Quantification of Minimal Residual Disease in Acute Myeloid Leukemia by Tryptase Monitoring Identifies a Group of Patients with a High Risk of Relapse

Wolfgang R. Sperr,1 Margit Mitterbauer,2 Gerlinde Mitterbauer,3 Michael Kundi,4 Ulrich Jäger,1 Klaus Lechner,1 and Peter Valent1

Abstract

Purpose: Recent data suggest that tryptase is produced by blast cells in a group of patients with acute myeloid leukemia (AML). In these patients, serum tryptase levels are elevated at diagnosis and decrease to normal (<15 ng/mL) or near normal values in those achieving complete hematologic remission (CR) after chemotherapy.

Patients: In this study, we examined the value of tryptase as a marker of minimal residual AML. In 61 patients with de novo AML exhibiting elevated serum tryptase (>15 ng/mL) at diagnosis, tryptase levels were measured serially during and after chemotherapy by a fluoroenzyme immunoassay.

Results: Of the 61 patients, 42 (68.9%) entered hematologic CR in response to induction chemotherapy. Twenty-nine of these 42 patients also entered biochemical remission (BR) defined by a decrease of tryptase levels to normal (<15 ng/mL). The remaining 13 patients exhibited elevated enzyme levels despite of hematologic CR. As assessed by multivariate analysis, the elevated tryptase in CR was found to be an independent prognostic variable concerning disease-free survival. Thus, AML relapses occurred in 15 of 29 patients with CR + BR (52%) and in 12 of 13 patients with CR without BR (92%), resulting in a significantly reduced probability of continuous CR for patients with CR without BR (P < 0.05). In all patients with continuous hematologic CR, tryptase levels remained constantly normal, whereas a recurrent elevation of tryptase in CR was invariably followed by a hematologic relapse.

Conclusion: A persistently elevated tryptase level in AML in CR is indicative of minimal residual AML and associated with a high risk of relapse.

Acute myeloid leukemia (AML) is a life-threatening hematopoietic neoplasm characterized by uncontrolled proliferation and accumulation of myeloid blast cells without significant maturation (1, 2). The prognosis and clinical picture in AML vary depending on the genes that underwent deregulation, cell type involved, and the specific biological properties of the clone (1–5). In distinct variants of AML, cytogenetic features are indicative of a favorable prognosis (6–8). If treated appropriately, the rate of cure in these patients is relatively high (8–11). In other patients, the outcome is poor or unpredictable. Adverse prognostic variables include a high burden of leukemic cells, distinct cytogenetic abnormalities, and a poor response to induction chemotherapy (8–10). In line with this notion, minimal residual disease has also been recognized as a poor prognostic sign in AML (12–15). Thus, today it is well appreciated that the availability of markers for monitoring and quantitating minimal residual disease in hematologic complete remission (CR) is of major clinical importance and helpful in planning treatment in individual patients with AML (7–11). In fact, several patients with evidence of minimal residual or recurrent AML may benefit from further aggressive therapy, from stem cell transplantation, or from maintenance therapy (8–15). Based on such approaches, treatment of AML has considerably improved during the past few years (7–11).

Several different techniques for monitoring minimal residual AML have been introduced in the past. The most specific technique seems to be PCR monitoring of breakpoint-related gene products (12–20). Other techniques have employed flow cytometric determinations or cytogenetic measurements (21–26). However, most of these techniques seem to have considerable limitations. First, only a subset of patients with AML can be monitored using a single genetic variable. In addition, most of these techniques, including PCR, are rather expensive and require serial bone marrow examinations.
Moreover, some breakpoint-specific markers seem to remain constantly positive despite of continuous remission and long-term survival. Evaluation of minimal residual disease in AML by flow cytometry may be another option but is a less sensitive technique compared with PCR monitoring.

Recent data suggest that tryptase is produced in AML blasts in a significant group of patients with AML (roughly 40%; ref. 27). These patients have elevated serum tryptase levels that can be measured quantitatively in blood samples using a highly sensitive immunoassay (27). We have recently shown that tryptase levels reflect the burden of AML cells and return to normal or near normal values in patients achieving a hematologic CR after induction chemotherapy (27). In the current study, we have done serial tryptase determinations during and after therapy to quantify minimal residual disease in a cohort of patients with de novo AML.

**Patients and Methods**

*Patient characteristics.* A total number of 168 consecutive patients with de novo AML who received at least one cycle of induction polychemotherapy were examined. All patients were seen between February 1998 and December 2003 at the University Hospital of Vienna (follow-up until August 2004). Sixty-one of the 168 patients (36.3%) had an elevated serum tryptase level (>15 ng/mL) at diagnosis. In this cohort of patients (n = 61), the median age was 59 years. Diagnoses were established according to criteria proposed by the French-American-British cooperative study group (28–31). Patients with tryptase + AML who received at least one cycle of induction chemotherapy consisted of daunorubicin (45 mg/m² i.v. per day, days 1-7, DAV 3+5+7 protocol). In case of blast cell persistence, a second cycle of chemotherapy (either DAV 2+5+5 or MiDAC, mitoxanthrone 12 mg/m² i.v. per day, days 3-5 and cytarabine, 2 × 1 g/m² i.v. per day, days 1-4) was administered. Consolidation treatment consisted of four cycles of high-dose or intermediate-dose cytarabine. Patients age ≤60 years received 2 × 3 g/m² ara-C i.v. per day on days 1, 3, and 5 (high-dose ara-C) per cycle. Patients over 60 years received 2 × 1 g/m² cytarabine i.v. per day on days 1, 3, and 5 (intermediate-dose cytarabine). Patients with AML M3 were treated according to the AIDA protocol (32). Allogeneic stem cell transplantation was done in 10 patients (five in first CR and five in second CR). Patients refractory to conventional chemotherapy were subjected to salvage chemotherapy (FLAG; ref. 33, or other protocols), experimental therapy, or to cytoreduction on demand using hydroxyurea.

**Measurement of serum tryptase.** The levels of total tryptase (α-protryptase + β tryptase; referred to as tryptase in this article) were determined in serum samples by a highly sensitive fluoroenzyme immunoassay (Pharmacia, Uppsala, Sweden; ref. 34). The detection limit of this assay was found to be 1 ng/mL. In healthy controls, serum tryptase levels ranged between <1 and 15 ng/mL with a median of ~5 ng/mL (27).

**Detection of CBF/3/MYH11 and PML/RARα by reverse transcription-PCR.** In patients with AML M4eo and inv(16), those with AML M2 and t(8;21), and those with AML M3 and t(15;17), the disease was monitored by reverse transcription-PCR specific for breakpoint-associated fusion gene products (i.e., CBF[3]/MYH11 [inv16], AML1/ETO [t(8;21)], and PML/RARα [t(15;17)]). Molecular monitoring of AML-related fusion genes was employed serially on bone marrow mononuclear cells (every 3 months) and peripheral blood mononuclear cells (monthly). Reverse transcription-PCR was according to published techniques (17, 18).

**Analysis of survival and risk of recurrent disease.** To determine an optimal positive/negative cutoff level for tryptase, the martingale residuals of the null model were analyzed with respect to tryptase according to the method proposed by Therneau et al. (35). Lowest fitting revealed a strong nonlinearity in calculation results. After reanalysis with the log-transformed tryptase values, a sigmoid-shaped relationship curve was found. The fitted function increases above zero at a tryptase value of 5 ng/mL (27).

**Table 1. Patients’ characteristics**

<table>
<thead>
<tr>
<th>All patients</th>
<th>Serum tryptase</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>(15)</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>1/1</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>57.5 (15-89)</td>
</tr>
<tr>
<td>White blood count (g/L)*</td>
<td>191 (0.3-311.7)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)*</td>
<td>9.4 (4.8-14.8)</td>
</tr>
<tr>
<td>Platelets (G/l)*</td>
<td>53.5 (5.0-1111.0)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (units/L)*</td>
<td>385.5 (116-6820)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)*</td>
<td>433 (31.2-1020)</td>
</tr>
<tr>
<td>Serum tryptase (ng/mL)*</td>
<td>9 (0-881)</td>
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</table>

<table>
<thead>
<tr>
<th>Cytogenetics (SWOG)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
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</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>23 (13.7)</td>
<td>3 (2.8)</td>
<td>20 (32.8)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>102 (60.7)</td>
<td>71 (66.3)</td>
<td>31 (50.8)</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>38 (2.6)</td>
<td>31 (29.0)</td>
<td>7 (11.5)</td>
</tr>
<tr>
<td>ND</td>
<td>5 (3.0)</td>
<td>2 (1.9)</td>
<td>3 (4.9)</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not done; SWOG, Southwest Oncology Group.

*Values express as median (range).
Kaplan-Meier estimates of cumulative percentage in CR were stratified into three groups: patients remaining at a tryptase level below 15 during the total observation period, patients who had values above 15 before CR but values below 15 after CR, and patients with values above 15 during the total observation period. Statistical analysis was done by Cox proportional hazard model with tryptase as time-dependent covariable and karyotype as factored variable. Additionally, the interaction term was tested for these covariables. For calculating relapse-free survival, patients who had died before a relapse occurred were censored. Comparison of groups with respect to continuous CR was done by pairwise log-rank tests with Bonferroni correction. To compare tryptase levels among patients’ subgroups defined by cytogenetics, the Kruskal-Wallis test was applied. Differences were considered significant when $P < 0.05$. A linear correlation was employed to test the relation between tryptase levels and other disease-associated variables (WBC counts, hemoglobin, platelets, lactate dehydrogenase, and fibrinogen).

**Results**

Tryptase levels at diagnosis. A total number of 168 consecutive patients with *de novo* AML who received at least one cycle of chemotherapy were examined. Sixty-one (36.3%) had an elevated serum tryptase level. Elevated tryptase was identified in patients with AML M0 (three of five), M1 (17 of 34), M2 (16 of 31), M3 (five of eight), M4 (7 of 35), M4eo (nine of nine), M5 (2 of 25), M7 (one of three), and AML not otherwise specified (one of seven). The highest tryptase concentration was found in one patient with AML-M4eo. Tryptase levels did not correlate with WBC counts, hemoglobin concentration, lactate dehydrogenase activity, or fibrinogen. Remarkably, however, patients with favorable cytogenetic abnormalities, including inv(16), t(8;21), and t(15;17), had significantly higher serum tryptase levels compared with those in the other cytogenetically defined risk groups ($P < 0.05$).

Response to induction treatment. In the entire group of patients ($n = 168$), the rate of CR was 68.5% ($n = 115$). In 23.8% ($n = 40$), no remission could be achieved and 7.7% ($n = 13$) of the patients died during or shortly after induction chemotherapy. There was no significant difference in the CR rates when patients with tryptase-positive AML (42 of 61, 68.9%) were compared with those with tryptase-negative AML ($P > 0.05$). Thus, an elevated serum tryptase level at diagnosis is not indicative of a poor prognosis.

A persistently elevated tryptase in acute myeloid leukemia is associated with a high rate of relapse. Tryptase monitoring was done in 42 patients with tryptase-positive AML in whom a hematologic CR was obtained. Twenty-nine of these 42 patients (69.0%) also entered a biochemical remission (BR) defined by a decrease of tryptase levels to normal ($<15$ ng/mL). The remaining 13 patients exhibited elevated enzyme levels despite hematologic CR. In 12 of them, relapses occurred after 2.5, 3.5, 4.6, 4.7, 4.8, 5.7, 10.0, 10.5, 11.6, 12.1, 14.6, and 19.5 months, respectively (median time to relapse, 10.0 months). Unexpectedly, 1 of the 13 patients with persistently elevated tryptase (AML M2) was found to be still in CR after 41.0 months. However, it was also found that in this patient, serum tryptase levels slowly decreased, and finally returned to normal levels ($<15$ ng/mL) after 19.9 months.

To document the prognostic significance of a persistently elevated serum tryptase level, CCR was determined in responding patients. In these analyses, it was found that patients exhibiting a persistently elevated serum tryptase level despite of hematologic CR showed a significantly reduced probability of CCR compared with patients with a BR ($P < 0.05$; Fig. 1). As assessed by multivariate analysis (Cox proportional hazard model), a persistently elevated serum tryptase was found to be an independent prognostic factor in these patients.

In particular, AML relapses occurred in 15 of 29 patients with CR in whom serum tryptase levels had returned to normal levels at CR = CR + BR (51.7%) and in 12 of 13 patients (92.3%) with a persistently elevated tryptase level at CR = CR without BR. These results indicate that detection of minimal

<table>
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<tr>
<th>Patient group</th>
<th>Tryptase level (ng/mL)</th>
<th>$n$ (%)</th>
<th>CR, $n$ (%)</th>
<th>NR, $n$ (%)</th>
<th>ED, $n$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>$&gt;15$</td>
<td>61 (36.3)</td>
<td>42 (68.9)</td>
<td>10 (16.3)</td>
<td>9 (14.8)</td>
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<tr>
<td></td>
<td>$&lt;15$</td>
<td>107 (63.7)</td>
<td>73 (88.2)</td>
<td>30 (28.1)</td>
<td>4 (3.7)</td>
</tr>
<tr>
<td>Favorable karyotype</td>
<td>$&gt;15$</td>
<td>3 (13)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>$&lt;15$</td>
<td>20 (87)</td>
<td>19 (95)</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Intermediate and unfavorable karyotype</td>
<td>$&gt;15$</td>
<td>38 (27.1)</td>
<td>21 (55.3)</td>
<td>10 (26.3)</td>
<td>7 (18.4)</td>
</tr>
<tr>
<td></td>
<td>$&lt;15$</td>
<td>102 (72.9)</td>
<td>69 (67.7)</td>
<td>29 (28.4)</td>
<td>4 (3.9)</td>
</tr>
</tbody>
</table>

* $P$ values were adjusted for multiple comparisons according to the method of Bonferroni.
residual AML in CR by tryptase measurement after induction chemotherapy is a prognostically important variable useful for assessing the risk of relapse.

In all patients who showed continuous hematologic CR, tryptase levels remained normal (<15 ng/mL). A recurrent elevation of tryptase in CR was invariably followed by a hematologic relapse (Fig. 2). Patients with tryptase-positive AML who did not respond to induction chemotherapy showed a persistently elevated serum tryptase level. In response to chemotherapy, a slight and transient decrease in tryptase was seen in these patients followed by a recurrent increase shortly after treatment (Fig. 2).

Comparison between tryptase monitoring and PCR monitoring in acute myeloid leukemia. To evaluate the sensitivity of tryptase monitoring, we compared tryptase levels to results obtained from PCR monitoring of AML by breakpoint-specific marker genes. For this purpose, eight patients with AML-M4eo and inv(16), seven patients with AML M2 and t(8;21), and four patients with AML-M3 and t(15;17) were examined.

Of the eight patients with AML-M4eo, three are still in hematologic CR. In these patients, serum tryptase levels were constantly normal and the CBF\(h\)/MYH11-PCR remained negative (observation period: 19.7, 30.4, and 62 months). In five of the M4eo patients, however, relapses occurred (at 4.6, 8.8, 11.3, 12.1, and 19.5 months). In one of them, the hematologic relapse was preceded by an increase in tryptase as well as by recurrence of CBF\(h\)/MYH11 transcripts (Fig. 3A). He then received salvage chemotherapy (MiDAC) followed by a stem cell transplant from an unrelated donor. In response to this treatment, serum tryptase levels again decreased to
reference range, and after a few months, the CBFβ/MYH11 transcripts were no longer detectable. Approximately 1 year after stem cell transplantation, the patient presented with an isolated central nervous system relapse (normal bone marrow) accompanied by measurable CBFβ/MYH11. After 9 months, a huge increase in tryptase was observed, and the PCR became positive (●). Shortly thereafter, a hematologic relapse was diagnosed. Following reinduction (HiDAC), he again achieved a CR and subsequently received a stem cell transplant from an unrelated donor. In response to this therapy, the patient became again PCR negative, and tryptase levels decreased to normal. However, an isolated central nervous system (CNS) relapse occurred a few months later. At that time, CBFβ/MYH11 was again detectable in the blood (not in the bone marrow [bm]), whereas tryptase levels remained normal. He then received intrathecal methotrexate and donor lymphocytes. In response to this treatment, the PCR became negative again.

In the seven patients with AML M2, relapses occurred in five patients (4.7, 7.2, 10.5, 13.7, and 14.6 months). In two of these patients, the elevated tryptase levels did not decrease back to normal levels but remained elevated (>15 ng/mL) and then further increased at the time of relapse. The AML1/ETO transcript was detectable at all time points in these two patients; one of them is depicted in Fig. 4A. In two patients, tryptase levels initially decreased back to normal range and AML1/ETO transcripts in the peripheral blood disappeared. However, at the time of relapse, tryptase was again elevated, and AML1/ETO transcripts were again detectable. In one patient, the relapse was not associated with a reincrease of tryptase. Two patients with AML M2 and t(8;21) are still in hematologic CR (26.4 and 41.0 months after start of therapy).

Imaging, Diagnosis, Prognosis
months). In one of these patients, tryptase levels were found to be normal (<15 ng/mL) at CR. In the other patient, however, the tryptase level remained elevated during and after consolidation (IDAC I-IV). After 11 months, serum tryptase levels increased significantly. At that time, a hematologic relapse was diagnosed. AML1/ETO transcripts were detectable in the bone marrow (bm) and peripheral blood (pb). B. tryptase levels in a patient with tryptase+ AML M2 who achieved a CCR. In contrast to the other patients with CR, serum tryptase levels did not return to normal range at the time of CR. However, tryptase slowly decreased over time, and after 19.9 months, tryptase levels were found to be normal. Interestingly, AML1/ETO transcripts became undetectable (○) in the peripheral blood after 11.6 months and in the bone marrow after 27.7 months.

Fig. 4. Serum tryptase levels in patients with AML M2. A, a 60-year-old patient with tryptase+ AML M2 and t(8;21) (AML1/ETO-positive) achieved hematologic CR after induction chemotherapy (DAV 3 + 5 + 7). However, tryptase levels (■) remained elevated during and after consolidation (IDAC I-IV). After 11 months, serum tryptase levels increased significantly. At that time, a hematologic relapse was diagnosed. AML1/ETO transcripts were detectable in the bone marrow (bm) and peripheral blood (pb). B, tryptase levels in a patient with tryptase+ AML M2 who achieved a CCR. In contrast to the other patients with CR, serum tryptase levels did not return to normal range at the time of CR. However, tryptase slowly decreased over time, and after 19.9 months, tryptase levels were found to be normal. Interestingly, AML1/ETO transcripts became undetectable (○) in the peripheral blood after 11.6 months and in the bone marrow after 27.7 months.

Discussion

Recent data suggest that serum tryptase levels are elevated in a group of patients with AML. In these patients, the enzyme is produced in AML blasts and reflects the burden of leukemic cells (27). In this study, we have serially determined serum tryptase levels in AML patients by a sensitive immunoassay before, during, and after chemotherapy. Our data show that tryptase is useful for quantitation and monitoring of minimal residual AML. Most significantly, a persistently elevated serum tryptase level in hematologic CR is a poor prognostic marker indicative of a high risk of recurrence of AML (relapse) in these patients.

Thus far, minimal residual AML has mainly been monitored by PCR techniques employing breakpoint-specific markers (12–20). However, these markers can only be used in a smaller group of patients. In addition, such monitoring is laborious and requires serial determinations of the bone marrow and quantitative PCR technology. For those patients
who do not have a molecular marker, other markers of minimal residual AML, such as cytogenetics (fluorescence in situ hybridization) or flow cytometry, have been proposed (21–26). However, these techniques may also have considerable limitations. Most importantly, these techniques may be less sensitive compared with PCR monitoring.

To test the hypothesis that tryptase is a marker of minimal residual AML, we compared tryptase levels with PCR monitoring in patients with AML M4eo carrying the inv(16), with AML1/ETO+ AML M2, and those with AML M3 carrying the t(15;17). In these patients, we found that tryptase is indeed a highly sensitive disease-related variable in AML. In fact, in four of five patients with AML-M4eo, the hematologic or/and molecular relapse was accompanied or even preceded by a recurrent increase in tryptase (loss of BR). Interestingly, in one patient, the “PCR relapse” was not accompanied by a recurrent increase in tryptase above normal range. This may be due to a higher sensitivity of the PCR test compared with tryptase in this case. One important aspect in this regard may be the extent of production of tryptase by clonal cells. In fact, in patients with a very high tryptase level at diagnosis, tryptase monitoring seemed equally (or even more) sensitive compared with PCR monitoring. By contrast, in patients with only slight elevation in tryptase at diagnosis, the enzyme did not seem to be a superior marker of minimal residual AML.

In patients with tryptase’ AML M2 and t(8;21), tryptase was also found to be a useful marker of disease monitoring. In fact, in both patients with recurrent disease, tryptase was found to increase at the time of relapse. In one patient with AML M2, a persistently elevated tryptase level was found despite continuous hematologic CR. However, during and after consolidation, tryptase slowly decreased in this patient, and after a latency period of 19.9 months, returned back to normal. This delay in the decrease of tryptase to normal is of particular interest, because it is well known from molecular analysis of AML1/ETO transcripts that it may take several months until residual AML cells disappear in these patients (19). In line with this notion, the AML1/ETO transcript in our patient also remained positive for several months in peripheral blood and bone marrow cells.

A remarkable aspect was that tryptase was particularly expressed in those patients who had “favorable” cytogenetic abnormalities including inv(16), t(15;17), or t(8;21). This is of interest because these patients may have a high chance to be cured (3, 6–10). With regard to tryptase expression, it may therefore be of considerable importance to know whether tryptase is persistently elevated in hematologic CR. Similar to a persistently detectable breakpoint fusion gene by PCR (12–18), a persistent or recurrent elevation of tryptase may significantly influence the treatment plan in these patients.

A persistently elevated tryptase level in AML may not necessarily be due to minimal residual disease. In fact, in patients with AML and an associated systemic mastocytosis (a rare condition), the elevated tryptase may derive from neoplastic mast cells but not from AML blasts (36, 37). Therefore, tryptase should not be used as a marker of AML in patients presenting with coexisting mastocytosis.

In summary, our data show that a persistently elevated tryptase in AML is also found to be a useful marker of disease monitoring. In fact, in both patients with recurrent disease, tryptase was found to increase at the time of relapse. In one patient with AML M2, a persistently elevated tryptase level was found despite continuous hematologic CR. However, during and after consolidation, tryptase slowly decreased in this patient, and after a latency period of 19.9 months, returned back to normal. This delay in the decrease of tryptase to normal is of particular interest, because it is well known from molecular analysis of AML1/ETO

References


Acknowledgments

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Clinical Cancer Research

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