Evidence of clonality in chronic neutrophilic leukaemia

J Böhm, S Kock, H E Schaefer, P Fisch

Background: Chronic neutrophilic leukaemia (CNL) is a rare myeloproliferative disorder of elderly patients characterised by sustained neutrophilia and splenomegaly. The diagnosis of CNL requires the exclusion of BCR/ABL positive chronic myelogenous leukaemia (CML) and of leukaemoid reactions (LRs). The differentiation between CNL and LR is problematic because both conditions share similar morphological features; it is also important because patients with CNL generally have a poor prognosis.

Aims: To determine whether CNL and LR could be distinguished on the basis of different clonality patterns.

Methods: Blood samples from 52 women were studied using the human androgen receptor gene assay (HUMARA).

Results: Monooclality was found in the neutrophils in all 17 patients with different myeloproliferative syndromes (MPSs), including those with CNL. In four of the patients with CNL, autologous T cells were also monoclonal, suggesting that they belonged to the neoplastic clone. This finding was in contrast to other MPSs in which T cells were almost always polyclonal. Of nine patients with clinically suspected LR, the neutrophils of five were polyclonal, whereas three patients had monoclonal neutrophils, suggesting that they might be in the process of developing an MPS. Among 26 healthy blood donors, 20 had polyclonal neutrophils and five showed skewed clonality patterns. One case of LR and one normal blood donor were scored “not informative” at the HUMARA locus.

Conclusions: Clonality studies of blood neutrophils using HUMARA aid in distinguishing female patients with monoclonal CNL from those with LR. For the diagnosis of CNL, monoclonality of the neutrophils should be demonstrated whenever possible.

Chronic neutrophilic leukaemia (CNL) is a rare myeloproliferative syndrome (MPS) of elderly patients showing sustained neutrophilia and splenomegaly. To date, only 143 cases of CNL have been reported in the literature, including 14 of our own cases reported recently. The diagnosis of CNL is based on the exclusion of chronic myelogenous leukaemia (CML) and of leukaemoid reactions (LRs). In contrast to CML, which is characterised by a BCR/ABL translocation, no definite molecular marker is known in CNL. The differential diagnosis between CNL and LR may be difficult or even impossible because both conditions share identical morphological features, including a raised neutrophil alkaline phosphatase (NAP) score. The spectrum of disorders capable of causing LR is so wide that clinicians may not be able to exclude all possible causes of LR. It is important to differentiate CNL from LR because the prognosis of patients with CNL is poor, even worse than that of those with CML.

“The diagnosis of chronic neutrophilic leukaemia is based on the exclusion of chronic myelogenous leukaemia and of leukaemoid reactions”

The human androgen receptor gene assay (HUMARA) for the analysis of clonality in tissues from female patients examines the inactivation patterns of the human androgen receptor gene on the X chromosome. This method relies on the length polymorphism of a human androgen receptor gene exon, which has restriction sites for methylease sensitive enzymes. Ideally, in polyclonal conditions such as LR, 50% of the cells show methylation of the maternal allele and 50% of the cells show methylation of the paternal allele. In contrast, neoplastic tissues such as leukaemic CML cells typically show complete methylation of one allele and demethylation of the other allele. In this study, we investigated blood samples of patients using HUMARA to determine whether CNL and LR could be distinguished on the basis of X chromosomal inactivation patterns.

MATERIALS AND METHODS

Selection of patients

In the archives of our department, which receives more than 10 000 bone marrow biopsies annually, we found five living female patients fulfilling the morphological and clinical criteria of CNL. These five patients belonged to a group of 14 CNL cases that we have reported previously. Clinically, these patients showed chronic neutrophilia, a variable degree of splenomegaly, and no thrombocytosis. The bone marrow was strongly hypercellular with expansion of the neutrophilic granulopoiesis, which was not left shifted. In the blood, moderate leucocytosis was present, with an excess of mature neutrophils and bands. In four of the cases the blood also contained myelocytes, but there were no blasts (table 1). In all our CNL cases, the NAP score was increased and the BCR/ABL translocation was excluded by reverse transcription polymerase chain reaction (PCR) and, in addition, by fluorescence in situ hybridisation (FISH). At the time of diagnosis, four of the patients with CNL had normal cytogenetics, but patient 4 showed an abnormal clone with trisomy 9 (20 of 21 metaphases). We performed clonality studies in blood samples using HUMARA in our five cases of CNL and compared the results with 12 patients who had untreated, newly diagnosed MPS, nine patients with clinically suspected LR, and 26

Abbreviations: CML, chronic myelogenous leukaemia; CNL, chronic neutrophilic leukaemia; FISH, fluorescence in situ hybridisation; HPRT, hypoxanthine phosphoribosyl transferase; HUMARA, human androgen receptor gene assay; LR, leukaemoid reaction; MPS, myeloproliferative syndrome; NAP, neutrophil alkaline phosphatase; PCR, polymerase chain reaction; PHA, phytohaemagglutinin
healthy blood donors (table 1). All the investigations were done according to the guidelines of our institute.

**Blood sample clonality assay**

The neutrophils of all 52 patients were enriched on a buffy coat by centrifuging 5 ml of blood at 800 g for 15 minutes, followed by the lysis of red blood cells. Lymphocytes were isolated for each patient (PNA Blood Minikit; Qiagen, Hilden, Germany). These DNA samples were digested overnight with the following primers: 5'-GCTGTGAAGGTTGCTGTTCCTCAT-3' (antisense) and 5'-GCTGTGAAGGTTGCTGTTCCTCATAF-3' (sense).

**RESULTS AND DISCUSSION**

We studied the clonality patterns of leucocytes, which consisted mainly of neutrophils and PHA expanded T cells, from patients with MPS and LR. The T cells were used as an internal control cell population because both neutrophils and T cells are derived from haemopoietic progenitor cells. The results of the HUMARA study are summarised in table 1, and representative findings in two patients with CNL and one normal blood donor are illustrated in fig 1. In all five cases of CNL, the leukaemic neutrophils displayed a monoclonal HUMARA pattern. Similarly, all seven patients with CML or atypical CML and all five patients with other MPS had monoclonal leucocytes. The PHA expanded T cells of four of five patients with CNL showed a monoclonal HUMARA pattern. This finding suggests that in CNL the T cells are frequently derived from the neoplastic clone. However, we cannot completely exclude the possibility that the finding of monoclonal T cells in four of the five CNL cases could represent an extreme form of “skewing”, meaning an unbalanced, “skewed” pattern of X chromosome inactivation in which cells with the inactivated maternal or paternal allele are predominant. Even though it is not always possible to differentiate clearly between true monoclonality and extreme skewing in the individual case, we think that such a concentration of cases showing extreme skewing in a small group of patients with CNL would be very unlikely. In contrast, in five of six patients with CML, in the patient with atypical CML, and in all five patients with other MPSs the T cells did not show a monoclonal HUMARA pattern, suggesting that they may be derived from residual normal progenitor cells. Thus, the different clonality patterns of the T cells may indicate that in CNL the neoplastic transformation occurs at an earlier stage of progenitor cell differentiation than in CML.

Most patients with the clinical diagnosis of LR showed polyclonal patterns for neutrophils and T cells, as expected. Three patients with clinically suspected LR had monoclonal neutrophils and polyclonal T cells, displaying a clonality pattern similar to the patients with MPS. Thus, these patients with

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age</th>
<th>BCR/ ABL</th>
<th>Hb</th>
<th>WBC</th>
<th>Differential blood count</th>
<th>Plt</th>
<th>N-clon/L-clon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CNL</td>
<td>52</td>
<td>Absent</td>
<td>140</td>
<td>30.0</td>
<td>Seg 70, Bn 3, My 9, Bas 1, Eo 2, Ly 10, Mo 5</td>
<td>182</td>
<td>M M</td>
</tr>
<tr>
<td>2</td>
<td>CNL</td>
<td>81</td>
<td>Absent</td>
<td>108</td>
<td>35.0</td>
<td>Seg 90, Bn 0, My 0, Bas 0, Eo 0, Ly 9, Mo 1</td>
<td>189</td>
<td>M P</td>
</tr>
<tr>
<td>3</td>
<td>CNL</td>
<td>37</td>
<td>Absent</td>
<td>143</td>
<td>36.2</td>
<td>Seg 64, Bn 15, My 13, Bas 0, Eo 0, Ly 7, Mo 7</td>
<td>273</td>
<td>M M</td>
</tr>
<tr>
<td>4</td>
<td>CNL</td>
<td>72</td>
<td>Absent</td>
<td>89</td>
<td>38.0</td>
<td>Seg 41, Bn 13, My 37, Bas 0, Eo 0, Ly 6, Mo 3</td>
<td>47</td>
<td>M M</td>
</tr>
<tr>
<td>5</td>
<td>CNL</td>
<td>63</td>
<td>Absent</td>
<td>123</td>
<td>24.6</td>
<td>Seg 60, Bn 6, My 17, Bas 5, Ery 2, Ly 9, Mo 1</td>
<td>210</td>
<td>M M</td>
</tr>
<tr>
<td>6</td>
<td>CNL</td>
<td>60</td>
<td>b2a2</td>
<td>98</td>
<td>83.5</td>
<td>Seg 28, Bn 14, My 39, Bas 1, Eo 1, Ly 10, Mo 7</td>
<td>718</td>
<td>M P</td>
</tr>
<tr>
<td>7</td>
<td>CNL</td>
<td>53</td>
<td>b3a1</td>
<td>103</td>
<td>46.1</td>
<td>Seg 30, Bn 4, My 12, Bas 0, Eo 1, Ly 13, Mo 7</td>
<td>361</td>
<td>M M</td>
</tr>
<tr>
<td>8</td>
<td>CNL</td>
<td>41</td>
<td>b3a2</td>
<td>98</td>
<td>239.0</td>
<td>Seg 45, Bn 5, My 39, Bas 3, Eo 0, Ly 2, Mo 6</td>
<td>619</td>
<td>M P</td>
</tr>
<tr>
<td>9</td>
<td>aCML</td>
<td>48</td>
<td>Absent</td>
<td>142</td>
<td>32.7</td>
<td>Seg 27, Bn 4, My 46, Bas 3, Eo 1, Ly 15, Mo 4</td>
<td>460</td>
<td>M P</td>
</tr>
<tr>
<td>10</td>
<td>CNL</td>
<td>64</td>
<td>b2a2</td>
<td>136</td>
<td>39.2</td>
<td>Seg 60, Bn 7, My 0, Bas 2, Eo 0, Ly 22, Mo 1</td>
<td>429</td>
<td>M M</td>
</tr>
<tr>
<td>11</td>
<td>CNL</td>
<td>58</td>
<td>b2a2</td>
<td>136</td>
<td>24.1</td>
<td>Seg 63, Bn 7, My 0, Bas 2, Eo 0, Ly 22, Mo 1</td>
<td>429</td>
<td>M M</td>
</tr>
<tr>
<td>12</td>
<td>CNL</td>
<td>65</td>
<td>b3a2</td>
<td>130</td>
<td>33.9</td>
<td>Seg 59, Bn 4, My 22, Ery 4, Bas 5, Eo 2, Ly 6, Mo 1</td>
<td>246</td>
<td>M P</td>
</tr>
<tr>
<td>13</td>
<td>CNML</td>
<td>83</td>
<td>Absent</td>
<td>100</td>
<td>37.2</td>
<td>Seg 63, Bn 0, My 0, Bas 0, Eo 0, Ly 29, Mo 28</td>
<td>191</td>
<td>M P</td>
</tr>
<tr>
<td>14</td>
<td>CNML</td>
<td>74</td>
<td>Absent</td>
<td>114</td>
<td>8.2</td>
<td>Seg 20, Bn 6, My 0, Bas 0, Eo 0, Ly 51, Mo 23</td>
<td>95</td>
<td>M P</td>
</tr>
<tr>
<td>15</td>
<td>PCV</td>
<td>61</td>
<td>Absent</td>
<td>91</td>
<td>15.4</td>
<td>Seg 80, Bn 4, Ery 4, Bas 0, Eo 0, Ly 10, Mo 2</td>
<td>44</td>
<td>M ND</td>
</tr>
<tr>
<td>16</td>
<td>CNML</td>
<td>73</td>
<td>Absent</td>
<td>105</td>
<td>12.2</td>
<td>Seg 78, Bn 0, My 0, Bas 1, Eo 4, Ly 16, Mo 1</td>
<td>124</td>
<td>M P</td>
</tr>
<tr>
<td>17</td>
<td>CNML</td>
<td>83</td>
<td>Absent</td>
<td>116</td>
<td>30.4</td>
<td>Seg 38, Bn 4, My 19, Bas 5, Eo 7, Ly 20, Mo 7</td>
<td>403</td>
<td>M P</td>
</tr>
<tr>
<td>18</td>
<td>LR (smoker)</td>
<td>39</td>
<td>Absent</td>
<td>140</td>
<td>14.0</td>
<td>Seg 63, Bn 2, My 2, Bas 1, Eo 4, Ly 25, Mo 3</td>
<td>Normal</td>
<td>M P</td>
</tr>
<tr>
<td>19</td>
<td>LR (diabetes)</td>
<td>50</td>
<td>Absent</td>
<td>88</td>
<td>10.4</td>
<td>Seg 61, Bn 0, My 0, Bas 0, Eo 3, Ly 32, Mo 4</td>
<td>348</td>
<td>M P</td>
</tr>
<tr>
<td>20</td>
<td>LR (fever)</td>
<td>63</td>
<td>Absent</td>
<td>99</td>
<td>18.1</td>
<td>Seg 87, Bn 1, Eo 2, Ly 8, Mo 22</td>
<td>518</td>
<td>M P</td>
</tr>
<tr>
<td>21</td>
<td>LR</td>
<td>67</td>
<td>ND</td>
<td>ND</td>
<td>21.5</td>
<td>ND</td>
<td>ND</td>
<td>P P</td>
</tr>
<tr>
<td>22</td>
<td>LR (diabetes)</td>
<td>93</td>
<td>ND</td>
<td>ND</td>
<td>20.1</td>
<td>ND</td>
<td>ND</td>
<td>M M</td>
</tr>
<tr>
<td>23</td>
<td>LR (smoker)</td>
<td>37</td>
<td>ND</td>
<td>ND</td>
<td>13.6</td>
<td>Seg 69, Bn 1, Ly 24, others 6</td>
<td>204</td>
<td>P P</td>
</tr>
<tr>
<td>24</td>
<td>LR (Sharp sy)</td>
<td>50</td>
<td>ND</td>
<td>90</td>
<td>7.0</td>
<td>Seg 80, Bn 1, Eo 90, Ly 7, Mo 6</td>
<td>207</td>
<td>M P</td>
</tr>
<tr>
<td>25</td>
<td>LR</td>
<td>53</td>
<td>Absent</td>
<td>147</td>
<td>13.8</td>
<td>Seg 56, Bn 2, Ly 35, Mo 7</td>
<td>354</td>
<td>NI NI</td>
</tr>
<tr>
<td>26</td>
<td>LR (smoker)</td>
<td>45</td>
<td>Absent</td>
<td>129</td>
<td>12.5</td>
<td>Seg 66, Bn 4, Eo 6, Ly 20, Mo 4</td>
<td>403</td>
<td>P P</td>
</tr>
</tbody>
</table>

*Diagnosis: aCML, atypical chronic myeloid leukaemia; CNM, chronic myeloproliferative disorders; CML, chronic myelogenous leukaemia; CMML, chronic myelomonocytic leukaemia; MPS, myelodysplastic syndrome; NI, not informative; ND, not determined. See text.*
By definition, a cell fraction is considered as monoclonal or "skewed" if the expression of the dominant allele exceeds 75%. Thus, the results of HUMARA clonality studies should only be interpreted together with the clinical, blood, and bone marrow findings. Among the patients with LR and blood donors there were two "non-informative" cases, where the analysis of clonality was impossible because the two different PCR amplified X chromosomal microsatellites were of approximately equal size.

"The different clonality patterns of the T cells may indicate that in chronic neutrophilic leukaemia the neoplastic transformation occurs at an earlier stage of progenitor cell differentiation than in chronic myelogenous leukaemia"

To our knowledge this is the first report of HUMARA clonality studies in CNL. This technique is naturally restricted to female patients and gives meaningful results in 80% to 90% of cases, owing to the high rate of heterozygosity at the HUMARA locus. However, the results may be blurred by the excessive skewing that is seen in normal blood donors. Thus far, the clonal nature of neutrophils and T cells. In five blood donors we found a skewed pattern of X chromosomal inactivation. This was seen in both the neutrophils and the T cells of these blood donors. The skewing has been interpreted as a natural phenomenon, detectable in some women upon aging. By definition, a cell fraction is considered as monoclonal or "skewed" if the expression of the dominant allele exceeds 75%. Thus, the results of HUMARA clonality studies should only be interpreted together with the clinical, blood, and bone marrow findings. Among the patients with LR and blood donors there were two "non-informative" cases, where the analysis of clonality was impossible because the two different PCR amplified X chromosomal microsatellites were of approximately equal size.

"The different clonality patterns of the T cells may indicate that in chronic neutrophilic leukaemia the neoplastic transformation occurs at an earlier stage of progenitor cell differentiation than in chronic myelogenous leukaemia"

To our knowledge this is the first report of HUMARA clonality studies in CNL. This technique is naturally restricted to female patients and gives meaningful results in 80% to 90% of cases, owing to the high rate of heterozygosity at the HUMARA locus. However, the results may be blurred by the excessive skewing that is seen in normal blood donors. Thus far, the clonal nature of neutrophils and T cells. In five blood donors we found a skewed pattern of X chromosomal inactivation. This was seen in both the neutrophils and the T cells of these blood donors. The skewing has been interpreted as a natural phenomenon, detectable in some women upon aging. By definition, a cell fraction is considered as monoclonal or "skewed" if the expression of the dominant allele exceeds 75%. Thus, the results of HUMARA clonality studies should only be interpreted together with the clinical, blood, and bone marrow findings. Among the patients with LR and blood donors there were two "non-informative" cases, where the analysis of clonality was impossible because the two different PCR amplified X chromosomal microsatellites were of approximately equal size.

"The different clonality patterns of the T cells may indicate that in chronic neutrophilic leukaemia the neoplastic transformation occurs at an earlier stage of progenitor cell differentiation than in chronic myelogenous leukaemia"

To our knowledge this is the first report of HUMARA clonality studies in CNL. This technique is naturally restricted to female patients and gives meaningful results in 80% to 90% of cases, owing to the high rate of heterozygosity at the HUMARA locus. However, the results may be blurred by the excessive skewing that is seen in normal blood donors. Thus far, the clonal nature of neutrophils and T cells. In five blood donors we found a skewed pattern of X chromosomal inactivation. This was seen in both the neutrophils and the T cells of these blood donors. The skewing has been interpreted as a natural phenomenon, detectable in some women upon aging. By definition, a cell fraction is considered as monoclonal or "skewed" if the expression of the dominant allele exceeds 75%. Thus, the results of HUMARA clonality studies should only be interpreted together with the clinical, blood, and bone marrow findings. Among the patients with LR and blood donors there were two "non-informative" cases, where the analysis of clonality was impossible because the two different PCR amplified X chromosomal microsatellites were of approximately equal size.

"The different clonality patterns of the T cells may indicate that in chronic neutrophilic leukaemia the neoplastic transformation occurs at an earlier stage of progenitor cell differentiation than in chronic myelogenous leukaemia"
REFERENCES


Evidence of clonality in chronic neutrophilic leukaemia

J Böhm, S Kock, H E Schaefer and P Fisch

doi: 10.1136/jcp.56.4.292

Updated information and services can be found at:
http://jcp.bmj.com/content/56/4/292

References
This article cites 26 articles, 5 of which you can access for free at:
http://jcp.bmj.com/content/56/4/292#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
- Immunology (including allergy) (1664)
- Molecular genetics (355)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/