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The frequency and characteristics of aberrant immunophenotypes in acute lymphoblastic leukaemia (ALL) in children – a multicenter study

Częstość występowania i charakterystyka nietypowych immunofenotypów w ostrych białaczkach limfoblastycznych u dzieci – analiza wieloośrodkowa

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SUMMARY

The immunophenotype of acute lymphoblastic leukaemia (ALL) cells is used for classification of leukaemia based on origin and maturation/differentiation stage of leukaemia cells. The aberrant expression of lineage restricted and line associated determinants on the surface and in cytoplasm of leukaemia cells (atypic immunophenotypes) is useful for detection of minimal residual disease (MRD) by flow cytometry. The multicenter studies of typical and atypical immunophenotypes of leukaemia cells enrolled 645 children with diagnosis of B cell origin (86.54%) and T cell origin (13.46%) ALL. Atypical immunophenotypes were noted in 204 cases (31.62%) with myeloid co-expression (CD13 and/or CD33, CD15) as the most frequent type of aberrant determinant expression. In B cell line-originating ALL, the frequency of atypical immunophenotypes decreased along with the maturation of B cells (ALL progenitor B – 46.65%, common ALL – 32.6%, ALL-pre B and ALL-B – 17.8%). In the group of T-ALL, atypical immunophenotypes were noted in 22 cases (25.2%). The analysis of aberrant expression of determinants showed an association between CD34 and myeloid co-expression (CD33, CD13) and a reverse association between CD34 and CD20.

The percentage and character of atypical immunophenotypes observed in ALL patients indicate the effectiveness of MRD detection by flow cytometry in above 60% of ALL cases in children.

KEY WORDS: Acute lymphoblastic leukaemia – Children – Atypic immunophenotypes – Multicolour cytometry

STRESZCZENIE

Immunofenotyp komórek ostrej białaczki limfoblastycznej (ALL) jest podstawą klasyfikacji uwzględniającej linię pochodzenia komórek białaczkowych, ich stopień dojrzałości i zróżnicowania. Zaburzenia immunofenotypu dotyczące determinant restrykcyjnych i związanych z różnicowaniem oraz dojrzewaniem komórek białaczkowych są wykorzystywane do poszukiwania choroby resztkowej (MRD) metodą cytometrii przepływowej. Wieloośrodkowe badania immunofenotypów komórek ALL z uwzględnieniem nietypowych immunofenotypów objęły 645 dzieci w większości przypadków (86.54%) wykazujących białaczkę z linii limfocyta B, w pozostałych (13.46%) – z linii limfocyta T. Nietypowe immunofenotypy obserwowano u 204 dzieci (31.62%). Najczęściej była to ko-ekspresja determinant mieloidalnych (CD13 i/lub CD33, CD15). W grupie ALL z linii limfocyta B częstość nietypowych immunofenotypów obniżała się w miarę dojrzewania i różnicowania komórek białaczkowych (ALL progenitor B – 46.65%, common ALL – 32.6%, ALL-pre B i ALL-B – 17.8%). W grupie ALL z linii limfocyta T nietypowe immunofenotypy obserwowano w 22 przypadkach (25.2%). Analiza zależności występowania determinant wykazała związek pomiędzy ekspresją CD34 a ko-ekspresjami CD13 i CD33 oraz odwrotną zależność pomiędzy ekspresją CD34 a CD20. Odsetek i charakterystyka nietypowych immunofenotypów wskazują na skuteczność poszukiwania MRD metodą cytometrii w co najmniej 60 % przypadków ALL.

SŁOWA KLUCZOWE: Ostra białaczka limfoblastyczna – Dzieci – Nietypowe immunofenotypy – Wielokolorowa cytometria

INTRODUCTION

The immunophenotypes of leukaemia cells are used for immunological classification of acute leukaemia. Moreover, they are regarded as a clinical risk factor associated with the prognosis. The expression of determinants on the surface and the presence in cytoplasm of leukaemia cells are commonly used for description of origin and the maturation/differentiation stages of leukaemia cells [1-10]. The atypic immunophenotypes are associated with or are an effect of deregulation of stem cell differentiation (lineage committed cells or early precursors) or maturation in later stages of given line ontogeny. The precise description of aberrant and/or atypic expression of determinants is useful in detection of minimal residual disease (MRD) [11–19].

The immunological classification of ALL includes the line of origin and leukaemia cells stages of maturation/differentiation. The expression of lineage-restricted determinants in cytoplasm and surface (e.g. CD22, CD19, CD79a for B cell line, CD3 for T cells) is the indicator for origin of leukaemia cells. The assay of leukaemia cells immunophenotype includes monoclonal antibody to lineage-restricted determinants and determinants associated with maturation and differentiation stages of lymphocyte lines cells. The most common list of monoclonal antibodies used for routine diagnosis of lymphoblastic leukaemia includes the following determinants: CD10, CD20, CD2, CD5, CD7, CD4, CD8, CD34, HLA-DR, CD33, CD13, CD14, CD15, heavy and light chains of immunoglobulins [1, 3, 5, 8, 13, 17–21].

The description of disturbances of leukaemia cells immunophenotypes and detection of minimal residual disease (MRD) requires multicoloured cytometry and a wide panel of monoclonal antibodies. This type of assay leads to better characteristics of a single leukaemia cell and differentiation of subpopulations within the leukaemic blasts population. It may help to find drug-resistant cells (e.g. showing the expression of multidrug resistance proteins) and to detect MRD. The following aberrant immunophenotypes are recognised: incomplete phenotype (lack of expression of determinant obligatory for normal ontogenesis, e.g. HLA-DR on B lymphocyte precursors), co-expression (cross-lineage determinants expression, e.g. CD33 on common ALL cells), overexpression (increased amount of determinant per cells, e.g. CD13, HLA-DR) or hypoexpression of determinant (decreased amount of determinant per cell). A rare form of aberrant immunophenotypes includes asynchronic immunophenotype, where the determinants from early and mature stage of differentiation are simultaneously present on leukaemia cell [1, 17, 19, 22].

The co-expression of determinants is a most frequent type of disturbances of leukaemia cells ontogeny. The multicoloured cytometry is commonly used as a reliable and fast method for detection of atypic immunophenotypes [11, 13, 14, 17, 18, 21–24]. The clinical significance of atypic immunophenotypes (lineage infidelity, co-expressions of determinants or other aberrations) of leukaemia cells is still a matter of discussion [9, 10, 16, 19]. Statistical observations based on a large group of patients and multicenter studies showed an association between the occurrence of atypic immunophenotypes and resistance to therapy, as well as an increased ratio of relapses. However, the significance of different types of atypic immunophenotypes (co-expression, incomplete immunophenotype, hyperexpression) did not show a correlation with prognosis and total survival [5, 7, 9, 10, 19, 25]. Nevertheless, the results of analysis of therapy effect accumulated data, long time survival and immunophenotypes of leukaemia cells may help in finding the optimal therapy for an individual patient ("patient-tailored" therapy). The observations of different immunophenotypes of leukaemia cells allow for a better understanding of single determinant's role in regulation of ontogeny process, e.g. myeloid determinant CD15 is often seen on acute lymphoblastic leukaemia cells in infants and to date, the clinical significance of this expression is unknown.

The purpose of this study is the analysis of frequency and characteristics of atypic imumnophenotypes of acute lymphoblastic leukaemia cells diagnosed in pediatric haematological departments in Poland. This analysis may help to find the optimal set of monoclonal antibodies for immunophenotypic classification and in consequence for detection of MRD based on aberrant expression of determinants.

PATIENTS AND METHODS

A total of 645 children with the initial diagnosis of ALL admitted to haematological departments in the years 2004–2009 were included into the study (the list of haematological departments is shown in List of participants).

METHODS

The leukaemia cells before the therapy were obtained from the bone marrow with the aspirate biopsy under local anaesthesia, or from the peripheral blood. Two methods, density gradient or lysis of erythrocytes (in the majority of analyses), were used for obtaining the leukaemia cells suspension. The immunophenotype of leukaemia cells was assayed after staining with the following monoclonal antibodies (Becton-Dickinson, DAKO):

HLA-DR, CD34, CD38, CD117, CD19, CD22, CD2, CD5, CD7, CD13, CD33, CD10, CD20, CD4, CD8, CD15, CD14, CD3, immunoglobulins heavy chains, kappa/lambda light chains, CD24, CD45 for surface expression of determinants and MPO, CD79a, CD22, CD3 and TdT (terminal deoxytranspherase) for cytoplasmic presence of these determinants.

The 3-colour or multicolour (4- and more colours – PE, FITC, APC, PerCP-Cy5 or others) staining was performed for the analysis of leukaemia associated immunophenotype and description of atypical immunophenotypes. The leukaemic cells population was selected based on SSC/FSC dot plot gate (two parameters) with additional, lineage-specific determinants (CD19 – B cell lineage, CD5, CD7 – T cell lineage) or CD45 expression in multicolour analysis. The acquisition of no fewer than 20000 events from each sample was used in different types of flow cytometers (FACS-Calibur, FACS-Canto (Becton-Dickinson), Epics, DAKO-Cytomation (DAKO, Denmark). The percentage of leukaemia cells with fluorescence higher than isotypic control for a given determinant, the expression of lineage-specific, leukaemia-associated determinants and aberrant expression of determinants (atypical immunophenotypes) were included into the analysis.

The atypical immunophenotypes were described based on double or multicolour staining of lineage-specific and aberrant determinants (e.g. CD13, CD33, CD15, CD2, CD5, CD7 on $CD19^+$ cells). The expression of a determinant was recognised when no less than 20% of leukaemia cells were positive based on higher fluorescence than isotypic control.

The following types of aberrant immunophenotypes were recognised:

- incomplete – the lack determinant obligatory for normal ontogeny on leukaemia cells (e.g. HLA-DR on $CD19^+$ cells),
- co-expression – cross-lineage expression of determinants (e.g. CD13, CD33 on $CD19^+$ cells),
- overexpression of determinants (e.g. HLA-DR, CD33),
- hypoexpression of determinants (e.g. CD10, CD45),
- asynchronic expression (e.g. CD34 and cytoplasmic or surface heavy or light chains of immunoglobulins).

Based on data from the studies of T cell ontogeny, the expression of CD10 and CD20 on ALL-T leukaemia cells was not considered as aberrant immunophenotype [19].

The cytoplasmic (MPO, CD79a, CD22, CD3, TdT) staining of fixed leukaemia cells was used as a routine procedure for description of leukaemia cells origin (lymphoid or myeloid) in all cases. The immunophenotypic classification of acute leukaemia was based on complex diagnosis, including cytoplasmic expression of lineage-specific determinants and surface expression of line-associated determinants. The following classification of lymphoblastic leukaemia from B and T cell line (Table 1) was used in all centres included into the study.

Table 1. Immunological classification of acute lymphoblastic leukemias from B cell and T cell lines
Tabela 1. Klasyfikacja immunofenotypowa ostrej białaczki limfoblastycznej z linii limfocyta B i T

Classification	Immunophenotype of acute leukaemia cells		Remarks
	Cytoplasm	Surface	
B cell line Early precursors: progenitor B – ALL-proB	CD79a, CD22, TdT	CD34, HLA-DR, CD19, CD22, CD38	In infants often co-expression of CD15
Precursors type: common ALL type I common ALL type II	CD79a, CD22, TdT	CD34, CD19, CD10, HLA-DR, CD22, CD38, CD19, CD10, HLA-DR, CD22, CD20, CD38, (CD34)	Some cases CD45-, frequently aberrant immunophenotypes
Transitional type: Transitional B cell ALL	CD79a, CD22, TdT	CD19, HLA-DR, CD22, CD20, heavy chain of immunoglobulin (sIg), (CD10)	Mainly in infants
ALL - preB	CD79a, CD22, IgM (μ)	CD19, HLA-DR, CD22, CD20,	
Mature B cell: ALL - B	CD79a, CD22, cIg(GAM)	CD19, HLA-DR, CD22, CD20, sIg(GAM), kappa or lambda	
T cell line Precursor type: ALL-preT	CD3, TdT	CD1a, CD2, CD5, CD7, CD34, HLA-DR, (CD4, CD8, CD38)	Weak expression of CD10, CD20
ALL-T	CD3, TdT	CD1a, CD2, CD4, CD5, CD7, CD8, CD3	Heterogeneity of expression

() – Expression weak or present in part of cases

RESULTS

Leukaemia associated and aberrant immunophenotypes

The study enrolled 645 patients below 18 years of age with the initial diagnosis of ALL. The origin of leukaemia cells from B-cell line was shown in the majority of patients (558 cases – 86.51%), from T cell line (ALL-T) – in the remaining patients (87 cases – 13.48%) (Table 2). Within the B cell origin leukaemia, the progenitor type (ALL-proB) was noted in 30 patients (4.6% of total ALL), common ALL type in 500 patients (77.51% of total ALL), while more mature types: pre-B and ALL-B were noted in 28 patients (4.34% of ALL). The common ALL patients were divided into 2 groups based on CD34 expression on leukaemia cells – present (370 patients – 74.0%) or absent (130 patients – 26.0%). The patients with T cell line leukaemia were considered as one group despite of expression of CD34 and HLA-DR (ALL-preT subtype). Patients with bi-line and bi-phenotypic types of leukaemia were excluded from this study.

The expression of CD34 was demonstrated on very early (committed cells) and precursor cells of B line ALL (392 out of 530 cases ALLproB and common ALL – 73.9%) and part of ALL-T cases (28 out of 87 assays – 32.1%) (Table 2). CD34 as stem cell marker is associated with early stages of cell ontogeny – CD19, CD10, CD22, HLA-DR for B and HLA-DR, CD2, CD5, CD7 for T cell lines. Along with the maturation of both cell lines, the expression of CD34 disappears, although in asynchronous immunophenotype, the preserved expression may be seen. The association between early stages of ontogeny and aberrant expression of determinants observed in our group of patients (Table 2) was in keeping with other studies of ALL in children. The highest percentage of aberrant immunophenotypes (mainly co-expression of myeloid determinants – CD13, CD33, CD15) was noted in ALL-proB type (46.6%) and common ALL CD34+ (37.9%). The maturation of B cells was associated with a decline of aberrant immunophenotypes number (17.8%) (Table 2). In ALL-T, a higher percentage of atypical immunophenotypes was observed in ALL-preT (HLA-DR/CD34+) subtype.

Table 2. Atypic immunophenotypes in studied group of patients
Tabela 2. Nietypowe immunofenotypy – rodzaje i częstość występowania w badanej grupie

Type of leukaemia	Number of patients	Atypical immunophenotypes number (%)	Aberrant expression of determinants (lack or additional expression)
ALL-proB CD34+ CD34–	30 22 6	14 (46.6)	Co-expression of CD2, CD13, CD33, CD15, CD13 and CD33
Common ALL CD34 + CD34 –	500 370 130	163 (32.6) 133 (37.9) 30 (28.03)	CD13, CD33, CD15, CD7, CD2, CD3, CD13 and CD33,
ALL-pre B and ALL-B	28	5 (17.8)	CD13, CD33, CD13 and CD33, CD15
ALL-T CD34+ (preT) CD34–	87 30 57	22 (25.28) 13 (46.4) 9 (15.2)	CD13, CD33, CD15
Total	645	204 (31.62)	

Associations between determinants

CD34 expression was independent of HLA-DR presence in B precursor types of leukaemia, so the number of HLA-DR+ cases was similar in CD34- and CD34+ groups of patients (Table 3). A weak reverse association between expression of CD34 and CD20 was noted, what resembled the normal process of B cell ontogeny. The difference in expression of CD22 between CD34+ and CD34- leukaemia cases was significant. High expression of CD22 in CD34+ leukaemia cells is observed in ALL-proB and common ALL leukaemia type. The CD22+ common ALL leukaemia cells were often CD20–. The surface expression of CD22 on early precursors of B cells is typical for leukaemic ontogeny and may be used for MRD detection. In normal ontogeny, the early precursors are CD22– on the surface and CD22+ in cytoplasm only.

Table 3. Association between expression of CD34 and HLA-DR, CD20, CD13/CD33 determinants in acute lymphoblastic leukaemia of B cell precursors (ALL-proB, common ALL)
Tabela 3. Zależność pomiędzy ekspresją determinant CD34 I HLA-DE, CD20 oraz CD13/Cd33 w ostrej białaczce limfoblastycznej prekursorów linii limfocyta B (ALL-proB, common ALL)

CD34	Absent (<20% of leukaemia cells) 131 analysis	Present (>20% of leukaemia cells) 392 analysis	Total number of cases	Comments
HLA-DR + (% of CD34)	104 (79.38%)	374 (95.40%)	478	Difference non-significant (n.s.)
CD20 + (% of CD34)	52 (39.8%)	114 (29.0%)	166	n.s.
CD22 + (% of CD34)	80 (61.0%)	306 (78.1%)	386	p= 0.0089 very significant
CD13 + (% of CD34)	11 (7.23%)	53 (13.53%)	64	n.s.
CD33+ (% of CD34)	13 (9.92%)	27 (6.71%)	40	n.s.
CD13/CD33+ (% of CD34)	3 (2.3%)	42 (10.44%)	45	n.s.
Myelo co-expression (total)	27 (20.60%)	122 (31.1%)	149	n.s.

The expression of CD13 was more often seen on CD34+ cells (53 cases – 13.5%) as compared to CD34– (11 cases – 7.23%), but the difference was not significant. A similar association was seen for expression of CD33 (27 cases on CD34+ leukaemia cells versus 13 cases of CD34– leukaemia cells).

The co-expression of myeloid determinants was noted in 149 cases of precursors B leukaemia cells with a weak association with expression of CD34 (31.1% in CD34+ cases versus 20.6% in CD34– cases) (Table 3).

In ALL-T, the expression of CD34 and HLA-DR are typical for early precursor T cell subtype (ALL-preT). The expression of HLA-DR was associated with CD34 expression (statistically very significant). However, about 10% cases of ALL-T without CD34 expression showed HLA-DR presence on the leukaemia cell surface. The expression of CD10 was higher in CD34– cases of ALL-T, what resembled the expression of CD10 on thymic stages of T cell ontogeny, when the expression of CD34 is absent. The expression of CD10 and CD34 on the same cells may be considered as the atypic immunophenotype of this leukaemia cell. In ALL-T, the atypic immunophenotypes (expression of myeloid determinants) were observed in association with the expression of CD34 (with a statistically significant difference between CD34+ versus CD34– cases), what denotes an association between atypical immunophenotypes and early stage of T cell differentiation (Table 4), similar to B cell precursors leukaemia.

Table 4. Association between expression of CD34 and HLA-DR, CD20, CD10, CD13/CD33 determinants in acute lymphoblastic leukaemia of T cell
Tabela 4. Zależność pomiędzy ekspresją determinant CD34 I HLA-DR, CD20, CD10 oraz CD13/CD33 w ostrej białaczce limfoblastycznej z linii limfocyta T

CD34	Absent (<20% of leukaemia cells) 57 analysis	Present (>20% of leukaemia cells) 30 analysis	Total number of cases	Significance of difference
HLA-DR+ (% of CD34)	5 (8.7%)	9 (30.0%)	14	p=0.0004 very significant
CD10 (% of CD34)	16 (28.0%)	5 (16.6%)	21	p=0.1223 n.s.
Co-expression of myeloid determinants (% of CD34)	7 (12.28%)	14 (46.6%)	21	p=0.0034 very significant

HLA-DR expression on leukaemia cells from B cell line was noted in 474 cases out of 558 cases (84.9%) and was associated with CD34, CD10 and CD19 expression. Lack of HLA-DR expression on B cell precursors (6 cases out of 558 – 1.07%) was regarded as atypical (incomplete) immunophenotype. In these 6 cases of HLA-DR- common ALL cells, the co-expression of myeloid determinants (CD13 and CD33) was noted in 2 cases. In ALL-T, the expression of HLA-DR was observed in 14 cases and the following immunophenotype were noted: HLA-DR and CD34 – 5 cases, HLA-DR, CD34 and myeloid co-expression – 3 cases, HLA-DR and myeloid co-expression – 2 cases. The immunophenotypes: HLA-DR, HLA-DR/CD34/CD10, HLA-DR/CD10, HLA-DR/CD10 and myeloid co-expression was observed in single cases of ALL-T.

CD20 expression was noted on leukaemia cells in 166 cases of B cell precursors leukaemia patients (31.7%). In progenitor B ALL (ALL-proB), CD20 was present on leukaemia cells in 2 cases and in both cases its expression was associated with co-expression of myeloid determinants. However, the majority of myeloid co-expression (both CD13 and CD33) was noted on B cell precursors leukaemia cells without the expression of CD20 (98 cases out of 136 myeloid-positive with CD20 assay – 72.05%). This very significant ($p < 0.0001$) reverse association supports the observation of a decrease of atypical immunophenotypes frequency along with B cell ontogeny.

CD13 and/or CD33 expression on B cell origin leukaemia cells is recognised as the most common atypic immunophenotype. In our group of patients, myeloid co-expression was noted in 46.6% of progenitor B ALL cases, 32.6% of common ALL cases and 25.2% of T-ALL cases. These results are in

keeping with other observations of higher frequency of aberrant myeloid expression in early stages of B cell maturation.

CONCLUSIONS

The frequency of atypic immunophenotypes declines with increasing maturation stages of lymphoid precursors.

The most common are co-expressions of determinants from myeloid line, what supports the indication for routine assay of lineage restricted determinants in cytoplasm.

Flow cytometry is an effective method in detection of MRD based on atypic immunophenotypes of blastic cells in more than 60% of ALL in children.

DISCUSSION

The study of 645 children with ALL showed the atypical immunophenotypes (aberrant expression of the determinant on the surface) of leukaemia cells in 31.62% of cases. Myeloid co-expression was the most frequent type of atypical immunophenotypes of ALL leukaemia cells. The analysis of CD34 expression on leukaemia cells showed a positive association with myeloid co-expression and a reversed association with CD20 expression in B cell origin ALL. In common type of ALL, the expression CD34 was independent of leukaemia-associated determinants (CD10, CD19, HLA-DR). The atypical immunophenotypes were associated with early stages of B or T cell maturation and decreased in frequency along with the differentiation and maturation of leukaemia cells.

Frequency of atypical immunophenotypes

The frequency of atypical immunophenotypes understood as the aberrant expression of determinants, was noted in about 30% of acute lymphoblastic leukaemia cases in different haematological studies [5, 19, 25]. However, for detection of MRD, the complex flow cytometry assays including the "classic" quantitative atypic immunophenotypes (co-expressions, asynchronic expression) and qualitative changes in expression of determinants are used. The "empty space" technique is recommended for detection of leukaemia cells within normal bone marrow cells in remission based on qualitative changes in expression of leukaemia associated antigens. MRD detection is possible in about 85-90% of ALL patients, including both types of analysis with flow cytometry [8, 11, 14, 17, 22, 23].

The association of CD34 expression and other determinants

The expression of CD34 on leukaemia cells is associated with higher therapy sensitivity of these cells in comparison to cases without this determinant, what might be an additional factor supporting a better prognosis [7, 29]. The expression of B cell leukaemia associated determinants (HLA-DR, CD10, CD19, CD20) was independent of CD34 expression and both (CD34+ and CD34–) subpopulations of acute lymphoblastic leukaemia cases were noted [19, 29]. This immunophenotype (CD34-, CD10+, CD19+, HLA-DR+) is absent in normal ontogeny of B lymphocyte precursors and may be used for MRD detection. The expression of CD20 in normal B cell ontogeny increases parallel to disappearance of CD34 and CD10 expression [1].

The occurrence of CD20 in later stages of B cell ontogeny on leukaemia cells is associated with lower frequency of co-expression of myeloid-determinants, what was noted in our subjects and in other groups of B cell line ALL patients [30]. The clinical significance of aberrant expression of determinants, mainly co-expression of myeloid antigens, as a prognostic factor is low due to aggressive therapy improving the remission rate and long time survival. However, the precise description of

leukaemia cell immunophenotypes at diagnosis is the basis for detection of MRD in first remission and follow-up. The assay of immunophenotypes of leukaemia cells in relapse allowed for finding the changes (decrease/increase) of determinants expression (shift) as an effect of chemotherapy and/or selection of resistant population of leukaemia cells. This shift of immunophenotypes, associated with drug resistance of such cells, made detection of MRD more difficult [12] and suggested clinical problems with therapy (second-line) of these patients.

Detection of MRD by flow cytometry

The combination of monoclonal antibodies used for diagnosis of ALL is critical for the detection of MRD. At least 3–4 colours cytometry was used as a routine method for diagnosis and classification of acute leukaemia in all centres. For MRD detection, the additional line-restricted determinant is assayed and this monoclonal antibody is added to all samples for selection of cells population e.g. CD19 for B cell line leukaemia. To obtain the best results with this technique the following ideas are proposed – first to used all panel of monoclonal antibodies with added the line-restricted as base for population selection [8, 11, 14, 18] or choose only 2 or 3 samples stained with 4-6 antibodies informative for given leukaemia cells [12, 17]. The combination of 6 colours is another proposition to improve this assay [22].

The MRD detection with flow cytometry is considered a very good and fast method but still with the possibility of both types of false (positive or negative) results. The advantage of molecular method is a higher level of sensitivity but a higher cost and longer time to obtain the results are the limitations [22,23,26-28]. Concurrent detection of MRD with flow cytometry and molecular assay (PCR, real-time PCR) showed a very high agreement between the results [26–28]. The use of both methods (multicolour flow cytometry, PCR, real-time PCR for detection of fusion gene transcripts or break-points and clonal immunoglobulins, T cell receptor gene rearrangements) for one patient in monitoring leukaemia cells presence in remission may help to avoid possible false results due to clonal evolution and shifts of immunophenotype after the induction therapy [22, 23].

The response to therapy measured by leukaemia cells clearance (cytoreduction) in peripheral blood and bone marrow provided prognostic information superior to the known classic “risk factors”. However, the most relevant information came from detection of MRD at the early phases of therapy, particularly in the end of remission-inductive protocol [11, 22, 23]. The precise date of MRD detection during the induction, consolidation therapy and first remission is still not standardised and various periods for MRD assays are proposed (e.g. 15^{th} day, 33^{th} day, +6, 14, 32, 56 weeks of therapy) [13, 17, 18, 28].

Detection of MRD includes the expression of determinants from other lines (qualitative assay) and intensity of expression of determinants associated with leukaemia cells but present in normal ontogeny (e.g. CD10, HLA-DR, CD19). The qualitative changes in the expression of CD10, HLA-DR on leukaemia cells are helpful in finding MRD in a case of typical leukaemia associated immunophenotype without quantitative changes (e.g. co-expression). The technique of empty space is commonly used in such analyses and is characterised by high sensitivity and reliability. However, despite an increase in the number of results of MRD detection and clinical significance, the standard monoclonal antibodies set, multicolour analysis procedures are under the discussion.

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