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Reproductive Hormone Profiles during Imatinib Therapy in Men with Chronic Myeloid Leukemia

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Abstract

Imatinib side effects related to testicular function have been reported in male patients with chronic myeloid leukemia. These include decreased testosterone levels, gynecomastia and impaired spermatogenesis. To further investigate testicular function in relation to imatinib treatment, a longitudinal study on reproductive hormone profiles was conducted in 17 male patients with chronic myeloid leukemia.

Blood samples were taken before and at one or more time points during imatinib therapy. Serum samples were analyzed for the hormones testosterone, estradiol, and luteinizing hormone (LH) to reflect testicular Leydig cell function. Sex hormone-binding globulin (SHBG) serum levels were measured to evaluate free testosterone, and serum levels of inhibin B and follicle stimulating hormone were measured to reflect spermatogenesis.

Out of the 17 patients included in the study, one patient developed gynecomastia after 7-10 months of therapy. Testosterone levels were generally low in the patients both before and during the study, and did not change in response to imatinib therapy. Conversely, SHBG levels decreased transiently at 3 and 6-9 months of therapy (p=0.002 and p=0.008, respectively). Estradiol levels decreased at 12-15 months of therapy (p=0.048). LH and hormones reflecting spermatogenesis were unchanged.

In conclusion, our longitudinal study of men with chronic myeloid leukemia showed a significant, but largely transient, decrease in SHBG levels in response to imatinib therapy. Testosterone levels were low in the men both before and during imatinib therapy.

Keywords: Chronic myeloid leukemia; Imatinib; Sex hormone-binding globulin; Testosterone; Gynecomastia

Introduction

Imatinib mesylate is a potent inhibitor of the oncogenic tyrosine kinase BCR-ABL1, and a targeted therapeutic agent in the treatment of chronic myeloid leukemia (CML). The small molecule also inhibits tyrosine kinases associated with cKIT and platelet derived growth factor (PDGF) receptors [1]. Though the drug is generally well tolerated [2], impaired testosterone production after imatinib therapy has been reported [3,4]. Reduced testosterone levels can, amongst other symptoms, result in gynecomastia, which has been reported as a side effect of tyrosine kinase inhibitor therapy [3,5-8]. Additionally, case reports of imatinib-related severe oligozoospermia have appeared [9,10]. Pathophysiologically, inhibition of cKIT and PDGF receptors is thought to be involved in these unintended testicular effects, in that both play crucial roles in Leydig and Sertoli cell development and function and in germ cell differentiation [11-13].

To further investigate potential testicular effects of imatinib therapy, we performed a prospective study characterizing the reproductive hormone profiles in male CML patients before and during imatinib therapy. Testicular Leydig cell function and androgen status were monitored via analysis of testosterone, estradiol, luteinizing hormone (LH), and sex-hormone binding globulin (SHBG) levels. In serum, testosterone binds to SHBG and albumin, meaning that only a fraction of testosterone circulates as free (and thus bioactive) testosterone. In normal men, LH, SHBG and estradiol increases with age, whereas testosterone is relatively stable until it decreases in the elderly [14]. In terms of gynecomastia, typical risk factors are increased SHBG, reduced testosterone or free testosterone, increased estradiol, or an increased estradiol/testosterone ratio [15-17].

Spermatogenesis and Sertoli cell function were evaluated through measurement of inhibin B and follicle stimulating hormone (FSH) serum levels. In normal men, FSH increases with age whereas inhibin B, like testosterone, is stable until it decreases in the elderly men [14].

Subjects and Methods

Participants

The investigation was a spin-off study to two clinical studies comparing, respectively, standard dose (400 mg/day) with high dose (800 mg/day) imatinib therapy [18], and imatinib and the combination of imatinib and pegylated interferon alpha-2B [19]. Patients in centers included in the spin-off protocol (hematology centers in Sweden, Finland and Denmark) were invited to participate in the present study, only if they had already agreed to be included in one of the mentioned

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clinical trials. For the latter clinical trial, only patients randomized for imatinib alone could participate in the spin-off study. Target sample size was 30 male patients. All subjects received imatinib throughout the study period.

Blood samples were taken just before therapy onset (baseline) and in connection with control visits during therapy (at 3, 6, 9, 12 and/or 15 months after start of imatinib therapy). Blood samples were taken from an antecubital vein and centrifuged after clotting. Serum was stored at -20°C until analysis. All samples were analyzed in the same laboratory, and samples from a given patient were analyzed in the same assay run. For ethical reasons, no semen samples were provided.

The relevant local ethical committees approved the study, procedures were in accordance with the Helsinki Declaration of 1975, and all participants gave their informed consent.

Hormone analyses

Testosterone and estradiol were measured using time-resolved fluoroimmunoassays (DELFIA; Wallac, Finland) with detection limits of 0.23 nmol/l and 15 pmol/l, respectively, and intra- and interassay coefficients of variation (CV) less than 6%. Inhibin B was analyzed by a double-antibody enzyme immunometric assay (Oxford Bio-Innovation Ltd., UK) with a detection limit of 20 pg/ml and intra- and interassay CVs less than 12 and 17%, respectively. LH, FSH, and SHBG were measured by time-resolved immunofluorometric assays (DELFIA).

The detection limits were 0.05 IU/l, 0.06 IU/l, and 0.23 nmol/l, respectively. In all three assays, intra- and interassay CVs were less than 8%. Normal reference range for FSH, LH, SHBG and Inhibin B were based on the methods described above. For testosterone and estradiol, normal ranges were based on radioimmunoassays (Coat-a-count, Siemens Healthcare Diagnostics Inc., LA for testosterone and Pantex, Santa Monica for estradiol).

Calculations

The ratios testosterone/LH, inhibin B/FSH, and estradiol/testosterone were computed. Free testosterone was calculated according to Vermeulen et al. [20], involving the measured testosterone and SHBG serum levels and a fixed albumin concentration of 43 g/l. Delta changes in hormone levels in response to imatinib therapy were calculated by subtracting baseline hormone levels from hormone levels measured at each time point during therapy.

Direct hormone assay comparisons showed that the testosterone Coat-a-count assay used for normal reference analysis resulted in hormone levels averagely 16% lower than the testosterone DELFIA assay used for patient analysis. For estradiol, the Pantex assay used for normal reference analysis gave on average 43% higher levels than the DELFIA assay used for patient analysis. Therefore, for testosterone and estradiol reference ranges, hormone levels were normalized according to the assay differences, making the reference range directly comparable to patients' hormone levels.

To compare the patients' hormone levels to those of a similar aged background population, we calculated age specific Z-scores for each patient and at each time point for the individual hormones. A Z-score (calculated as the difference between the population's mean hormone level and the patient's hormone level divided by the standard deviation of the population) between -2 and +2 can be defined as being within the reference range of the background population. A group of 938 healthy Danish men aged 20-75 years defined the reference range, and from this group, men aged in the range +/- 5 years of a given patient's age, were used to calculate the Z-score for this patient. For testosterone and estradiol Z-scores, normalized hormone levels were used due to the assay differences described above, and to obtain the normal distribution required for Z-score calculations, all hormone values were transformed. Z-scores were only used for illustrative and descriptive (not statistical) purposes.

Average monthly percentage changes in hormone levels were calculated from the slopes of regression lines made for each patient and each hormone.

Statistics

For statistical purposes, the time points for, respectively, 6 and 9 months, and 12 and 15 months, were grouped, using the mean of the hormone values when both were measured. The possible influence of imatinib therapy on serum hormone levels or ratios was analyzed using the non-parametric Wilcoxon matched pairs signed rank sum test, comparing hormone levels at different time points of imatinib therapy with pre-therapy hormone measurements. This is identical to testing the delta changes in hormone levels against zero using the same Wilcoxon analysis). A trend analysis through serial time points was also conducted for each hormone as follows: a linear regression line, based on ln transformed hormone values, was conducted for each subject, and the beta-values (slopes) for these lines were tested against zero in a one-sample *t*-test. Both Wilcoxon matched pairs signed rank sum tests and the trend analyses were made on all included patients as well as on the subgroup of patients with a complete data set (blood samples taken at 0, 3, 6-9 and 12-15 months).

Potential abnormalities in hormone profiles of a patient developing gynecomastia in response to imatinib therapy, or receiving a different dose of imatinib therapy, were analyzed in a one-sample *t*-test, testing regression line slopes for the remaining patient group against the slope for the patient in question. The p-values of 0.05 were considered significant. The software program PASW statistics 18 was used for data processing and statistical analysis.

Results

A total of 31 patients were included. Of these, 17 had blood samples taken immediately before and at one or more time points after therapy onset and were thus included in the analyses. Nine of the 17 patients had a complete data set, with blood samples taken at 0, 3, 6-9 and 12-15 months. Fourteen patients were excluded from analysis due to missing pre-treatment samples or because no samples were taken after onset of imatinib therapy.

The included 17 patients were aged 22-67 years (median 49 years) and had a body mass index of 20.7-39.7 kg/m^2 (median 27.6 kg/m^2). Imatinib doses were 400 mg/day, except for patient #15 who received 800 mg/day. Patient #11 (64 years) complained about soreness around the nipples after 7 months and had clinical signs of gynecomastia after 10 months of therapy. This patient also took spironolactone, and had been taking this for years without side effects. There were no reports of gynecomastia in the other 16 patients.

Line plots of age specific Z-scores for LH, FSH, testosterone, inhibin B, estradiol, and SHBG serum levels for each patient are depicted

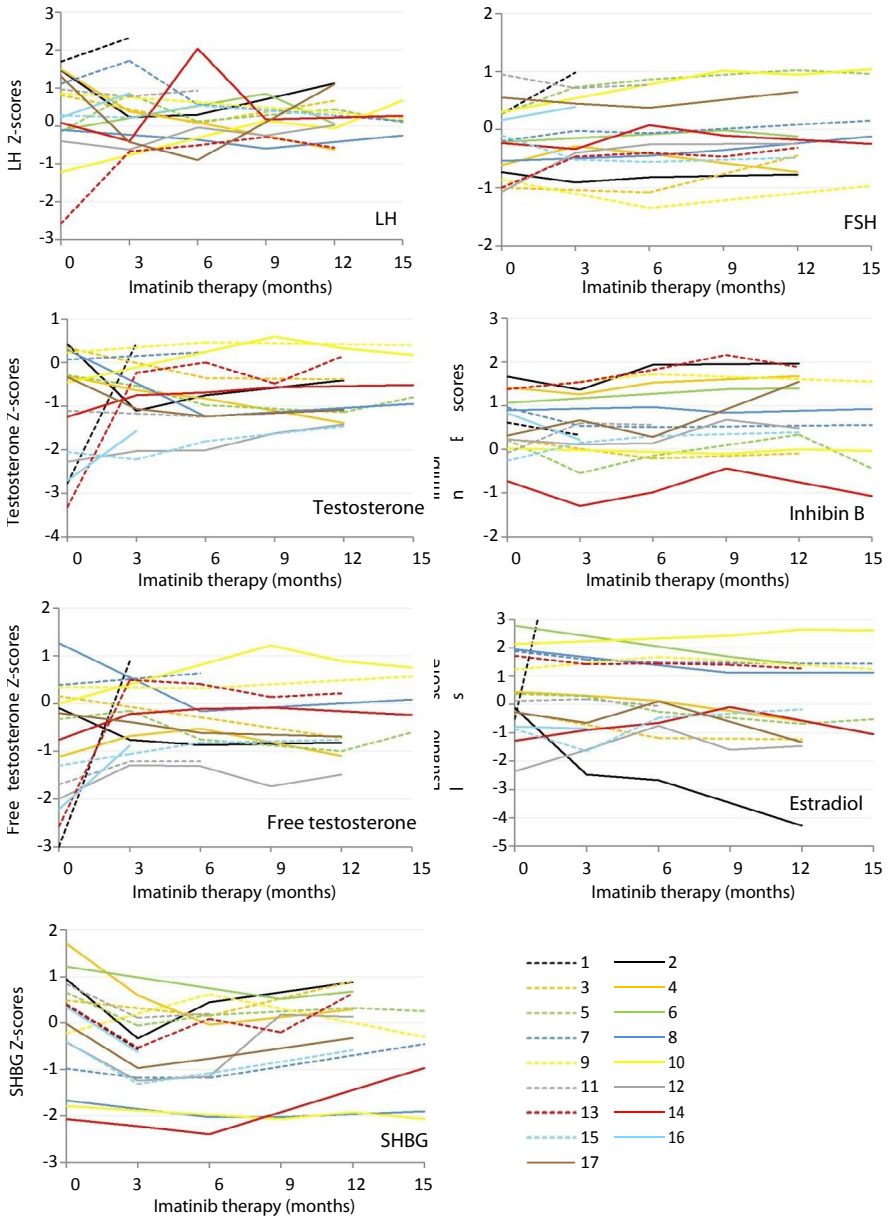

Figure 1: Individual line plots of age-specific Z-scores before and during imatinib therapy for the hormones LH, FSH, testosterone, inhibin B, free testosterone, estradiol, and SHBG in 17 men with CML. Each line represents one patient. The Z-scores were calculated using hormone data from healthy Danish men aged +/- 5 years of the individual patient's age, and serve to illustrate how the patients' hormone levels lie, as compared to an age-matched background population. Z-scores between -2 and 2 are considered to be within the reference range of the age-matched background population. Abbreviations: LH: luteinizing hormone; FSH: follicle stimulating hormone; SHBG: sex hormone-binding globulin.

in figure 1. For LH, FSH, inhibin B and SHBG, the vast majority of the patients' Z-scores lay within -2 and +2, thus illustrating that overall the patients are within the reference range of an age-matched background population for these hormones, both before and during imatinib therapy. Patients' testosterone and free testosterone levels were generally in the lower part of the reference range. Four patients (#1, #12, #13 and #16) had testosterone and free testosterone Z-scores at or below the lower limit of the reference range at baseline, which moved into the reference range in response to therapy. For estradiol, most patients were within the reference range, whereas one patient (#12) had very low levels which increased to some extent in response to therapy, and another patient (#2) experienced a marked decrease in estradiol during imatinib therapy.

Hormone levels and delta changes in hormone levels at the different time points are summarized in (Table 1) and monthly percentile changes are given in (Table 2). The Wilcoxon analysis of all 17 patients showed that SHBG levels decreased significantly at 3 and 6-9 months ($p<0.01$ for both), whereas no significant change could be found at 12-15 months of therapy. Free testosterone was borderline significantly increased ($p=0.05$) after 3 months of therapy, and estradiol and the

		Hormone levels before and during imatinib therapy				Hormone changes in response to imatinib therapy		
	Ref. range	0 months	3 months	6-9 months	12-15 months	Δ 3 months	Δ 6-9 months	Δ 12-15 months
N		17	12	15	14	12	15	14
LH (IU/L)	1.6;7.7	3.9 (1.0;11.0)	3.7 (2.3;15.8)	3.8 (2.1;7.1)	3.7 (2.4;6.1)	-0.3 (-3.0;4.8)	-0.1 (-3.7;2.7)	-0.2 (-4.0;2.0)
FSH (IU/L)	1.7;13.1	4.5 (2.4;13.0)	4.9 (2.3;13.1)	5.1 (2.3;11.4)	5.2 (2.5;9.7)	0.6 (-2.0;5.1)	0.4 (-1.6;3.5)	0.8 (-0.8;3.4)
T (nmol/L)	10.4;32.6	15.2 (6.3;23.7)	13.2 (8.5;23.6)	14.8 (9.4;27.9)	14.6 (11;23.1)	-0.3 (-9.5;17.3)	0.1 (-10.3;14.8)	-0.9 (-8.8;15.1)
Free T (pmol/L)	203;629	283 (90;612)	292 (194;517)*	306 (176;859)	285 (177;646)	53 (-99;428)*	36 (-287,507)	18 (-250;294)
E_2 (pmol/L)	58;182	77 (35;198)	63 (32;133)	78 (30;176)	66 (13;188)*	-4 (-36;11)	-9 (61;19)	-20 (-73;29)*
Inh B (ng/L)	45;294	198 (95;347)	181.5 (67;320)	190 (98;426)	228 (77;385)	-25 (-61;73)	15 (-65;104)	34 (-58;140)
SHBG (nmol/L)	18;72	39 (16;69)	27 (15;45)*	33 (16;57)*	33.8 (17;74)	-12 (-25;-1)*	-7 (-35;14)*	-1 (-30;10)
T / LH ratio	2.1;11.7	3.2 (0.6;7.1)	3.6 (1.5;8.1)	4.1 (1.7;8.0)	3.8 (2.5;10.1)	0.6 (-2.3;2.1)	-0.5 (-2.1;3.0)	-0.1 (-2.0;2.5)
E_2 / T ratio	2;8	6 (3;21)	5 (2;7)	6 (2;11)	4 (1;9)*	0 (-15;1)	0 (-14;4)	0 (-15;2)*

Median (range) for hormone levels and their ratios before and during imatinib therapy, and delta changes in hormone levels in response to treatment. Asterisks indicate a statistical difference between hormone levels at a given time point and hormone levels at 0 months. Reference ranges (9-95 percentiles) are based on data from 938 men aged 20-75 years from the general population. Abbreviations: Ref. range: reference range; LH: luteinizing hormone; FSH: follicle stimulating hormone; T: testosterone; E2: estradiol; inh B: inhibin B; SHBG: sex hormone-binding globulin

Table 1: Hormone levels for 17 patients with CML before and during imatinib therapy.

% per month	9 pt. with full data sets	All 17 pt.	All 17 pt. 0-6 mths.	Pt. #11	Pt. #15
LH	-0.1 (±3.9)	1.6 (± 5.0)	1.0 (±9.8)	-0.2	0.1
FSH	1.0 (±1.9)	2.2 (± 4.5)	2.4 (±5.9)	-2.2[b]	-1.5[c]
T	1.2 (±3.5)	4.9 (± 14.1)	4.8 (±16.1)	-1.0	2.0
Free T	1.2 (±3.3)	7.3 (± 20.4)	8.9 (±21.9)	3.7	2.1
E_2	-1.8 (±4.4)	-1.6 (± 3.4)	-0.8 (±5.1)	-1.0	3.1[c]
Inh B	1.6 (±1.8)[a]	0.3 (± 3.9)	-0.6 (±4.6)	7.5[b]	2.5
SHBG	0.7 (±2.4)	-1.0 (± 5.0)	-3.9 (±5.5)	-5.0	0.2

Percentile hormone changes per month are presented as mean (±SD) for, respectively, the subgroup of 9 patients with a complete data set, all 17 patients, all 17 patients at 0-6 months, patient #11, who developed gynecomastia, and patient #15, who received high-dose imatinib. a: Inh B levels increased significantly in response to imatinib therapy in the subgroup of nine patients with a complete data set. b: Patient # 11 was compared to all patients 0-6 months and differed significantly in terms of FSH and Inh B levels. c: Patient #15 was compared to patients with a complete data set and differed significantly in terms of FSH and estradiol levels. Abbreviations: pt: patients; mths: months; LH: luteinizing hormone; FSH: follicle stimulating hormone; T: testosterone; Free T: free testosterone; E2: estradiol; inh B: inhibin B; SHBG: sex hormone-binding globulin.

Table 2. Monthly percentile changes (mean (±SD)) in reproductive hormone levels in CML patients in response to imatinib therapy.

estradiol/testosterone ratio were significantly reduced after 12-15 months of therapy ($p<0.05$ for both). No statistical differences were found for serum levels of LH, FSH, testosterone, and inhibin B at 3, 6-9, or 12-15 months of imatinib therapy as compared to baseline levels.

Neither did the testosterone/LH nor the inhibin B/FSH ratio change at any time point as compared to before therapy. Trend analyses made on all 17 patients revealed no significant changes for any of the hormones.

When only the nine patients with full data sets were analyzed, similar, but not completely identical, results were obtained. Again SHBG levels were significantly reduced at 3 and 6-9 months ($p<0.01$ and $p=0.01$, respectively). Estradiol serum levels and the estradiol/testosterone ratio were borderline significantly reduced at 12-15 months ($p=0.05$ for both). Changes in free testosterone levels did not reach statistical significance ($p=0.08$ at 3 months) and no statistical differences were found for LH, FSH, testosterone, inhibin B, the testosterone/LH ratio or the inhibin B/FSH ratio at any time point. Trend analyses made on the nine patients with full data sets showed that inhibin B levels increased significantly in response to imatinib therapy ($p=0.03$), whereas all other hormones showed no linear changes.

Patient #11, who developed gynecomastia, had only blood samples taken at 0, 3 and 6 months. Therefore, linear regression lines involved in the analysis of his hormone levels as compared to the other patients' hormone levels were based only on these time points. Patient #11 differed significantly from the rest of the group in terms of FSH and inhibin B, with an average monthly increase of 7.5% in inhibin B levels (group mean was a decrease of 0.6% per month), and an average monthly decrease in FSH levels of 2.2 percent (group mean was an increase of 2.4% per month). No statistical differences were observed for the other hormones (Table 2).

Patient #15, who is the only one who received high-dose imatinib, had a complete data set and was therefore compared to the rest of the subgroup with complete data sets. He differed significantly in terms of FSH and estradiol, with an average monthly decrease of 1.5 percent in FSH levels (group mean was an increase of 1.0%), and an average monthly increase of 3.1 percent in estradiol levels (group mean was a decrease of 1.8% per month). No statistical differences were observed for the other hormones (Table 2).

Discussion

The present small, but longitudinal study enables for the first time a thorough evaluation of the reproductive hormonal profile in CML patients in response to imatinib therapy.

A significant, but transient, decrease in SHBG levels was observed after 3-9 months of imatinib therapy. Since SHBG levels increased again towards pretreatment levels by the end of the study period, the changes were non-linear and thus not detected in the trend analysis. Imatinib-induced changes in SHBG levels have, to our knowledge, not been reported previously, and the cause for a decrease in this hormone

is not immediately clear. Considering, however, that SHBG is produced in the liver, the findings may be linked to a general hepatic effect. Cases of hepatotoxicity have previously been linked to imatinib [21,22], but overall, liver enzymes are rarely affected in response to imatinib therapy. No information is provided on the hepatic function of the CML patients in the present study, and thus we can only speculate of any correlation between imatinib-induced SHBG decrease and liver function. Even though patients´ SHBG levels largely stayed within the reference range of the reference range of the background population throughout the study period, changes could in theory cause side effects due to clinically relevant changes in free testosterone levels. However, in terms of gynecomastia, reduced SHBG levels should serve to *lower*, rather than increase the risk of gynecomastia [17].

Of notion, the one patient who developed gynecomastia in the present study also experienced decreased SHBG levels, suggesting that his gynecomastia was not related to this binding factor. He received spironolactone as well and had a relatively high age and low age-adjusted testosterone and free testosterone levels. These factors are all known to increase the risk of developing breast tissue [23,17]. Whether imatinib therapy still was the decisive factor, we cannot determine.

Not only the patient developing gynecomastia, but all the included CML patients tended to have low testosterone and to some extent low free testosterone levels, both before and during therapy. Low (free) testosterone levels in the patients may reflect the general CML pathology, and is, as mentioned, a risk factor *per se* for developing gynecomastia [15]. Indeed, rather low baseline serum testosterone and free testosterone levels were also found in an Italian study reporting cases of gynecomastia in CML patients on imatinib therapy [3]. In general, development of gynecomastia, fatigue, erectile dysfunction or other symptoms of androgen insufficiency in patients with CML should always lead to an andrological examination.

In the present study, the low baseline testosterone levels did not change significantly in response to treatment, and as a consequence of the reduced SHBG levels, free testosterone even tended to increase transiently around 3 months of therapy. Any minor therapy-induced changes in albumin, the other binding protein determining the level of free testosterone, are not expected to serve an impact on free testosterone levels [20].

In contrast to our findings of unchanged or even slightly increased free testosterone levels, the Italian study reported reduced free testosterone levels in response to imatinib therapy, the decrease being most pronounced in the patients developing gynecomastia [3]. However, six of seven patients developing this side effect in the Italian study received high dose imatinib (6-800 mg/day), whereas all but one patient in the present study received only 400 mg/day. In this respect, it was interesting to note that the single patient in our study who received high dose imatinib differed significantly from the rest of the group in that his estradiol levels increased in response to treatment, which can be a risk factor for gynecomastia [17]. Unfortunately, estradiol levels were not analyzed in the Italian study [3]. Together, however, these findings may suggest that dose is an important factor in relation to possible hormonal side effects of imatinib, and that signaling pathways involved in the unintended testicular effects are primarily stimulated at higher imatinib doses.

In the large majority of patients receiving 400 mg/day of imatinib, estradiol levels were unchanged in the initial treatment period, but fell to significantly lower levels after 12-15 months of therapy. Secondarily, also the estradiol/testosterone ratio was significantly lower at this time point. While the majority of the patients stayed within the normal range, the estradiol levels of a single patient (#2) dropped to a Z-score below -5 for unknown reasons. None of these changes can be related to an increased risk of gynecomastia, which is often provoked by increased estradiol or estradiol/testosterone ratio [17].

Thus, in general, the observed imatinib-related changes in Leydig cell related hormonal profiles served to lower rather than increase the risk of gynecomastia. Indeed, our data showed signs of a clear imatinib-induced improvement in androgen status in some patients, which may have occurred secondary to an overall improvement in health status. Serum levels of LH, which were rather heterogeneous before imatinib therapy, appeared to stabilize around the mean of the reference range in response to treatment. And though it was not reflected in the statistical analyses, the three patients with the lowest baseline testosterone and free testosterone age-adjusted Z-scores experienced a marked improvement of androgen status in response to therapy onset.

Also the levels of the hormones reflecting Sertoli cell function and spermatogenesis were indicative of a good reproductive function. Patients' FSH levels were normal and inhibin B levels even tended to lie in the upper part of the reference range, normally reflecting well functioning spermatogenesis [24]. Actually, the subgroup analysis of patients with a complete data set showed that inhibin B levels increased in response to therapy. Interestingly, the patient developing gynecomastia appeared with hormone levels suggesting a stimulation of the Sertoli cells, giving increased inhibin B levels, which in turn had a negative feedback on the pituitary, resulting in decreased FSH levels. These changes are not immediately related to his gynecomastia and actually reflect a good and perhaps therapy-induced improved spermatogenesis.

Overall, our data of hormone levels reflecting well functioning spermatogenesis are in line with reports of successful pregnancies in couples where the male partner received tyrosine kinase inhibitor therapy [25-27]. Case reports have, however, also documented Sertoli cell dysfunction and severe oligozoospermia in pubertal patients receiving imatinib [9,10,28]. Notably, the spermatogenesis process still undergoes maturation during puberty, involving signaling processes different from those activated in the adult testis [29]. Thus, even though imatinib therapy in adulthood may not affect fertility, the risk of such side effects in children and adolescents should be further investigated.

It was beyond the scope of the present study to correlate common imatinib-related adverse events like hematologic or gastrointestinal side effects [2] with the reproductive hormone profiles. To the best of our knowledge, such correlations have not been reported previously.

The present study is limited by a relatively limited number of participants and lack of complete data sets for all included patients. Also, the well-known phenomenon of diurnal variation in hormone levels [30,31] may blur the detection of possible imatinib-induced changes in reproductive hormone profiles. A higher number of patients and morning blood samples would have been preferable. Still, this longitudinal study is, to the best of our knowledge, the largest of its kind.

In conclusion, our longitudinal study of men with CML showed a significant, but largely transient, decrease in SHBG levels in response to imatinib therapy. No marked therapy induced changes could be observed for any of the other reproductive hormones, and no signs

of imatinib-related impairment of androgen status were observed. In general, however, testosterone levels tended to be low in the patients both before and during imatinib therapy.

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Disclosure

Co-authors Inge Høgh Dufva and Ole Weis Bjerrum are also investigators in other studies sponsored by Novartis and Ole Weis Bjerrum is a Novartis advisory board member. Kimmo Porkka has received honoraria and research funding from Bristol Myers-Squibb, Novartis and Pfizer.

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