Biphenotypic Acute Leukemia: A Report of Two Cases

[1]RZ Azma, [1]NH Hamidah, [1]C-F Leong, [1]O Ainoon, [2]Fadilah SAW, [3]S-K Cheong, [1]Salwati S, [1]Sharifah N A

Department of [1]Pathology and [2]Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur
[3]Clinical School, International Medical University, Seremban.

ABSTRAK

Kami melaporkan dua kes leukemia akut bifenotipik yang didiagnos di Hospital Universiti Kebangsaan Malaysia (HUKM), ciri-ciri klinikal, hematologi dan tindakbalas penyakit ini terhadap kemoterapi. Kedua-dua pesakit adalah wanita pertengahan umur yang menunjukkan pembengkakan hati dan limpa serta bilangan sel darah putih yang tinggi. Sum-sum tulang menunjukkan kepadatan sel dimana 90% terdiri dari sel-sel leukemia pelbagai saiz. Analisa sitokimia menunjukkan lebih daripada 3% adalah positif terhadap ujian peroksidase dan sel-sel leukaemia yang lebih kecil menunjukkan positiviti secara blok terhadap ujian *periodic acid-schiff* (PAS). Ujian imunofenotip menunjukkan ko-ekspresi dwi penanda sel CD 33 dan CD 19, CD 19 dan CD34, intra CD22, intra TdT dan intraMPO. Analisa sitogenetik daripada salah seorang pesakit tersebut menunjukkan kehadiran kromosom Philadelphia yang disahkan dengan ujian Fluorescence *In Situ* Hybridisation (FISH). Analisa molekular juga menunjukkan kehadiran protein fusi BCR-ABL. Kedua-dua pesakit menerima rawatan kemoterapi yang terdiri daripada daunorubicin dan cytosine arabinoside. Namun begitu, pesakit yang mempunyai kromosom Philadelphia BCR-ABL tidak berjaya mencapai remisi morfologi selepas kemoterapi induksi. Memandangkan prognosis buruk pesakit leukemia ini, pemindahan sel-sel stem periferi dirancangkan untuk kedua-dua pesakit ini.

Kata kunci: leukemia akut bifenotipik, immunofenotip, faktor-faktor prognosis, sitogenetik, rawatan kemoterapi

ABSTRACT

We report two cases of biphenotypic acute leukaemia diagnosed in Hospital Universiti Kebangsaan Malaysia (HUKM), their clinical, haematological characteristics and response to chemotherapy. Both patients are middle-aged ladies who presented with hepatosplenomegaly and high white cell count, mainly composed of blast cells. Their bone marrow aspirations were hypercellular comprising of more than 90% heterogenous blast cells. Cytochemical analyses show more than 3% positivity towards peroxidase, with smaller blasts showing block positivity towards PAS. Immunophenotypically, the blasts showed dual expression of CD 33 and CD 19, CD 19 and CD34, intra CD22, intra TdT and intraMPO. One of the patients showed presence of the Philadelphia chromosome on cytogenetic analysis which was confirmed by Fluorecsence *In Situ* Hybridisation (FISH). Molecular analysis also showed presence of the BCR-ABL fusion protein. Both patients were given combination chemotherapy consisting of daunorubicin and cytosine

Address for correspondence and reprint requests: *Dr. Raja Zahratul Azma bt. Raja Sabudin, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Jalan Yaacob Latif, Bandar Tun Razak, 56000 Kuala Lumpur, Malaysia. E-mail: zahratul@mail.hukm.ukm.my*

arabinoside.However, the patient with positive Philadelphia chromosome BCR-ABL did not achieve morphological remission after induction chemotherapy. In view of the poor prognosis of this disease, both the patients were planned for upfront peripheral blood stem cell transplantation.

Keywords: biphenotypic acute leukaemia, immunophenotyping, prognostic factors, cytogenetic, treatment

INTRODUCTION

Biphenotypic acute leukaemia (BAL) is a rare disease, which occurs in 5 to 10% of acute leukaemias. BAL can arise de novo or it may be secondary to previous cytotoxic therapy. As with other types of acute leukaemia, patients with BAL usually present with symptoms resulting from cytopenias. The blast count at diagnosis does not differ from that in acute myeloid leukemia (AML) or acute lymphoid leukaemia (ALL). BAL can present at any age, including children, although it is more common in adults. The blasts cell morphology are variable; some cells may display myeloid differentiating features such as azurophilic granules or Auer rods; others may have lymphoid or undifferentiated morphology. In some cases, two blast populations may be seen, consisting of a population resembling myeloblast with another population of smaller lymphoid-looking blasts. Immunophenotyping is mandatory to establish the diagnosis of BAL. These blasts co-express myeloid and T or B lineage specific antigens. Diagnosis is based on a scoring system adopted by the European Group of Immunological Classification of Leukaemias (EGIL) (Bene et al 1995), and it has been included in the WHO classification of haemopoietic malignancies as acute leukaemia of ambiguous lineage (Brunning et al 2001). There is no specific cytogenetic abnormality in BAL. Studies done by Carbonell F et al (1996) had shown that the most common cytogenetic abnormalities were t(9;22)(q34;q11) and abnormalities of chromosome 11, particularly 11q23. BAL is very difficult to treat with many cases being resistant to induction chemotherapy; and those which achieved morphological remission had a higher risk of relapse. The prognosis of BAL in adults is worse than AML or ALL and most are associated with over-expression of P glycoprotein (Pgp) (Legrand et al 1993).

CASE REPORT

CASE 1

AS, a 45-year-old policewoman was admitted to HUKM with severe back pain of one day. She also gave a history of loss of appetite and loss of weight of two months duration. She was diagnosed to have hypertension, ischaemic heart disease and hyperlipidaemia. On physical examination, she was pale, with enlarged liver and spleen. No lymph node enlargement or hypertrophy of the gums was observed. No bleeding tendency was noted. Vital signs were stable. Examination of other systems were normal. Laboratory investigation and results are shown in Table 1.

The patient was given induction chemotherapy consisting of cytosine arabinoside for seven days and daunurobicin for three days. Bone marrow assessment at day 28 post chemotherapy showed that she has achieved marrow remission. She was given the same regime for her first consolidation treatment and for her second and third consolidation; the chemotherapy regimen was changed to mitoxantrone for two days and cytosine arabinoside for four days. Throughout the

post chemotherapy period, she developed severe myelosuppression with neutropenic sepsis, thrombocytopenia and anaemia. She was planned for allogeneic peripheral blood stem cell transplantation but while waiting for the work out, she had a relapse. She was then given salvage chemotherapy which consists of Fludarabine, cytosine arabinoside and idarubicin (FLAG-IDA) with growth factor support. Unfortunately, 10 days post chemotherapy, she developed severe neutropenic sepsis with shock and she succumbed to her death.

CASE 2

RO, a 30-year-old Malay lady was referred to HUKM from Putrajaya Hospital with a history of fever of two weeks duration, blurring of vision of her left eye, fatigue and lethargy. On admission, she was very pale. There were multiple ulcers over her lips and palate, and she had gum hypertrophy. Multiple bilateral cervical lymphadenopathy were also noted. Vital signs were stable. Fundus examination showed presence of left retinal haemorrhages with dilated vessels. Cardiovascular and respiratory systems were normal. Abdominal examination showed hepatosplenomegaly measuring 4 cm and 3 cm below the costal margins respectively. Laboratory investigations and results are shown in Table 1.

This patient was also given induction chemotherapy consisting of cytosine arabinoside for seven days and daunorubicin for three days. In the post chemotherapy period, the patient developed persistent pancytopenia, complicated with neutropenic sepsis. A month after chemotherapy, her peripheral blood showed presence of residual blasts. Bone marrow aspiration confirmed the persistence of disease. A second induction chemotherapy was started with a regimen consisting of cyclophosphamide, dexamethasone, doxorubicin and vincristine. However, during chemotherapy, she developed severe upper gastro-intestinal bleeding and succumbed to death.

TABLE 1: Laboratory findings in two cases of Biphenotypic Acute Leukemia (BAL)

	CASE 1	*CASE 2*
FBC	*WBC: 37.4 x 10^9/l* *Hb: 9.2g/dl* Platelets: 54 x 10^9/l	*WBC: 123 x 10^9/l* *Hb: 5.4g/dl* Platelets: 63 x 10^9/l
Peripheral blood smear	*Heterogenous population of blast cells (> 90%)*	*Heterogenous population of blast cells (> 90%)*
Bone marrow aspiration	*Hypercellular marrow (> 90% blasts). Heterogenous population of blasts (Fig. 1):* *i. Large blasts: abundant to moderate cytoplasm, prominent nucleoli* *ii. Small blasts: high N/C ratio, inconspicuous nucleoli.*	*Hypercellular marrow (> 90% blasts). Heterogenous population of blasts (Fig. 2):* *i. Large blasts: abundant cytoplasm with azurophilic granules, prominent nucleoli* *ii. Small blasts: high N/C ratio, scanty basophilic cytoplasm, indistinct nucleoli*
Cytochemistry of bone marrow	*Large blast cells are mainly positive for peroxidase and some small blasts are positive for PAS (block positivity)*	*Some blasts are positive with peroxidase stain, some are positive with non-specific esterase and some with specific esterase. Some of the blasts also showed block positivity with PAS stain.*
Immunophenotyping	*Gated cells co-expressed CD 19 and CD33, CD 13 and CD10, HLA DR and CD34, intra TdT, intra22 and intraMPO (Fig. 3)*	*Gated cells co-expressed CD 19 and CD33, CD 13 and CD10, HLA DR and CD34, intra TdT, intra22 and intraMPO*
Cytogenetic analysis (FISH)	*Unsatisfactory*	*Presence of Philadelphia chromosome (Fig. 4)*
Molecular analysis	*No BCR-ABL fragment detected*	*Major BCR-ABL fragment detected (Fig. 5).*

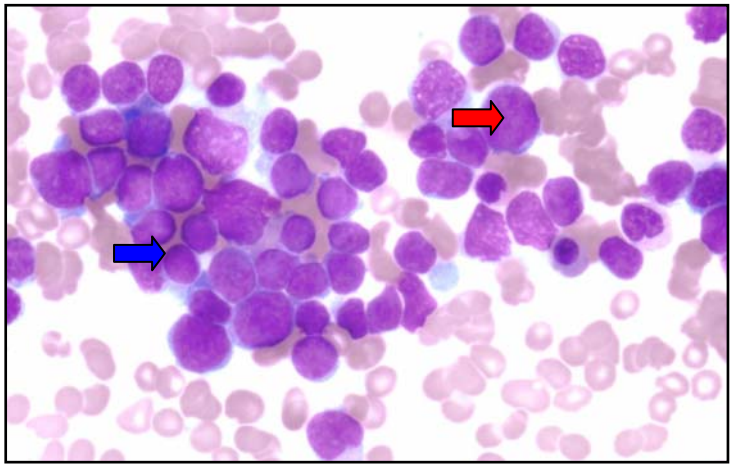


FIGURE 1: Bone marrow aspiration of case 1 showing a heterogenous population of large (red arrow) and small (blue arrow) blasts cells. (MGG stain x 400)

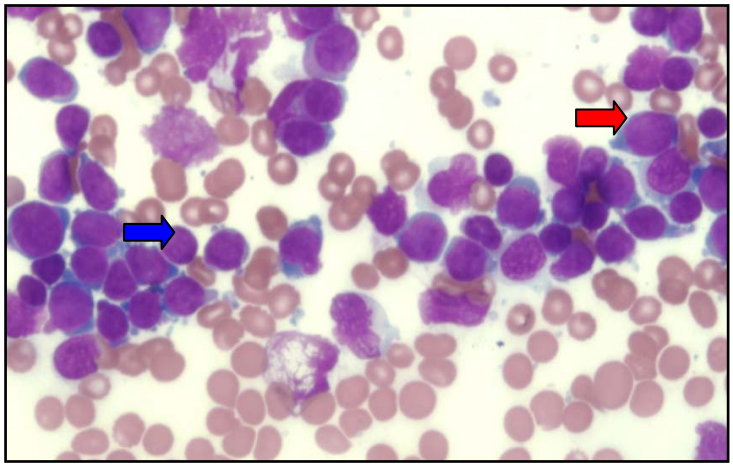


FIGURE 2: Bone marrow aspiration of case 2 showing a heterogenous population of large (red arrow) and small (blue arrow) blast cells. (MGG stain x 400)

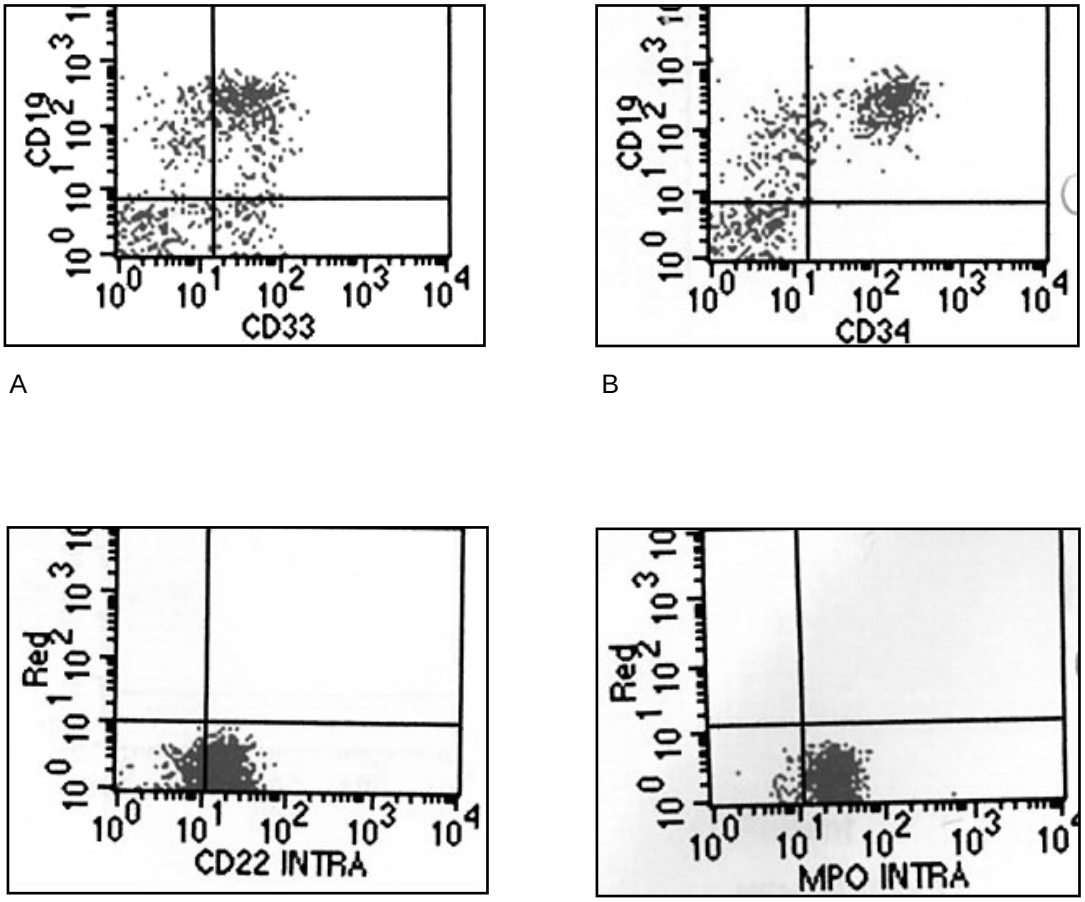


FIGURE 3: Immunophenotyping of case 1 showed co-expression CD 19 and CD33, CD 13 and CD10, HLA DR and CD34, intra22 and intraMPO of gated cells.

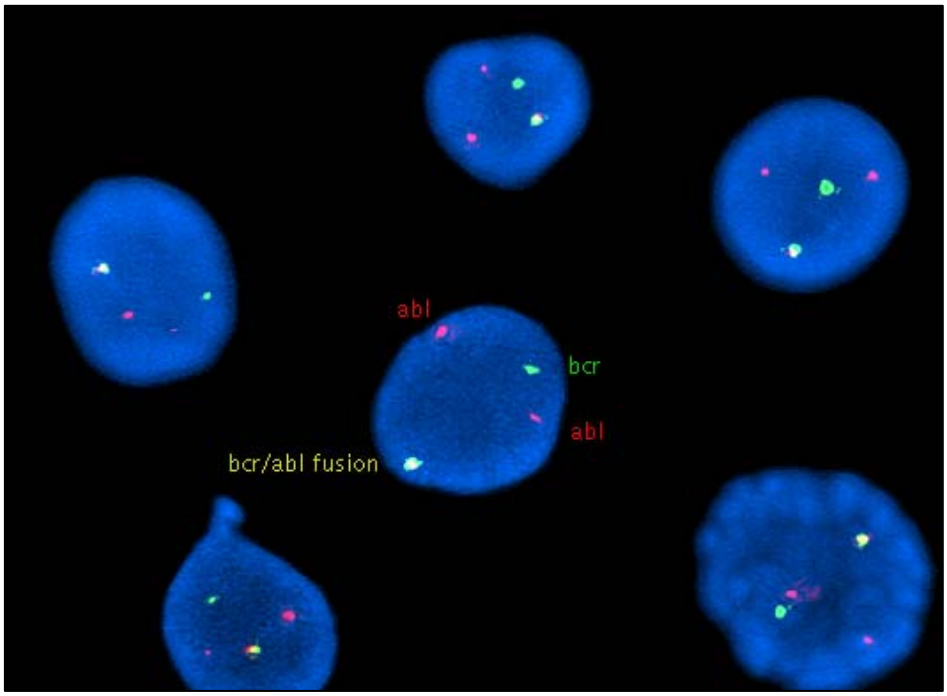
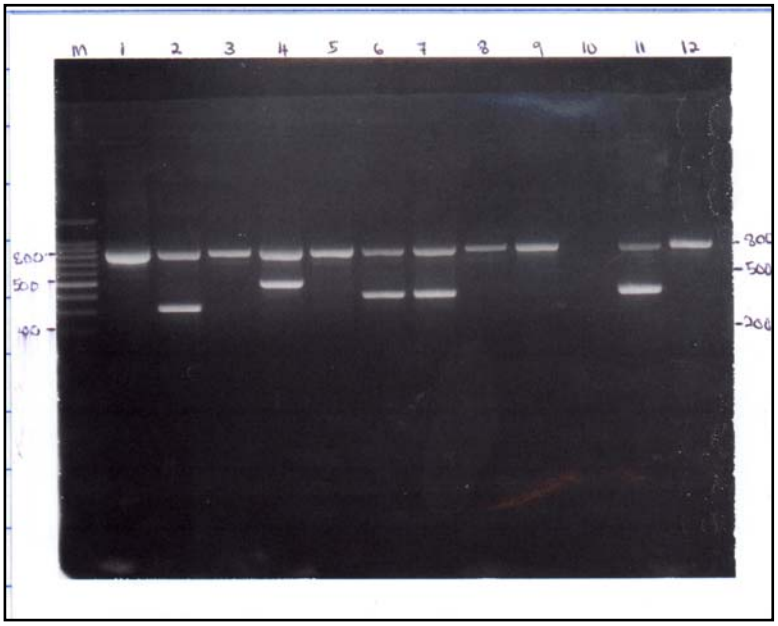


FIGURE 4: FISH analysis of case 2 showed presence of BCR-ABL fusion gene.



1. Normal patient
2. Patient
10. negative control
11. K562 (positive control for CML)

FIGURE 5: Molecular analysis by RT-PCR of case 2 showed presence of major *BCR-ABL* fragment.

DISCUSSION

Biphenotypic acute leukaemia (BAL) is a form of acute leukaemia of ambiguous lineage. It is a rare entity which is difficult to define previously, due to lack of consistent diagnostic criteria. The characterisation of these disease based on morphology alone can be difficult. Blasts are often undifferentiated, with no distinguishing characteristics. However, some cases have been described based on the detection of two distinct populations of blasts which vary in size. Our two cases showed two distinct populations of blasts morphologically. The larger blast cells with moderate to abundant cytoplasm are positive for peroxidase stain and the smaller blast cells showing scanty cytoplasm, are negative for peroxidase stain.

Immunophenotyping is the most important diagnostic tool in characterizing BAL. Diagnostic criteria have been defined including those outlined by EGIL (Bene, M.C. et al 1995) and WHO (Brunning RD et al 2001). The diagnosis is based on a scoring system for markers proposed by the EGIL, which defines the minimum number of phenotypic characteristics necessary to be considered true biphenotypia. Patients who do not meet these criteria will be diagnosed as either AML with lymphoid aberrant markers or ALL with myeloid aberrant markers. Markers that have been used and considered to be most specific to differentiate the three cell lines are shown in Table 2. Each marker has a different score. The score encompasses the number and degree of specificity of the markers expressed by the leukaemic cells. The diagnostic criteria are (i) there should be at least a score of 2 from each lineage, and (ii) both myeloid and lymphoid markers are expressed on the same cells in order to diagnose BAL. This is to differentiate biphenotypic acute leukaemia from bilineage acute leukaemia. In these two cases, the blast cells expressed intra CD22, and intra MPO which fulfilled criteria (i) and the co-expression of CD 19 and CD 33, CD 13 and CD 10 in both cases fulfilling criteria (ii) which confirmed the diagnosis of BAL. In contrast, bilineage acute leukaemia has two distinct population of leukaemic cells. The larger cells will express myeloid markers while the smaller cells will express B or T-lymphoid markers. Previous studies of BAL have suggested that these leukaemias may arise by malignant transformation of a pluripotent stem cell. The finding of a significantly greater percentage of biphenotypic cases with CD34 expression compared to non-biphenotypic cases in a study done by Legrand O et al (1998), supports the concept that this transformation process occurs at a step close to the haemopoietic progenitor cell. In the same study, the percentage of BAL patients with unfavourable karyotype was higher compared to AML (60% versus 20%). The high percentage of unfavourable karyotypes especially abnormalities of chromosome 11q23 and t(9;22) has been shown by a few studies (Carbonell et al 1996, Hanson et al 1993). One of the patients had an unfavourable karyotype, the Philadelphia chromosome, and molecular analysis also showed presence of BCR-ABL gene. The study by Legrand O et (1998) al also showed that most BAL patients have P-glycoprotein (Pgp) overexpression which correlates with poor treatment outcome. Treatment outcome of BAL with standard AML protocol has been proven to be unfavourable and yet there are no standard induction protocol for BAL; even those patients who attained remission has a higher risk of relapse. The majority of the patients received chemotherapy for AML and once the patients attained morphological remission, upfront stem cell transplantation will offer a better outcome. Both of our patients received the AML induction regime; patient 1 went into marrow remission and was planned for allogenic peripheral blood stem cell transplantation. Unfortunately, there was

TABLE 2: Scoring system for markers proposed by the European Group for the Immunologic Classification of Leukaemia (EGIL).

Score	B-lymphoid	T-lymphoid	Myeloid
2	CytCD79a Cyt IgM CytCD22	CD3(m/cyt) Anti-TCR	MPO
1	CD19 CD20 CD10	CD2 CD5 CD8 CD10	CD117 CD13 CD33 CD65
0.5	TdT Cd24	TdT CD7 CD1a	CD14 CD15 CD64

* Based on the EGIL Scoring system for Biphenotypic Leukaemia, both case 1 and case 2 have more than 2 scores for both B-lymphoid and myeloid which is consistent with biphenotypic acute leukaemia.

no matched sibling donor available, and she relapsed about 10 weeks after the fourth course of chemotherapy. The second patient did not achieve marrow remission. Legrand O et (1998) al have documented that the outcomes of treatment in patient with BAL are worst when compared to AML or ALL. The causes of poor prognosis in patients with acute BAL appear to be related to intrinsic cell characteristics (increase of unfavourable karyotypes, overexpression of Pgp and immature cell markers). Therefore, more studies on the induction chemotherapy protocols for this disease is important not only to improve the treatment outcome but also to minimise the severe adverse effects of the treatment protocols.

REFERENCES

Bene, M.C., Castoldi, G., Knapp, W., Ludwig, W.D. et al. Proposals for the immunological classification of acute leukaemias. European Group for the Immunological Characterization of Leukaemias (EGIL). *Leukaemia.* 1995; 9: 1783-86.

Brunning, R.D., Matutes, E., Borowitz, M., Flandrin, G., Head, D., Vardiman, J., Bennett, J. 2001. In: WHO Tumours of haemopoietic and lymphoid tissues. *IARC Press, Lyon, 106 – 107.*

Carbonell, F., Swansbury, J., Min, T. et al Cytogenetic findings in acute biphenotypic leukaemia. *Leukaemia* 1996; *10: 1283 – 7.*

Hanson, C. A., Abaza, M., Sheldon, S., Ross, C.W. et al. Acute biphenotypic leukaemia: immunophenotyping and cytogenetic analysis. *British Journal of Haematology* 1993; *84, 49 – 60*.

Legrand, O., Perrot, J.Y., Simonin, G., Baudard, M., Cadiou, M., Blanc, C., Raymond, S., Viguie, F., Marie, J.P., Zittoun, R. Adult biphenotypic acute leukaemia: an entity with poor prognosis which is related to unfavourable cytogenetics and P-glycoprotein over-expression. *British Journal of Haematology* 1998; *100:147 – 155.*