Early Assessment of Minimal Residual Disease Identifies Patients at Very High Relapse Risk in NPM-ALK-positive Anaplastic Large Cell Lymphoma

Christine Damm-Welk\(^1,6\), Lara Mussolin\(^2,3,6\), Martin Zimmermann\(^1\), Marta Pillon\(^2\), Wolfram Klapper\(^4\), Ilske Oschlies\(^4\), Emanuele S.G. d’Amore\(^5\), Alfred Reiter\(^1\), Wilhelm Woessmann\(^1,7\), Angelo Rosolen\(^2,7\)

\(^1\)NHL-BFM Study Center, Department of Pediatric Hematology and Oncology, Justus-Liebig University; Feulgenstr. 12; 35392 Giessen, Germany
\(^2\)Department of Pediatric Hematology and Oncology, University of Padova, Padova, Italy and \(^3\)Istituto di Ricerca Pediatrico Fondazione Città della Speranza, Padova, Italy
\(^4\)Institute of Pathology, Hematopathology section and Lymph node registry, Christian-Albrechts-University Kiel, Germany
\(^5\)Pathology Unit, Ospedale San Bortolo, Vicenza, Italy
\(^6\)These authors contributed equally to this work
\(^7\)Co-senior authors

Correspondence should be addressed to:
Christine Damm-Welk, PhD, or Willi Woessmann, MD
Justus-Liebig-University, Department of Pediatric Hematology and Oncology
Feulgenstr. 12, D-35392 Giessen, Germany
T: ++49/641/99-43421
F: ++49/641/99-43429
christine.damm-welk@paediat.med.uni-giessen.de
wilhelm.woessmann@paediat.med.uni-giessen.de

Running title: MRD in NPM-ALK positive ALCL
Key points

Early MRD-positivity in NPM-ALK positive anaplastic large cell lymphoma correlates with a very high relapse risk and inferior survival.

Abstract

Detection of minimal disseminated disease (MDD) at diagnosis correlates with relapse risk in children with ALK-positive anaplastic large cell lymphoma (ALCL). We investigated whether minimal residual disease (MRD) positivity by qualitative RT-PCR for \textit{NPM-ALK} during treatment identifies patients at highest relapse risk. Blood and/or bone marrow of 180 patients with NPM-ALK-positive ALCL treated with BFM-type protocols was screened for \textit{NPM-ALK} transcripts at diagnosis. 103 were MDD-positive. MRD before therapy course two could be evaluated in 52 MDD-positive patients. MRD-positivity correlated with non-common histology. The cumulative incidence of relapses (CIR) of 26 MDD-positive/MRD-positive patients (81±8\%) was significantly higher than CIR of 26 MDD-positive/MRD-negative (31±9\%) and 77 MDD-negative patients (15±5\%) (p<.001). Five-year survival of MDD-negative and MDD-positive/MRD-negative patients was 91±3\% and 92±5\%, respectively, compared to 65±9\% of MDD-positive/MRD-positive patients (p<.001). Early evaluation of MRD in NPM-ALK positive ALCL identifies patients with a very high relapse risk and inferior survival.
Introduction

The relapse rate of children with anaplastic large cell lymphoma (ALCL) reaches 25-35% with current chemotherapy. More than 95% of childhood ALCL express anaplastic lymphoma kinase (ALK) fusion proteins, 90% of which carry the translocation t(2;5)(p23;q35) resulting in the specific fusion gene Nucleophosmin (NPM)-ALK\textsuperscript{5,6}. NPM-ALK-expressing cells can be detected in bone marrow (BM) or blood (PB) by RT-PCR in 50-60% of patients with NPM-ALK positive ALCL as a sign of minimal disseminated disease (MDD)\textsuperscript{7,8}. MDD turned out to be a significant prognostic factor in addition to the histological subtype and clinical risk factors\textsuperscript{10,11}. In vivo response to chemotherapy measured by minimal residual disease (MRD) has been established as the strongest prognostic factor for children with acute lymphoblastic leukemia\textsuperscript{12-14}. In Burkitt’s leukemia, early detection of MRD could identify patients at highest risk of relapse as well\textsuperscript{15}. We, therefore, investigated whether detection of minimal residual disease (MRD) by qualitative RT-PCR for \textit{NPM-ALK} in MDD-positive patients in BM or PB early during treatment allows identification of patients at highest risk of relapse in ALCL.
Patients and methods

Eligibility
ALCL-patients treated according to the protocols NHL-BFM 95, LNH97 or ALCL99 (Italian and German patients) between 8/1998 and 12/2008 were potentially eligible. Three patients with completely resected stage I disease were excluded since they received no prephase and only three courses of chemotherapy. Eligibility was confirmed by demonstration of NPM-ALK positivity of the tumor by either positive NPM-ALK PCR and/or positive two-color FISH for t(2;5) and/or nuclear and cytoplasmic staining for ALK. BM or PB had to be available at diagnosis for MDD analyses by PCR from consenting patients/parents. From those patients with a positive MDD result, PB or BM before the second course of chemotherapy was requested after informed consent for MRD-studies. Ethical approval of the studies was obtained from national and local review boards, respectively. Informed consent was obtained in accordance with the Declaration of Helsinki from all patients.

Patients
180 patients fulfilled the inclusion criteria. This cohort is an extension of the previously published series of the AIEOP- and BFM-groups. Patients were stratified according to stage (St. Jude’s system) and the involvement of risk organs. Staging procedures included BM aspiration cytology and spinal tap. BM involvement was defined by cytologically detectable ALCL cells, irrespective of numbers. The BFM-type protocols consisted of comparable cytoreductive prephase and chemotherapy courses. All patients received six 5-day chemotherapy courses over a period of four to six months. 27 patients received Vinblastine maintenance therapy in the trial ALCL99 (11 with MRD available).
The clinical characteristics of the patients are shown in supplementary table 1.

**Qualitative PCR for NPM-ALK**

Total RNA was isolated from mononuclear or nuclear BM or PB samples by standard methods. cDNA synthesis was performed using 1µg total RNA, random hexamers and superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The qualitative PCR with a sensitivity of 10^-5 was performed as previously described^7-9^.

**Statistical analysis**

Analysis of event-free survival (EFS) and overall survival (OS) was performed using the Kaplan-Meier method with differences compared by the log-rank test. Cumulative incidence functions for relapse were constructed following the method of Kalbfleisch and Prentice^18^. Functions were compared with Gray's test^19^.

The prognostic effect of MRD on treatment outcome was compared with other known prognostic factors in patients with ALCL using Cox regression analysis^20^. All analyses were performed using SAS (SAS-PC, version 9.1, Cary, NC: SAS Institute Inc.). Data were updated as of June 2012.
Results and Discussion

Five-year OS and EFS for the whole group of 180 patients with MDD measurement were 84±3% and 65±4%, respectively. The cumulative incidence of relapse (CIR) was 32±4%. As in our previous report with a limited patient number\(^8\), MDD in BM and PB was highly concordant (data not shown). Therefore, both media were accepted for MDD- and MRD-assessment.

Minimal disseminated disease (MDD)

MDD was detected in BM, PB or both in 103 of 180 patients (57%) with NPM-ALK positive ALCL, which was comparable to our earlier reports\(^7-9\). MDD-positivity significantly correlated with mediastinal or visceral involvement, stage and histological subtype (supplementary table S1) confirming our earlier observations in a large patient cohort\(^7-9\).

46 of 103 MDD-positive patients relapsed compared to 10 of 77 patients without detectable MDD (CIR 46±5% versus 15±5%, \(P<.001\)). MDD-positive patients had a 5-year EFS and OS of 51±5% and 79±4%, respectively, compared to 83±5% and 91±3%, respectively, for MDD-negative patients (\(P<.0001\) for EFS and .016 for OS).

Minimal residual disease (MRD)

PB and/or BM before the second course of chemotherapy were available for MRD-measurement from 52 of 103 patients with detectable MDD. 26 were MRD-positive and 26 MRD-negative. MRD-positivity among MDD-positive patients significantly correlated with non-common histological subtype but not with clinical characteristics (Table 1).

For control purposes we measured MRD from 23 MDD-negative patients. None of these patients turned MRD-positive.
CIR of the 26 MDD-positive/MRD-positive patients was significantly higher (81±8%) than CIR of the 26 MDD-positive/MRD-negative (31±9%) and 77 MDD-negative patients (15±5%) (p<.001) (Figure 1A). CIR of the 51 MDD-positive patients without available material for MRD evaluation was 33±7%. The five-year EFS of MDD-positive/MRD-positive patients was significantly lower compared to MDD-positive/MRD-negative or MDD-negative patients (Figure 1B). Five-year survival of MDD-negative and MDD-positive/MRD-negative patients was 91±3% and 92±5%, respectively, whereas survival of MRD-positive patients was 65+9% (p<.001) (Figure 1C). In addition to MDD and MRD, both clinical risk factors (mediastinal, visceral or skin involvement), non-common histological subtype and ALK-antibody titers ≤1/750 were associated with a significantly higher risk of relapse in univariate analysis. In multivariate analysis including the covariables MDD, MRD, clinical risk factors, histological subtype and ALK-antibody titers the hazard ratio for EFS was 1.83 for MDD (95% confidence interval, CI, 0.55-6.12, p=.3), 6.00 for MRD (CI 2.01-17.92, p=.001), 1.1 (CI 0.28-4.33, p=.90) for clinical risk factors, 3.66 for non-common histology (CI 1.54-8.68, P=.003) and 3.25 for low ALK-antibody titers (CI 1.38-7.68, P=.007).

This is the first observation of prognostic impact of MRD measured early during treatment in ALK-positive ALCL. Clinical, pathological (histological subtype) and biological characteristics measurable at diagnoses (MDD, ALK-antibody titers) distinguish patients with low and high relapse risk in ALCL7-10. MRD with the strong impact in multivariate analysis allows identifying patients with chemoresistant disease. The correlation of MRD-positivity with non-common subtype is in line with observations that the histological subtype is associated with other high-risk characteristics and remains an independent prognostic factor even in studies including MDD and ALK-antibody titers8-10. The observation that MRD measured
early - after only three weeks of chemotherapy - allowed detection of patients with a very high relapse risk is indicative of a different lymphoma-biology which is also reflected by the histological subtype. Taking into account that the pre-existing immune response against ALK in part reflected by the titer of ALK antibodies may play a major role in final control of ALCL\textsuperscript{9,21,22}, it may be speculated that the absence of MRD in ALCL may in part be influenced by host factors as well. Given the strong association of MRD-positivity with relapse risk early MRD-assessment could be envisioned as a surrogate marker for the endpoint EFS among MDD-positive patients in future clinical trials. Early treatment response might even serve as eligibility criteria for early phase clinical studies.
Acknowledgements

The study was supported by a grant from the Deutsche Jose Carreras Leukämie-Stiftung (DJCLS08/09) to WW and WK. CDW and WW were additionally supported by the Forschungshilfe Peiper, LM, MP and AR by Fondazione Città` Della Speranza, Associazione Italiana contro le Leucemie (AIL) and by Camera di Commercio di Venezia.

Authorship and Disclosures

CDW, LM, AR, AR and WW designed and coordinated the study. CDW and LM performed the molecular analyses and collected samples. WK, IO and E.S.G.d’A performed the histopathological analyses. MP and MZ analyzed the data. CDW, LM, AR and WW wrote the paper. All authors approved the final version of the manuscript.

The authors reported no potential conflicts of interest.
References


Table 1. Association of minimal residual disease (MRD) assessed before therapy course 2 by qualitative PCR for *NPM-ALK* in bone marrow or blood with clinical risk factors and histological subtype of the ALCL among minimal disseminated disease (MDD) positive patients.

<table>
<thead>
<tr>
<th></th>
<th>MDD positive</th>
<th>MRD Negative</th>
<th>MRD Positive</th>
<th>MRD n.a.</th>
<th>P***</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>103</td>
<td>26</td>
<td>26</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>81</td>
<td>21 (81%)</td>
<td>20 (77%)</td>
<td>40</td>
<td>.73</td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>5 (19%)</td>
<td>6 (23%)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Mediastinum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38</td>
<td>8 (31%)</td>
<td>3 (12%)</td>
<td>27</td>
<td>.09</td>
</tr>
<tr>
<td>Yes</td>
<td>65</td>
<td>18 (69%)</td>
<td>23 (88%)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Visceral organs*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>57</td>
<td>18 (69%)</td>
<td>11 (42%)</td>
<td>28</td>
<td>.05</td>
</tr>
<tr>
<td>Yes</td>
<td>46</td>
<td>8 (31%)</td>
<td>15 (58%)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>25 (100%)</td>
<td>25 (96%)</td>
<td>43</td>
<td>.32</td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>1 (4%)</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neg</td>
<td>89</td>
<td>22 (85%)</td>
<td>21 (81%)</td>
<td>46</td>
<td>.71</td>
</tr>
<tr>
<td>Pos</td>
<td>14</td>
<td>4 (15%)</td>
<td>5 (19%)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Stage**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.31</td>
</tr>
<tr>
<td>I</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>2 (8%)</td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>70</td>
<td>19 (73%)</td>
<td>19 (73%)</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>23</td>
<td>5 (19%)</td>
<td>7 (27%)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.004</td>
</tr>
<tr>
<td>Common</td>
<td>39</td>
<td>17 (68%)</td>
<td>6 (26%)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>ALK AB titer</td>
<td>Not common</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>--------</td>
<td>--------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>≤750</td>
<td>26</td>
<td>7</td>
<td>10 (59%)</td>
<td>9 .09</td>
<td></td>
</tr>
<tr>
<td>&gt;750</td>
<td>49</td>
<td>15 (68%)</td>
<td>7 (41%)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>28</td>
<td>4</td>
<td>9</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

MDD, minimal disseminated disease; MRD, minimal residual disease; BM, bone marrow; CNS, central nervous system; ALK AB titer, ALK antibody titer; n.a., not available

* liver, spleen, lung.

** St. Jude’s staging system.

*** for the comparison MRD-positive with MRD-negative patients
Figure legend

Figure 1. Outcome of ALCL patients according to minimal residual disease (MRD) in bone marrow or blood measured by qualitative PCR results for *NPM-ALK* before the second course of chemotherapy. (A) Cumulative incidence of relapse and Kaplan-Meier estimates of (B) 5-year event-free survival and (C) overall survival of the 183 patients with initial qualitative PCR result for *NPM-ALK* in bone marrow and/or blood (minimal disseminated disease, MDD).
Early assessment of minimal residual disease identifies patients at very high relapse risk in NPM-ALK-positive anaplastic large cell lymphoma

Christine Damm-Welk, Lara Mussolin, Martin Zimmermann, Marta Pillon, Wolfram Klapper, Ilske Oschlies, Emanuele S.G. d'Amore, Alfred Reiter, Wilhelm Woessmann and Angelo Rosolen