Acute Megakaryoblastic Leukaemia: Experience of Diagnostics at Three Clinical Centres

ABSTRACT

Background. Acute megakaryoblastic leukaemia (AMkL) is a rare hematologic clonal malignancy, characterised by the accumulation of abnormal myeloid blasts with megakaryoblastic differentiation in the bone marrow and/or extra-marrow hematopoiesis sites with marked clinical heterogeneity in children and adult populations. Owing to a low occurrence in the daily clinical practice, AMkL presents a difficulty for immunophenotypic diagnostics.

Purpose. We present 36 cases of children and adult AMkL observed from 2007 through 2014 at the Federal Scientific Clinical Center of Children Hematology, Oncology and Immunology in memory of Dima Rogachev, Moscow, Russia (FSCCCHOI), Pavlov First Saint-Petersburg State Medical University, Russia (PFSPbMU), and Research Center for Paediatrics and Children Surgery, Almaty, Kazakhstan (SCPCS).

Methods. A retrospective analysis of clinical and multicolour flow cytometry data of AMkL was diagnosed in two Russian and one Kazakhstan clinical centers during 8 years has been performed, and the applicability of the modern diagnostic criteria was evaluated.

Results. From 2,867 cases of acute leukaemia, we identified 36 patients with AMkL (1.26% of all cases) including 30 children and 6 adults with disease age onset ranged from 2 days to 75 years. Trisomy 21 was detected in 19.4% of the samples. In the group of children under 3 years (23), trisomy of chromosome 21 was detected in 7 patients (30.4%).

Conclusion. Most typical immunophenotypical features of AMkL and its rare variants allow diagnosing it in ethnically diverse populations. We describe specific details of the sample preparation and interpretation of multicolour flow cytometry data.

KEYWORDS. acute megakaryoblastic leukaemia, multicolour flow cytometry, immunophenotype, CD marker expression, Down syndrome

INTRODUCTION

Acute megakaryoblastic leukaemia (AMkL) is a rare malignancy of a clonal nature characterised by the accumulation of abnormal myeloid blasts with megakaryoblastic differentiation in the bone marrow and/or at extra-marrow hematopoiesis sites with marked clinical heterogeneity in children and adult populations. Owing to low rate of occurrence in the daily clinical practice, it remains a malignancy, presents a difficulty for immunophenotypic diagnostics. Additionally, statistical data are difficult to compare. The frequency of AMkL does not exceed 1–10% of all acute myeloid leukaemia (AML) cases in adults, 3–10% of AML cases in children and 20% AML cases in newborns. This disease was first described by Dr Von Boros and coll. in 1931 and was included in FAB and EGIL classification as AML M7. Most cases of AMkL were described in children under 1–3-year-old, including those with Down syndrome. The incidence of AMkL in children with Down syndrome is 500 times greater than the one in children that do not have a trisomy of chromosome 21. Its diagnostic code according to the international classification is ICD-O 9910/3 (a high-level malignancy).

Clinical manifestations of AMkL are nonspecific compared with other acute leukaemias and are often associated with cytopenia and signs of dysplasia. The main clinical signs of AMkL are severe hepato- and spleno-megaly, severe bone pain, tingling, and petechia. The main clinical signs of AMkL are severe hepato- and spleno-megaly, acute leukaemia, multicolour flow cytometry, immunophenotype, CD marker expression, Down syndrome.

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anaemia, thrombocytopenia and moderate leukocytosis in the peripheral blood (PB). Extra bone marrow lesions in children occur in the bones and abdomen. The prognosis is obviously poor.

The morphology of AMKL is not specific for leukaemia: the diagnosis can be established based on finding of at least 20% of blasts (with at least half of them of megakaryocytic lineage) in the bone marrow. Low numbers of blasts can be characteristic of the disease onset. The AMKL is frequently accompanied by myelofibrosis, which complicates obtaining bone marrow aspirates of good quality (up to the “dry” smear), which necessitates using PB for accurate diagnostic. Differentiated megakaryocytes are morphologically similar to myeloblasts with separation of cytoplasm or lymphoblasts. More differentiated megakaryocytes have medium or large size (12–18 microns) and are present together with less differentiated blasts that have high nuclear-cytoplasmic ratio. The nuclei of megakaryocytes have round and slightly irregular shape or indentations with delicate mesh of chromatin, and contain one to three nucleoli. Chromatin distribution and nuclear hyperchroma is typical uniform. The cytoplasm is basophilic, often forms protrusions or pseudopodia. Auer rods are absent. Dysplastic micro-megakaryocytes, whose nuclei are not lobed and show dense chromatin structure, can be observed, while the dysplastic changes in granulocytic and erythroid lineages are rare.

Similar to some other hematological diseases, in some cases tumour cells cannot be detected, which makes morphological diagnostics of AMKL challenging because of the high rate of false-negative results. Specifically, biological samples from patients with AMKL may be falsely evaluated as being free of tumour cells if the leukemic blasts have paratrabecular arrangement, or are organised into conglomerates and are thus not captured in the material sent to evaluation.

Cytochemistry analysis of the leukemic blasts typically reveals the absence of myeloperoxidase (MPO), chloroacetate esterase, and lipids, while acid phosphatase (AP), acid nonspecific esterase, α-naftilacetate esterase (a-NE) and PAS can be seen in the diffuse or coarsely granular form.

AMKL is not associated with specific genetic abnormalities. In adults, various chromosomal alterations, including 3q21q26; −5/del(5q); −7/del(7q); inv(12) (p10) can be seen. In children, mostly normal karyotype with multiple chromosomal breakages is observed. However, identification of t(1; 22) (p13; q13) (RBM15-MKL1) in children is confirmative of the childhood AMKL. This form of the disease is mostly found among infant girls (first 6 months of life) and children up to 3 years. The expression of a fused gene RBM15-MKL1 exerts a modulating effect on chromatin organisation, NOx-induced differentiation and extracellular signaling pathways. De novo AMKL with multi-lineage dysplasia is characterised by multiple non-specific chromosomal breakages, including −7/7q−; −5/5q−. In rare cases, megakaryoblastic variant of chronic myeloid leukaemia blast crisis can be observed.

Contrary to the morphological diagnostics, multicolour flow cytometry allows identifying megakaryocyte lineage blasts quickly and accurately, and differentiating between the AMKL and acute lymphoblastic leukaemia, other variants of AML and bone marrow metastases of small cell cancer. According to the current WHO classification, AMKL megakaryocytes are cancer stem cells with some level of commitment to megakaryocytic differentiation. The megakaryocyte markers (CD31, CD41a, CD61, CD62, and coagulation factor VIII) are linearly associated with AMKL, with CD41a and CD61 forming a complex of glycoproteins gpIIb/gpIIIA.

It is assumed that evaluation of CD42b expression allows distinguishing platelets that adhere to the nucleated cells from megakaryoblasts: CD42b is expressed on platelets and not on megakaryoblasts. Consequently, a combination of high level of CD41a and CD61 expression with low level of CD42b characterises megakaryoblasts, and distinguishes them from myeloblasts with adhered platelets (Fig. 1). CD61 is normally expressed on skin mast cells and osteoclasts, however, this does not affect the interpretation of flow cytometry data, since neither of these cell types are normally captured in the samples from patients (bone marrow, peripheral blood, cerebrospinal fluid). In up to 18% of cases of paediatric AMKL, the cells are positive for glycophorin A. Markers of B-cell lineage are almost always negative.

### MATERIALS AND METHODS

Our study is based on the database of laboratory of immunology at FSCCHOI for the period of 2012–2014, where the data on 731 paediatric patients with confirmed diagnosis of AL are stored. Additionally, the data on 1623 patients of different ages with confirmed diagnosis of AL was obtained from the database of clinical immunology and molecular diagnostics laboratory at PSSPbMU for the period of 2007–2014 and the data on 97 adult patients and 416 paediatric patients with AL was obtained from the database of the flow cytometry laboratory at SCPCS covering the period of 2010–2014. Prior to accessing the data, the informed consent of the patients or their parents/guardians was obtained.
Almost all samples from the patients FSCCCHOI and SCPCS and from 9 out of 11 samples of PFSPbMU (male patients 2 days and 73-year-old correspondingly) were represented by the bone marrow. Additionally, cerebrospinal fluid and bone marrow samples were obtained from a 49-year-old female patient with the suspicion of neuroleukaemia.

Sample preparation was performed by stain-lyse-wash method at FSCCCHOI and SCPCS, or by stain-lyse-no washes method at PFSPbMU. The flow cytometry analysis was performed at FSCCCHOI on BD FACSCantoII (BD Bioscience, San Jose, CA, USA), or at PFSPbMU on PartecPAS (Partec GmbH) (2007) and FC500 Beckman Coulter (Beckman Coulter, Fullerton, CA, USA) (2007–2014), at SCPCS on FacsCalibur (BD Bioscience, San Jose, CA, USA) (2010–2012) and BD FACSCantoII (BD Bioscience, San Jose, CA, USA) (2012–2014). Quality controls were performed on a daily basis using calibration beads and control material as recommended by the manufacturers. Compensation controls were performed on a monthly basis in accordance with the guidance on the quality of each laboratory. Data analysis was performed with FloMax (Partec GmbH), CXP (Beckman Coulter, Fullerton, CA, USA), Infinicyte (Cytognos, Salamanca), CellQuestPro and FACSDiva (BD Bioscience, San Jose, CA, USA) software. Participating laboratories regularly participate in the programs of external quality assessment: Federal Service of External Quality Control (FSCCCHOI and PFSPbMU) and Central European Quality Control Program (PFSPbMU and SCPCS).

RESULTS

Acute megakaryoblastic leukaemia incidence is quite rare in adult and paediatric hematology practice. According to our pooled data, which included 2,867 cases of primary diagnosis of leukaemia, incidence of childhood and adult AML M7 is 1.26% (36 cases). In the predominantly European populations (Russian medical centers of Moscow and St. Petersburg), the frequency of the disease (1.02%) differs from the one in the predominantly Kazakh population (2.3%) (Table 1). Demographic indicators of patients are presented in Table 2.

The age of patients were ranged widely from 2-day-old male to 73-year-old male. Male prevalence among paediatric patients (16:11) were particularly evident in a less than 3-year-old age group (10:3). Trisomy of chromosome 21 was detected in 7 patients who were younger than 3 years old (53.8%).

Immunophenotypic pattern varied, which was challenging for interpretation, but the statistically significant difference between AMKL pattern of children and adult patients (Table 3) was not observed. The obligatory

### Table 1: AMKL incidence according to the databases from three diagnostic centers.

<table>
<thead>
<tr>
<th>Diagnostic centers</th>
<th>Acute leukemias, primary diagnostics (n)</th>
<th>AMKL</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>PFSPbMU</td>
<td>1623</td>
<td>13</td>
<td>0.8</td>
</tr>
<tr>
<td>FSCCCHOI</td>
<td>731</td>
<td>11</td>
<td>1.5</td>
</tr>
<tr>
<td>SCPCS</td>
<td>514</td>
<td>12</td>
<td>2.3</td>
</tr>
<tr>
<td>In total</td>
<td>2867</td>
<td>36</td>
<td>1.26</td>
</tr>
</tbody>
</table>

### Table 2: Age and gender of studied AMKL patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Gender</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>16</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>5</td>
<td>4</td>
<td></td>
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</tbody>
</table>

### Table 3: Data of AMKL blast immunophenotyping.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Expected expression</th>
<th>Children</th>
<th>Adults</th>
<th>In total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/%</td>
<td>n/%</td>
<td>n/%</td>
<td></td>
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</table>

- **Marker of hematological origin**
  - CD45: + 13/48.1 | 5/55.5 | 18/50.0
  - CD34: ± 8/29.6 | 3/33.3 | 11/35.0
  - CD38: ± 9/33.3 | 6/66.7 | 15/41.7
  - CD117: + 13/48.1 | 3/33.3 | 16/44.4
  - HLADR: − 4/14.8 | 3/33.3 | 7/19.4

- **Line-unrestricted markers of precursor cells**
  - CD13: ± 9/33.3 | 3/33.3 | 12/33.3
  - CD33: + 18/66.7 | 8/88.9 | 26/72.2
  - CD15: − 2/7.4 | 3/33.3 | 5/13.9
  - CD65: 1/3.7 | 1/3.3 | 2/7.4

- **Myeloid markers**
  - CD11b: + 2/7.4 | 2/5.6
  - CD66: 1/3.7 | 1/3.3 | 2/5.6

- **Megakaryocytic markers**
  - CD41: + 23/85.2 | 9/100 | 32/88.9
  - CD42a: 17/63.0 | 3/33.3 | 20/55.5
  - CD42b: 8/29.6 | 4/44.4 | 12/33.3
  - CD61: + 22/81.5 | 8/88.9 | 30/83.3
  - CD62P: 5/18.5 | 1/11.1 | 6/16.7

- **Markers of other lineages**
  - CD2: + 2/7.4 | 2/5.6
  - CD4: ± 11/40.7 | − | 11/30.6
  - CD7: + 10/37.0 | 2/22.2 | 12/33.3
  - CD16: 2/7.4 | 2/5.6
  - CD11b: 2/7.4 | − | 2/5.6
  - CD66: 1/3.7 | − | 1/2.8
  - CD123: 5/18.5 | − | 5/13.9
  - CD25: + 1/11.1 | 1/2.8
  - CD71: 1/3.7 | 1/11.1 | 2/5.6
  - GlyA: 2/7.4 | − | 2/5.6
flowcytometric evaluation of the bone marrow or PB from patients with suspected acute leukaemia included an assessment of SSC/CD45 dot plot, where the position of major cell populations would be quite stable. The appearance of cell populations outside the conventional dot plot positions of lymphocytes, monocytes and granulocytes, in most cases, were considered as an evidence of oncohaematological pathology (Fig. 2). CD42b antigen presence was revealed in third children patients and more than 40% of adult presence.

Example 1. Bone marrow sample of 33-month-old male patient 1 was evaluated and was found to contain an additional population, constituting up to 70.0% of nucleated cells. The cells were medium or large-sized with an intermediate level of granularity and low levels of CD45 expression. Lymphocytes constituted 4.7%. The size of the blasts roughly matched the one of the monocytes, while the level of CD45 expression was reduced in comparison with other nucleated cells. The expression of megakaryocytic markers was detected on all cells belonging to the blast population. Blasts were homogeneously positive for CD41a, CD42a, CD61, CD34 and CD17 expressions. Their myeloid origin was confirmed by detecting CD33 expression. Total blasts immunophenotype (CD3+CD34+CD41a+CD42a+CD61+CD45low to dimCD56+CD117+CD123+) was compared with the expected expression of various differentiation markers in AML M7 (Table 3). Bright expression of myeloid antigen CD33 and megakaryocytic antigens CD41a, CD42a and CD61 on the surface of blast cells combined with the lack of MPO expression proved the diagnosis of AMkL.

Example 2. Peripheral blood of 2-day-old male patient 2 was analysed for diagnostic purposes. Flow cytometry data showed a sharply deformed cell pattern in the PB, which was presented by a single cluster (87.0% of nucleated cells) of medium-sized cells with low and intermediate level of granularity and very low level of CD45 expression (negative to dim). This data were in accordance to the morphology data that showed total blastosis. MPO expression was not detected by cytochemistry or flow cytometry. The blast immunophenotype was CD33+CD34+CD38-CD45low to dimCD56+CD61+. Bright expression of myeloid antigen CD33 and megakaryocytic antigen CD61 on the surface of blast cells coupled with the absence of MPO expression proved the diagnosis of AMkL.

DISCUSSION

Currently, leukaemia is regarded as a malignant proliferation of immature hematopoietic precursors that are morphologically identified as blast cells with an obvious AMkL-specific immunophenotypical blast profile and are not the result of inflammation. Leukaemia-associated blast cells as abnormal counterparts of normal hematopoietic precursors may be positive for panleukocytic marker CD45. Its expression level is unconditionally lower on the blast cells as compared to functionally mature leukocytes.

Origination of leukaemia blast cells from normal hematopoietic precursor (HP) cells causes the expression of HP markers CD34, HLA-DR and CD117. The proliferative potential of the blasts, i.e., tumour mass accumulation rate is associated to some extent by the
expression of the CD38. Myeloid lineage of AMKL

blasts in most cases is confirmed by expression CD13

and CD33 (bright). It is obvious that AMKL diagnosis

cannot be established without detecting the expression

of at least one of the platelet markers CD41, CD42b, CD61, CD36 on the cell surface, or in the cytoplasm. Aberrant immunophenotype is further characterised by
the expression of T-cellular antigens CD7, CD4 and of
other markers of different lineages.

In AMKL, missing MPO and CD14 expression sug-
gests the presence of incompletely differentiated gran-
ulocytes and monocytes. According to our data and
previous study, the cells are mostly CD45 and HLA-DR-negative, especially in children, or the expression level is significantly lower than on the normal counterpart cells.

Contrary to Wang et al. (2014), our compari-
sion of immunophenotype characteristic for AMKL in
children and adults with trisomy of 21 chromosomes
did not identify statistically significant discrepancies.
According to Wang, in children with AMKL with tri-
somy for 21th chromosome, CD13, CD33 and CD36-
positive cells are more frequently detected when
compared to children without Down syndrome, and
CD56 is more frequently detected when compared to
the adult patients. Notably, AMKL as a leukaemia of
a myeloid lineage is not always confirmed by the
expression of pan-myeloid markers. According to
pooled data from three of our diagnostic centers,
CD13 and CD33 were seen in 33.3% and more than
66% respectively, arguing against regarding this as
rare finding.

Immunophenotypic profile of AMKL has spe-
cific features as compared to the other types of AML.
In particular, the evaluation of the expression of mega-

karyocytic markers should be performed carefully, since
in many pathologic states platelets can adhere to larger
cells (monocytes and neutrophils), thus conferring
false positivity to them. Finding CD61 on the surface
of nucleated cells by flow cytometry is normally an
artefact caused by adhering thrombocytes or their frag-
ments to nucleated cells. Thrombocytic differentiation with
monocytes and neutrophils is a common occurrence in
some individuals, supposedly caused by the interaction
of CD62P with CD15 on activated platelets and CD15+ cells. Regular observations in our laboratories also fre-
cently detect CD42b on megakaryoblasts. Accordingly,
before megakaryocytic marker evaluation is attempted,
the cells should be washed 3–4 times with 15–20 ml
of buffer to remove the adhered platelets. Thus, sample
preparation should include steps similar to those used
when preparing the cells for detecting cytoplasmic
markers and immunoglobulins.

Taking into account, the probability of thrombocytic
satellitism and the clinical significance of diagnosis,
it is not advisable to analyse samples that were stored
for some time, and samples that were obtained with
violation of transportation rules or after the onset of
treatment.

In summary, testing blast cells to find AMKL-specific
properties permits to establish the immunophenotypical
diagnosis of AMKL within one working day.

Our study demonstrates that the significant AMKL-
specific cell surface markers are mostly CD42a, CD61
and CD4 and that their evaluation assists in establishing
a diagnosis of AMKL. Our data allows hypothesising that
CD42b cannot be used to distinguish leukemic blasts from
the cells with platelet satellitism, because its expression is
one of manifestations (signs?) of tumour aberrancy.

REFERENCES


2. Athale UH, Razzouk BI, Raimondi SC, Tong X, Behm FG, Head
DR, et al. Biology and outcome of childhood acute mega-

2001;97:3727–3732.

3. Bennett J, Catovsky D, Daniel MT, Flandrin G, Head
HR, Sultan C. Proposals for the classification of the acute leuke-
mias. French–American– British (FAB) co-operative group. Br J

van’t Veer MB. Proposals for the immunological classification of
acute leukemias. European group for the immunological charac-


5. Giri S, Pathak R, Prouet P, Li B, Martin MG. Acute megakaryocytic
leukemia is associated with worse outcomes than other types of


A, Reinhardt K, Dworzak M. Blast cell deficiency of CD11a as
a marker of acute megakaryoblastic leukemia and transient
myeloproliferative disease in children with and without Down

8. Weinberg O, Seetharam M, Ren L, Seo K, Ma L, Merker JD, Gotlib J,
Zehnder JL, Arber DA. Clinical characterisation of acute myeloid
leukemia with myelodyplasia-related changes as defined by
the 2008 WHO classification system. Blood. 2009;113(9):
1906–1908.

9. Bozkurt SU, Berrak SG, Tugtepe H, Canpolat C, Palanduz S,
Tecmir T. Acute megakaryoblastic leukemia mimicking small
round cell tumour with novel t(1;5)(q21;p13). APMIS. 2008;

Correia C, Bizarro S, Almeida M, Teixeira MR. Acute megakary-
2009;113(9):141–149.

11. Duchayne E, Fenneteau O, Pages MR, Saintry D, Arnoulet C,
Dastugue N, Garand R, Flandrin G. Groupe Français d’héma-
tologie cellulaire; Groupe Français de cytogénétique héma-

Correia C, Bizarro S, Almeida M, Teixeira MR. Acute megakaryo-
blastic leukemia with a four-way variant translocation originating
the RBM15–MKL1 fusion gene. Pediatr Blood Cancer. 2011;56:
846–849.