years were included. Of these patients, 29 were treated for metastatic disease and 6 for unresectable primary disease. Two patients were treated with 800 mg imatinib daily, the remaining with 400 mg daily. The median follow-up was 43 months (range 4–114). Patient characteristics are summarized in Table 1. Table 2 shows the baseline levels of phosphate, calcium(corr) and parathyroid hormone.

Serum phosphate and calcium (Figure 1) levels declined during imatinib treatment. The median decrease in phosphate at 4 weeks was 0.23 mmol/L (SD 0.15). Six patients (17%) developed hypophosphatemia during the first six months of treatment; one within 4 weeks and five within 3 months after start of imatinib. Twenty-four patients (69%) de-

veloped hypocalcaemia within 6 months. The median calcium reduction at 4 weeks was 0.15 mmol/L (SD 0.11). At the same time, parathyroid hormone was rising, and 19 patients (54%) developed hyperparathyroidism. The median increase in parathyroid hormone at 4 weeks was 2.25 pmol/L (SD 3.37). PTHrp was below the detection limit in all patients in all samples. For TTP analysis, one patient was excluded because of non-measurable lesions. We found that the median TTP in patients who developed hypophosphatemia was shorter (8.4 months) compared to patients in which phosphate levels remained in the normal range (25.3 months), $p = 0.016$ (Figure 2).

In the validation cohort 31 patients (19 male, 12 female) with a median age of 60 years were included. Of these patients, 23 were treated for metastatic disease and 8 for unresectable primary disease. The median follow-up was 53 months (range 8–108). Patient and tumor characteristics are summarized in Table 1. One patient was left out of the analyses, because the plasma sample after one week of treatment was missing. Baseline phosphate levels were in the normal range in 30 patients. Of note is that the median baseline calcium level was below normal. A significant discrepancy in baseline values of phosphate and calcium levels was observed between both cohorts. For the calcium levels this difference persisted at all later time points, whereas for phosphate levels this dissimilarity remained only at 1 week. Nevertheless, we found a similar decrease in phosphate levels as in the test cohort (Figure 3): the median decrease at 4 weeks was 0.17 mmol/L (SD 0.19). Ten patients (33%) developed hypophosphatemia.

For six patients from the validation cohort, the baseline CT scan was not available. In those cases we used the TTP as noted in the patient record. In the validation cohort, we found no difference in TTP for patients treated in the palliative setting with imatinib between them who developed hypophosphatemia or hypocalcaemia and those who did not.

	Test cohort *n* = 35		Validation cohort *n* = 31	
Sex (male / female)	23 / 12		19 / 12	
Age median (range)	62 (23–81)		60 (46–81)	
Disease stage	*n*	%	*n*	%
Primary disease only	6	20	8	26
Metastatic disease	29	80	23	74
Primary tumor location	*n*	%	*n*	%
Stomach	13	37	14	45
Small bowel	13	37	7	22
Colon	3	9	4	13
Rectum	2	6	3	10
Unknown/other	4	11	3	10
Imatinib dose (daily)				
400 mg (*n*)	33		31	
800 mg (*n*)	2		-	
Best response according to RECIST 1.1 on CT scan	*n*	%	*n*	%
CR	2	6	-	-
PR	18	51	24	77
SD	10	29	6	20
PD	4	11	1	3
NM	1	3	-	-
Follow-up median (range) in months	43 (4–114)		53 (8–108)	

Table 1: Patient characteristics.
CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, NM = non-measurable, TTP = time to progression, SD = standard deviation

	Test cohort *serum*	**Validation cohort** *plasma*
Phosphate(mmol / L)	1.06 (SD 0.13)	0.96 (SD 0.17)
Calcium(corr) (mmol / L)	2.34 (SD 0.11)	2.17 (SD 0.09)
PTH (pmol / L)	5.80 (SD 2.56)	

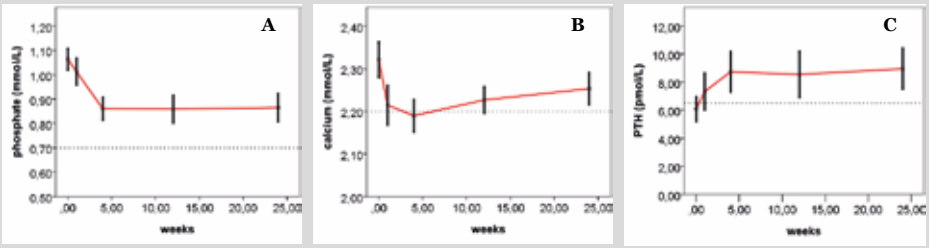

Figure 1: Mean values of serum phosphate (A), albumin-adjusted serum calcium (B) and serum parathyroid hormone (C) in the test cohort.
The error bars represent the 95% confidence interval. Dotted line in Figure A and B is the lower limit of normal; dotted line in Figure C is the upper limit of normal.

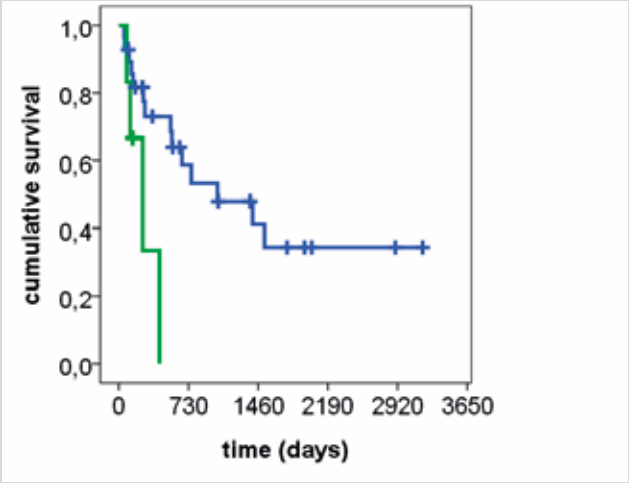

Figure 2: Kaplan-Meier curve of TTP for patients with a GIST developing hypophosphatemia (green, *n* = 6) versus patients in which serum phosphate remained within normal range (blue, *n* = 28) after 3 months of treatment with imatinib, *p* = 0.016.

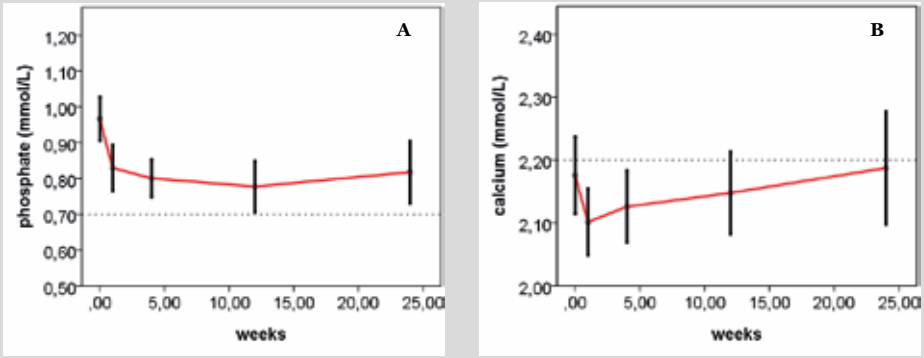

Figure 3: Mean values of plasma phosphate (A) and albumin-adjusted plasma calcium (B) of the validation cohort.
The error bars represent the 95% confidence interval. * = *p*-value < 0.05 versus baseline. Dotted line in both figures is the lower limit of normal.

Discussion

We found a significant decrease in calcium and phosphate levels after only one week of imatinib treatment in patients with GIST. Simultaneously parathyroid hormone increased and after 4 weeks a new equilibrium seemed to have been established. However, development of hypophosphatemia or hypocalcaemia was not associated with time to progression in our study.

Moreover, 7 patients with unresectable primary disease only developed resectable disease after initiation of imatinib, underwent surgery and could quit imatinib therapy. Two of them had developed hypophosphatemia. Although this concerns a very small sample size, it underscores that hypophosphatemia is not a reliable marker for imatinib ineffectiveness.

The changes in calcium and phosphate metabolism during imatinib treatment we found are in line with other studies [5–7, 13, 14]. In our cohorts, a plateau was reached after 4 weeks, which is earlier than described before [6, 7].
The combination of an elevated parathyroid hormone and hypocalcaemia is consistent with a diagnosis of secondary hyperparathyroidism, in this case likely as an off-target effect of imatinib therapy. Vitamin D deficiency or increased fibroblast growth factor 23 (FGF23) levels can be alternative or additional explanations for the observed anomalies, but others found no changes in FGF23 or vitamin D during imatinib therapy in patients with CML [15].

While growth failure is described in children treated with imatinib for CML [16], there are no reports of clinically relevant complications in adult patients due to hypocalcaemia and hypophosphatemia with prolonged imatinib therapy. The decline of calcium and phosphate in our cohorts was significant, but in absolute levels was not far below the lower

limit of normal and treatment was not necessary. Long-term follow-up of imatinib showed that serum calcium and phosphate remained lower than baseline, but the values did not reach limits associated with reduced mineralization [17]. The decline in serum levels is thus usually mild and asymptomatic, often even within the normal range. Remarkable is the observation that bone density is increased compared to baseline after 24 months imatinib treatment, contrary to most conditions with secondary hyperparathyroidism but in line with the suggestion of a calcium shift into the bone.

Since the introduction of targeted therapies, there is an unmet need for upfront (e.g. genetic tumor profile) or early predictive (normally treatment induced) markers to detect primary resistance because those therapies are expensive and can induce serious side-effects. Therefore, much effort is put in research on biomarkers and clinical markers of response. We performed no mutation analyses for the genes encoding KIT or PDGFRα since those upfront predictive markers are not validated to guide first-line therapy choice.

Examples for early predictive markers are acneiform rash in association with response to cetuximab [18] and hypertension in association with efficacy of bevacizumab [19]. Hypomagnesaemia is identified as predictive off-target biomarker for efficacy of cetuximab treatment in colorectal cancer [20]. These examples illustrate that adverse events occurring in separate organs, apparently unrelated to the tumor, can predict tumor response to therapy. We could not confirm in the validation cohort that imatinib treated GIST patients who developed hypophosphatemia within the first 6 months of therapy have a worse prognosis than the patients who did not develop hypophosphatemia. Interestingly, the result of our test cohort seems in contrast with the observation in patients with CML: they had a better prognosis when they develop hypophosphatemia [7].

A drawback of our study is that the interval between CT scans more than one year after start of imatinib was not standardized and, as a consequence, differed between patients. CT scanning is currently the best available and reproducible method to measure lesions selected for response assessment.

Another point of criticism is the discrepancy we found between baseline calcium and phosphate levels in the two cohorts. This discrepancy can be authentic, but the claimed assay properties of equal outcome for serum and plasma may be incorrect. Another possibility for this discrepancy is a mutated calibration of the analyzer between measurements. Moreover, the quality of the samples might be affected by difference in duration of storing. In conclusion, in our retrospective analysis we cannot confirm the predictive value of imatinib induced hypophosphatemia and hypocalcaemia for efficacy in GIST patients. Prospective studies with imatinib should include these inexpensive and simple biomarkers in their analyses to validate properly for efficacy.

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