## Erythrophagocytosis in de novo-Philadelphia-positive acute leukemia of ambiguous lineage

**Erythrophagocytosis by neoplastic cells in acute leukemia has been most frequently associated with FAB M4 and M5 subtypes, with the t(8;16) and with C-MOZ rearrangements, however it is exceptional in acute lymphoblastic leukemia and has not been previously reported in Philadelphia-positive (Ph+) acute leukemia. We herein present a case of Ph+ acute leukemia of ambiguous lineage in which erythrophagocytosis is an outstanding feature. The implications between the different postulated leukemogenic pathways and the hypothesized mechanism of erythrophagocytosis are concisely reviewed and discussed.**

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Erythrophagocytosis by neoplastic cells in acute leukemia has been most frequently associated with monocytic leukemias and with cytogenetic abnormalities involving (8)(p11).[1,2] It has also been described in other FAB myeloid subtypes[3,4,5] and exceptionally in acute lymphoblastic leukemia.[6] We herein present a case of Philadelphia positive acute leukemia of ambiguous lineage in which erythrophagocytosis is an outstanding finding both in peripheral blood and bone marrow.

A 41 y.o. male was admitted with acute leukemia suspicion. He complained of lumbar pain and fever (temperature >38,5°C) one week before admission. Physical examination did not reveal any abnormality. Most relevant blood biochemistry values were: Alkaline phosphatase: 718 IU/L, lactate dehydrogenase: 1902 IU/L and C-Reactive protein 140 UI/L; ionogram, and both liver and renal parameters were within normal ranges. Coagulation tests were as follows: prothrombin time 76% (INR 1,2); activated partial thromboplastim time: 24 sec.; fibrinogen: 2.0 gr/L. Hematologic values were the following: Hb: 126 g/L, platelet: $52.0\times10^9$/L, WBC: $10,0\times10^9$/L with a differential showing 50% blasts. A chest radiography and an ultrasonographic study of abdomen did not show abnormalities. Several microbiologic and serologic tests were irrelevant. The exam of peripheral blood films revealed undifferentiated blasts and notably a few images of erythrophagocytosis (Figure 1). A bone marrow (BM) aspirate revealed massive infiltration by mid-sized to large undifferentiated blasts with high nuclei/cytoplasm ratio; the rounded nuclei presented delicate immature chromatin and one or two nucleoli. The slightly basophilic cytoplasm did not show granules. Erythrophagocytosis images were frequently observed (Figure 2). Cytochemical studies did not show positivity either for myeloperoxidase, chloroacetate naphtol esterase or butirate esterase. PAS positivity was observed in 62% of blasts. Surface immunophenotypic study revealed 82% of cells with positivity for: CD34, CD19, CD10, CD22, CD20, CD45(with a myeloid profile) and CD123; partial positivity was observed for CD33(20%), CD13(51%), CD15(20%) and CD2(32%). Cytoplasmic immunophenotypic analysis showed TdT+(>80%), CD22+(>80%), cμ±(18%), CD3- and Myeloperoxidase+. All immunophenotypic studies were repeated with the same results. A BM biopsy showed massive infiltration. Immunohistochemical studies confirmed positivity for pan-B markers (CD19 and CD20), myeloid markers (myeloperoxidase and CD33) and a conspicuous Ki67 expression. Phenotypic analysis is summarised in Table 1.

**Table 1.**

| *CD* | *FC* | *IHC* |
|---|---|---|
| CD19 | >80% (++) | Positive (++) |
| CD20 | >80% (+) | Positive (+) |
| CD22 | >80% (++)(S. and Cyt.) | NP |
| CD79a | NP | Positive (+) |
| HLA-DR | >80% (++) | NP |
| CD10 | >80% (++)(MFI: 184) | Positive (++) |
| CD34 | >80% (++) | Positive (++) |
| CD45 | >80% (+)(MFI: 51) | Positive (++) |
| CD15 | 20%(+) | NP |
| CD13 | 50%(++) | NP |
| CD33 | 20%(+) | Positive (+) |
| CD11b | Negative | NP |
| CD65 | Negative | NP |
| CD2 | 32%(+) | Positive (+) |
| CD3 | Negative (Cyt.) | Negative |
| CD7 | Negative | NP |
| CD56 | Negative | Negative |
| MPO | >60% (++) | Positive (++) |
| CD123 | >80% (+) | NP |
| TdT | >80% (+) | Positive (++) |
| Ki67 | NP | Positive (++) |

++: MFI ≥100 (CF); : MFI >20 and <100 (CF); NP: not performed. MFI: mean fluorescence intensity. S: surface. Cyt: cytoplasmic.

**Figure 1. A red blood cell phagocyted by a blast cell in peripheral blood (May-Grümwald Giemsa, x1000).**

Cytogenetics showed a complex karyotype: 51,XY, +5, +8, t(9,22)(q34;12), +13, +21, +22, der(22)t(9;22)(q34;q11.2). Interphase FISH using an extra signal (ES) dual colour probe for Philadelphia chromosome (VYSIS) disclosed an atypical pattern with three fusion signals consistent with a supernumerary Philadelphia chromosome associated with mBCR/ABL rearrangement (Figure 3). Quantitative real-time polymerase chain reaction (RT-PCR) assays for BCR-ABL with TaqMan technology performed at diagnosis on BM sample showed the presence of the p190 transcript (normalized copy numbers (NCN): 2671, BCR-ABL/GUS ratio: 26,7%). Furthermore, immunoglobulin heavy chain gene and gamma-chain-T-cell-receptor rearrangements were observed.

Although a final diagnosis of Philadelphia-positive acute leukemia of ambiguous lineage was established, the patient was treated using PETHEMA Ph+ 2003 protocol for Philadelphia-positive acute lymphoblastic leukemia that includes imatinib administration.

Figure 2. The bone marrow aspirate revealed massive infiltration by mid-sized to large undifferentiated blasts with high nuclei/cytoplasm ratio, a rounded nuclei and delicate immature chromatin . Two bone marrow blast cells side by side showing images of erythrophagocytosis (May-Grümwald Giemsa, x1000).

Figure 3. Interphase FISH using a ES probe for Philadelphia chromosome showed an atypical pattern with three yellow fusion signals (red arrows), one orange signal and one green signal.

The patient finished both the induction and consolidation schedules without major complications. Five months after diagnosis he underwent a peripheral blood stem cell transplantation (PBSCT) from a non-related HLA-matched (10/10) donor. MRD was undetectable (0,0 NCN, BCR-ABL/GUS ratio: 0,000) by means of RT-PCR analysis both prior to PBSCT and by day +90 after procedure. Nine months after diagnosis the patient has a Karnofsky of 100%.

In the acute leukemia setting, erythrophagocytosis is a rare finding reported in less than 1% of cases.[5] In this regard, erythrophagocytosis in acute myeloid leukemias has been related with several FAB subtypes most notably the M4 and M5. In these particular subtypes it has been associated with abnormalities involving the C-MOZ gene, located at (8)(p11), that most frequently originate the t(8;16)(p11;p13) translocation that gives rise to the C-MOZ/CBP chimeric transcript.[1] Erythrophagocytosis has also been associated with FAB M0, M1, M2 and M7 subtypes, and with the t(16;21)(p11;q22), the t(10;17)(p13;p12), as well as with deletion of the long arm of chromosome 20 (20q-) involving q11.[3,4,5] Deletion of the long arm of chromosome 20 at q12q13 was also described in the only case of acute lymphoblastic leukemia with erythrophagocytosis reported so far.[6]

The precise mechanism by which the leukemic blasts phagocyte erythrocytes has not been precisely defined. It has been postulated that aberrantly premature expression of the complement receptors CR1 and CR3 and the IgG receptors FcR and gp150 by the leukemic blasts might contribute to this event.[4] Involvement of some cytokines (i.e. TNF and IL2) has been also postulated.[5] In our case, and similar to the concomitant expression of myeloid, B-cell and T-cell line markers, an aberrant expression of Fc and/or complement receptors leading to opsonization of eryhthrocytes to Ph+ blast cells seems an attractive hypothesis but it can only be speculated at.

As regards the t(9;22), erythrophagocytosis is exceptional in this setting, as only one case has been reported in a blastic crisis of chronic myeloid leukemia. As this case dates back to 1977, no phenotypic or molecular data were recorded.[7] While the BCR/ABL chimeric transcript has been reported to carry out its leukemogenic effect through JAK/STAT, SAPK/JUN and C-MYC activation pathways,[8] that of C-MOZ/CBP chimeric gene, frequently associated to erythrophagocytosis, seems to occur due to aberrant acetylation through mistargeting of HAT activity leading to inhibition of transcription,[1] thus both mechanisms are not apparently related to eachother.

A number of poor prognosis factors coincided in our patient. Firstly Ph+ AL per se, secondly a complex karyotype associated to the Philadelphia chromosome and finally erythrophagocytosis, the latter linking itself to a dismal clinical outcome.[5] Interestingly, imatinib treatment, peripheral blood stem cell transplantation or both seem to have overcome all these factors, although caution is mandatory due to the short period of follow up considered.

To conclude, this case is the first observation of erythrophagocytosis in de novo Ph+ AL. The observation of erythrophagocitosis in a Ph+ AL raises the question that the prematurely aberrant phenotypic expression of the antigen/receptors that hypothetically lead to phagocytosis of erythroid cells overrides different cell lines and a number of cytogenetic abnormalities and leukemogenic pathways.

FO and MMO: diagnostic procedures, laboratory tests and the final diagnosis; conception of the study and writing of the manuscript. CC, MJM and VV: responsible for clinical management, conception of the study and writing of the manuscript. MG: molecular tests.

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*All authors declare they have no potential conflict of interest regarding the issues considered in this paper.*

*The herein presented case has been treated according to a nation-wide standard protocol for Ph+ALL (PETHEMA LLA Ph+ 2003), therefore considerations about protection of human rights, specific informed consent for experimental treatments and registration in clinical trials do not apply.*

*This paper contains original images and data that have not been submitted elsewhere; in addition we state that no other paper regarding the clinical or biologic data of this patient is currently being prepared. Information regarding this patient has been sent to PETHEMA and EBMT databases.*

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