Clinical significance of occult central nervous system disease in adult acute lymphoblastic leukemia: a multicenter report from the Campus ALL Network

Maria Ilaria Del Principe,1 Elisa Buzzatti,1 Alfonso Piciocchi,2 Fabio Forghieri,3 Massimiliano Bonificio,4 Federica Lessi,5 Silvia Imbergamo,6 Enrico Orzuolo,1 Giovanni Rossi,7 Nicola Fracchiola,8 Silvia Trappolini,9 Benedetta Neri,10 Chiara Sarlo,11 Adriano Venditti,1 Robin Foà15 and Anna Rita Guarini16

1Ematologia Dipartimento di Biomedicina e Prevenzione, Università degli Studi di Roma “Tor Vergata”, Roma; 2GIMEMA Data Center, Roma; 3Ematologia Dipartimento di Scienze Mediche e Chirurgiche, Università degli Studi di Modena e Reggio Emilia, Azienda Ospedaliera di Modena, Modena; 4Dipartimento di Medicina, Sezione di Ematologia, Università Verona, Verona; 5Ematologia ed Immunologia Clinica, Azienda Ospedaliera di Padova, Padova; 6UO Ematologia, Azienda Ospedaliero-Universitaria Pisana, Pisa; 7U.O. di Ematologia e Trapianto di Cellule Staminali, IRCCS “Casa Sollievo della Sofferenza”, San Giovanni Rotondo, Foggia, Ospedale Maggiore Policlinico, Milano; 8Clinica di Ematologia, AOU Ospedali Riuniti di Ancona, Ancona; 9Ematologia, Ospedale Sant’Eugenio, Dipartimento di Biomedicina e Prevenzione, Università degli Studi di Roma “Tor Vergata”, Roma; 10Ematologia, Policlinico Universitario-Campus Biomedico, Roma; 11Divisione di Ematologia, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Pavia; 12Ematologia e Trapianto di Cellule Staminali, Ospedale Vito Fazzi, Lecce; 13Ematologia, Dipartimento di Onco-Ematologia, Fondazione Policlinico Tor Vergata, Roma; 14Ematologia, Dipartimento di Medicina Traslazionale e di Precisione, Università “Sapienza”, Roma and 15Dipartimento di Medicina Molecolare, Università “Sapienza”, Roma, Italy

ABSTRACT

In acute lymphoblastic leukemia (ALL), flow cytometry (FCM) detects leukemic cells in patients’ cerebrospinal fluid (CSF) more accurately than conventional cytology (CC). However, the clinical significance of FCM positivity with a negative cytology (i.e., occult central nervous system [CNS] disease) is not clear. In the framework of the national Campus ALL program, we retrospectively evaluated the incidence of occult CNS disease and its impact on outcome in 240 adult patients with newly diagnosed ALL. All CSF samples were investigated by CC and FCM. The presence of ≥10 phenotypically abnormal events, forming a cluster, was considered to be FCM positivity. No CNS involvement was documented in 179 patients, while 18 were positive by modified conventional morphology with CC and 43 were occult CNS disease positive. The relapse rate was significantly lower in CNS disease negative patients and the disease-free and overall survival (OS) were significantly longer in CNS disease negative patients than in those with manifest or occult CNS disease positivity. In multivariate analysis, the status of manifest and occult CNS disease positivity was independently associated with a worse OS. In conclusion, we demonstrate that in adult ALL patients at diagnosis FCM can detect occult CNS disease at high sensitivity and that the status of occult CNS disease positivity is associated with an adverse outcome. (Registered at clinicaltrials.gov identifier: NCT03803670).

Introduction

Over the last two decades, improved response rates have been reported in adult patients with acute lymphoblastic leukemia (ALL).1-3 In this context of a superior systemic disease control, central nervous system (CNS) involvement has become
an ever more influential limitation to the achievement of a long-term cure and a main cause of mortality. At diagnosis, about 5-10% of adult ALL patients have CNS involvement, which translates into a shorter overall survival (OS) compared to that of patients without CNS involvement.

Conventional cytology (CC) examination of the cerebrospinal fluid (CSF) remains the gold standard for the diagnosis of CNS involvement in ALL; CC is estimated to have a >95% specificity. However, it has a relatively low sensitivity (<50%), resulting in frequent false negative determinations. Such a low sensitivity is due to the poor cellularity of CSF and to the difficulties in distinguishing benign from malignant cells on morphologic grounds only.

Flow cytometric (FCM) immunophenotyping is a valuable tool for the diagnosis and staging of hematologic disorders involving lymph nodes, blood, bone marrow and other body fluids. Current FCM assays allow detection of phenotypically abnormal cells up to the limit of at least 0.01% (1 target cell in 10⁴ events), representing, therefore, a very effective tool for minimal residual disease monitoring in acute leukemia. Indeed, several recently published experiences have demonstrated the superior sensitivity of FCM over CC for the detection of CNS disease in patients with ALL and non-Hodgkin lymphoma. These studies have also contributed to establish a new standard that is the so-called “occult CNS disease” (OCNSD), namely the status of FCM positivity and CC negativity. None of these reports has, however, clarified whether a condition of OCNSD has an additional prognostic role compared to the well-established negative impact of CC positivity. We therefore conducted a multicenter, retrospective study in the framework of the national Campus ALL program aimed at improving the management of adult ALL patients. The aims of the present study were: (i) to evaluate the incidence of OCNSD in a large series of adult patients with ALL; and (ii) to assess the impact of OCNSD on the clinical outcome of these patients.

Methods

Study design and patients

Our retrospective analysis included patients seen between January 2007 and December 2017 at 13 Italian hematologic centers. Cases were documented using a case report form. Variables included the following data: age, sex, ALL onset, genetic/cytogenetic features, B/T phenotype, white blood cell count (WBC) at diagnosis and at the time of lumbar puncture (LP), lactate dehydrogenase (LDH), chemotherapy, date of complete remission (CR), CSF cell count and chemistry, CC and FCM results, date of systemic and/or CNS relapse, allogeneic stem cell transplant (ASCT), date of death or the last follow-up. Personal information was treated in a confidential manner and all sensitive data were analyzed anonymously. Samples were collected at diagnosis. In CONSORT-era positive = MCNSDpos) (Table 1). No case proved to be positive by both FCM and CC (manifest CNS disease positive = MCNSDpos) and 18 (7%) were MCNSDpos (Table 1). There was no significant difference in distribution among the three categories. Cytogenetic/genetic status of FCM positivity and CC negativity. None of these reports has, however, clarified whether a condition of OCNSD has an additional prognostic role compared to the well-established negative impact of CC positivity. We therefore conducted a multicenter, retrospective study in the framework of the national Campus ALL program aimed at improving the management of adult ALL patients. The aims of the present study were: (i) to evaluate the incidence of OCNSD in a large series of adult patients with ALL; and (ii) to assess the impact of OCNSD on the clinical outcome of these patients.

Cell counts and conventional cytology

Cytospins for CC examination were prepared as previously described in detail. CC positivity was defined as unequivocal, morphological evidence of leukemic blast in the CSF and/or a CSF WBC count ≥5×10⁴/µL with less than 10 erythrocytes/µL. A cocktail of 6-8 monoclonal antibodies was used (Online Supplementary Table S1). On average, 1,080 events were acquired (range 0-210,000). In agreement with the recommendations for the analysis of rare events, a cluster of at least 10 phenotypically abnormal events was regarded as proof of CSF infiltration (Figure 1). Traumatic LP were excluded from the analysis.

Flow cytometry analysis

All centers involved were selected on the basis of a strict adherence to a standardized approach relying on the same procedures (time elapsed from collection to processing, number of fluorochromes, number of acquired events and analysis). Samples for FCM analysis were locally processed within 60 minutes from harvest, as described elsewhere. A cocktail of 6-8 monoclonal antibodies was used (Online Supplementary Table S1). On average, 1,080 events were acquired (range 0-210,000). In agreement with the recommendations for the analysis of rare events, a cluster of at least 10 phenotypically abnormal events was regarded as proof of CSF infiltration (Figure 1). Traumatic LP were excluded from the analysis.

Statistical analysis

The statistical analysis is described in the Online Supplementary Appendix.

Ethical considerations

Approval of the local institutional review board and ethics committee was obtained at all participating sites. The trial was registered at clinicaltrials.gov identifier: NCT03803670.

Results

Patients’ characteristics

The clinical and laboratory characteristics of the 240 patients are summarized in Table 1. At diagnosis, 179 (75%) CSF samples were negative by both FCM and CC (CNSneg), while 45 (18%) were OCNSD positive (positive by FCM and negative by CC=OCNSDpos) and 18 (7%) were positive by both FCM and CC (manifest CNS disease positive = MCNSDpos) (Table 1). No case proved to be FCM-negative and CC-positive.

The characteristics of patients belonging to the three groups are listed in Table 1. There was an equal male:female ratio among CNSneg, OCNSDpos and MCNSDpos patients. There was no significant difference in median age, median WBC count, B/T lineage, LDH levels between the three patient categories. Cytogenetic/genetic data were available in 178 of 240 cases (74%) and no difference in distribution among the three categories was
observed. On the other hand, the status of OCNSD\textsuperscript{pos} and MCNSD\textsuperscript{pos} was significantly associated with a high CSF cellularity ($P<0.001$) (Table 1) and the levels of CSF proteins ($P=0.023$) (Table 1). One hundred and seventy-one patients (71%) were treated within or according to GIMEMA protocols, 37 (15%) with the Hyper-CVAD/MTX-ARAC regimen, and 32 (14%) according to the NILG ALL10/07 protocol. Considering the heterogeneity of the chemotherapy regimens utilized, we analyzed our series dividing the patients into three groups on the basis of the intensity of the treatment received. Accordingly, 91 patients (37.9%) underwent a conventional treatment, 120 (50%) an intensified pediatric-inspired regimen, and 29 (12.1%), qualified as unfit or frail, were treated with a reduced intensity schedule (Table 1).

**Outcome**

Of the 232 evaluable patients, 198 (85%) achieved a CR with no significant differences between the three CNS status-based groups ($P=0.3$). Of these 198 patients, 116 (59%) experienced a relapse; in 18 of 116 (15%), disease recurrence occurred in the CNS alone or was combined with a hematologic relapse. The relapse rate was significantly higher in OCNSD\textsuperscript{pos} and MCNSD\textsuperscript{pos} patients than in CNS\textsuperscript{neg} patients ($P=0.001$) (Table 2). The 3-year disease-free survival (DFS) was also significantly longer in CNS\textsuperscript{neg} patients compared to OCNSD\textsuperscript{pos} or MCNSD\textsuperscript{pos} patients: 39\% (95\%CI: 31-48) versus 21\% (95\%CI: 4.5-33.9) respectively ($P=0.005$) (Table 2). On the contrary, there was no difference in 3-year DFS between OCNSD\textsuperscript{pos} and MCNSD\textsuperscript{pos} patients ($P=0.3$) (Figure 2).

The 3-year overall survival (OS) in CNS\textsuperscript{neg}, OCNSD\textsuperscript{pos} and MCNSD\textsuperscript{pos} patients was 53\% (95\%CI: 45.5-61.5), 31\% (95\%CI: 19.2-50.3) and 22\% (95\%CI: 9.4-52.7), respectively ($P<0.0001$) (Table 2). There was no difference in 3-year OS between OCNSD\textsuperscript{pos} and MCNSD\textsuperscript{pos} patients ($P=0.2$) (Figure 3).

**Multivariate analysis**

The clinical impact of the CNS status on OS was also challenged in the multivariate Cox proportional hazard analysis applied to models including age, transplant, sex, WBC count and treatment received. Multivariate analysis confirmed that the OCNSD\textsuperscript{pos} (HR=1.82, 95\%CI: 1.15-5.92; $P=0.01$) or MCNSD\textsuperscript{pos} status (HR=3.23, 95\%CI: 1.76-5.92; $P<0.0001$), defined at the time of diagnosis, were factors that independently impacted on OS together with the treatment regimens (intensified vs. conventional vs. reduced intensity for age) (Table 3).

**Discussion**

This retrospective study shows that FCM offers better technical support than CC in detecting leukemic cells in the CFS of adult patients with ALL, and documents the
clinical impact of OCNSD on the outcome of these patients. By introducing FCM analysis, the detection power improved to such an extent that evidence of CNS involvement increased from 7% to 25% of ALL cases at diagnosis. This analysis confirms previous reports that demonstrated the superior sensitivity of FCM over CC.10,12,13,22,23 In a large retrospective study of 326 CSF samples collected from patients affected by diffuse large B-cell and Burkitt lymphomas, a CSF involvement was detected by FCM in 53 (15%) diffuse large B-cell lymphomas and in 9 (11%) Burkitt lymphomas.24 FCM allows detection of a hematologic disease in CSF specimens even when the cellularity is very low.9,25 This peculiarity has been confirmed in pediatric ALL patients where FCM was able to

Table 1. Clinical characteristics of patients according to the central nervous system (CNS) status.

| Level          | ALL   | CNSneg | OCNSDpos | MCNSDpos | P       |
|----------------|-------|--------|----------|----------|---------|
| N              | 240   | 179    | 43       | 18       |         |
| Sex, N (%)     |       |        |          |          |         |
| F              | 103   | 76     | 20       | 7        | 0.835   |
| M              | 137   | 103    | 23       | 11       |         |
| Age, years, median (range) | 45 (17-80) | 45 (17-80) | 46 (17-72) | 36.50 (18-73) | 0.302 |
| Lineage, N (%) |       |        |          |          |         |
| B              | 184   | 140    | 34       | 10       | 0.088   |
| T              | 56    | 39     | 9        | 8        |         |
| WBC x 10^9/L, median (%) |        |        |          |          | 0.799 |
| Cytogenetic, N (%) |       |        |          |          |         |
| Abnormal       | 118   | 91     | 20       | 7        | 0.860   |
| Normal         | 65    | 52     | 9        | 4        |         |
| Treatment, N (%) |       |        |          |          |         |
| Conventional   | 91    | 70     | 15       | 6        | 0.400   |
| Intensified    | 120   | 85     | 23       | 12       |         |
| Reduced        | 29    | 24     | 5        | 0        |         |
| LDH, U/L, median (range) |        |        |          |          | 0.806 |
| CSF-WBC per mm³, median (%) |        |        |          |          | <0.001 |
| CSF protein, mg/dL, median (range) |        |        |          |          | 0.023 |

ALL: acute lymphoblastic leukemia; N: number; F: female; M: male; CNSneg: cerebrospinal fluid (CSF) samples negative by both flow cytometry (FCM) and conventional cytology (CC); OCNSDpos: CSF samples positive by FCM and negative by CC; MCNSDpos: CSF positive by both FCM and CC; WBC: white blood cells; LDH: lactate dehydrogenase.

Figure 2. Disease-free survival (DFS) based on central nervous system (CNS) status. Kaplan-Meier plot comparing DFS of cerebrospinal fluid (CSF) samples negative by both flow cytometry (FCM) and conventional cytology (CC) (CNSneg), occult CSF samples positive by FCM and negative by CC (OCNSDpos), and CSF positive by both FCM and CC (MCNSDpos). Overall P value = 0.005.
substantially improve recognition of occult CSF involvement. In agreement with pediatric reports, the CNS status of our adults did not correlate with risk factors associated with the risk of relapse, such as WBC count at onset, B/T phenotype or cytogenetic/genetic features. In pediatric ALL, FCM positivity alone in the absence of a positive CC seems to affect clinical outcome. Similar observations have been made in patients with high-risk non-Hodgkin lymphomas and Burkitt lymphomas, in whom FCM positivity of CSF was associated with a significantly higher risk of CNS relapse and a worse prognosis.

In adult ALL patients, the role of OCNSD is less clear because of the limited number of studies based on small series of patients. By analyzing 168 CSF samples collected from 31 patients with ALL, Subira et al. reported a concordance between FCM and CC except for ten samples. All patients found to be FCM negative remained free from CNS disease. In a small population of 38 adults with ALL or lymphoblastic lymphoma, we previously observed that the median OS of patients with FCM single positivity was intermediate between double positive or negative patients.

The uncertain clinical significance of the FCM analysis

| Level                     | ALL | CNS<sup>neg</sup> | OCNSD<sup>pos</sup> | MCNSD<sup>pos</sup> | P |
|---------------------------|-----|-------------------|---------------------|---------------------|---|
| N                         | 240 | 179               | 43                  | 18                  |   |
| Hematologic response, N (%)|     |                   |                     |                     |   |
| CR                        | 198 (85.3) | 152 (87.4) | 32 (80.0) | 14 (77.8) | 0.317 |
| No CR                     | 34 (14.7) | 22 (12.6) | 8 (20.0) | 4 (22.2) |   |
| ASCT, N (%)               |     |                   |                     |                     |   |
| No                        | 88 (44.9) | 65 (44.2) | 17 (47.2) | 6 (46.2) | 0.944 |
| Yes                       | 108 (55.1) | 82 (55.8) | 19 (52.8) | 7 (53.8) |   |
| Relapse, N (%)            |     |                   |                     |                     |   |
| No                        | 78 (40.2) | 70 (47.0) | 7 (22.6) | 1 (7.1) | 0.001 |
| Yes                       | 116 (59.8) | 79 (53.0) | 24 (77.4) | 13 (92.9) |   |
| Relapse site, N (%)       |     |                   |                     |                     |   |
| CNS                       | 16 (16.8) | 8 (12.7) | 7 (31.8) | 1 (10.0) | 0.099 |
| BM                        | 79 (83.2) | 55 (87.3) | 15 (88.2) | 9 (90.0) |   |
| OS 3 years                | Estimate % | 46.4 | 52.9 | 31.1 | 22.2 | <0.001 |
| (95%CI)                   | (40.1-53.8) | (45.5-61.5) | (19.2-50.5) | (9.4-52.7) |   |
| DFS 3 years               | Estimate % | 34.3 | 38.6 | 20.6 | 21.4 | 0.005 |
| (95%CI)                   | (27.9-42.2) | (31.4-48) | (10.2-41.3) | (7.3-58.4) |   |

ALL: acute lymphoblastic leukemia; N: number; CNS<sup>neg</sup>: cerebrospinal fluid (CSF) samples negative by both flow cytometry (FCM) and conventional cytology (CC); OCNSD<sup>pos</sup>: CSF samples positive by FCM and negative by CC; MCNSD<sup>pos</sup>: CSF positive by both FCM and CC; ASCT: allogeneic stem cell transplant; CR: complete remission; BM: bone marrow; OS: overall survival; DFS: disease-free survival; CI: confidence interval.
of CSF is confirmed by the discordant position of the current guidelines. In fact, while the National Comprehensive Cancer Network (NCCN) guidelines do not indicate that FCM analysis of the CSF should be part of the initial work-up, more recent American pocket guide for the clinician recommends (although not strongly) performing this examination at diagnosis. Based on our large multicenter report, occult CNS status does indeed have a significant impact on outcome. In fact, patients with OCNSD had a worse DFS and OS compared to those who were OCNSD negative. The superimposable duration of OS of OCNSD and MCNSD patients indicates that even the presence of a few cells in the CNS sanctuary has a clinical impact; these few cells can only be detected by using approaches more sensitive than CC, such as FCM. The pronounced neurotropism of ALL can be responsible for disease recurrence once the leukemic cells, having survived systemic chemotherapy within the CNS sanctuary, migrate to the circulation. Thus, the availability of highly sensitive methods capable of accurately defining whether or not the CSF is colonized by leukemic cells not only offers a refined diagnostic/prognostic work-up, but also helps to personalize CNS prophylaxis through the early identification of patients who may benefit from more aggressive approaches.

With the limitations of its retrospective nature, the results of our study demonstrate that, in adult ALL patients, FCM can more precisely identify and quantify the number of patients with CNS involvement at diagnosis and that this impacts significantly on the clinical course and outcome of the disease, thus enabling a further refinement of the current diagnostic risk-stratification process. This refined CNS evaluation should become a routine tool for the work-up of ALL patients at presentation. Further and larger prospective studies are needed to further standardize the procedures and promote optimal clinical application of this technique.

Disclosures
This study was carried out as part of the routine clinical work-up of patients. The authors declare no competing financial interests.

Contributions
MIDP, AV and AG designed the study; interpreted data, wrote the manuscript; AP analyzed data and performed statistical analysis; EB, FF, MB, FL, SI, EO, GR, NF, ST, BN, CS, PZ, MD, MC, GD, MS, GP provided patients information, collected clinical data and contributed to data analysis; MAC and CC obtained flow cytometry data; IDS interpreted data and contributed to data analysis; RF wrote and revised the manuscript. All authors approved the manuscript.

Acknowledgments
The authors would like to thank all participants of the Campus ALL program. Partly supported by Associazione Italiana Ricerca sul Cancro (AIRC), Special 5x1000 Program Metastases (21498), Milan (Italy) to RF.

References
1. Thomas X, Boiron JM, Huguet F, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of LALA-94 trial. J Clin Oncol. 2004;22(20):4075-4086.
2. Kantarjian HM, O’Brien S, Smith TL, et al. Results of treatment with Hyper-CVAD, a dose-intensive regimen in adult acute lymphoblastic leukemia. J Clin Oncol. 2000;18(3):547-561.
3. Thomas X, Le GH. Central nervous system involvement in adult acute lymphoblastic leukemia. Hematology. 2006;11(3):295-302.
4. Lazarus HM, Richards SM, Chopra R, et al. Medical Research Council (MRC)/National Cancer Research Institute (NCRI) Adult Leukaemia Working Party of the United Kingdom and the Eastern Cooperative Oncology Group. Central nervous system involvement in adult acute lymphoblastic leukemia at diagnosis: results from international ALL trial MRC UKALL XII/E5993. Blood. 2006;108(2):65-72.
5. Jabbour E, Thomas D, Cortes J, Kantarjian H, O’Brien S. Central nervous system prophylaxis in adults with acute lymphoblastic leukemia. Cancer. 2010;116(10):2290-2300.
6. Larson RA. Managing CNS disease in adults with acute lymphoblastic leukemia. Leuk Lymphoma. 2010;51(3):13.
7. Bromberg JE, Breema DA, Kraan J, et al. CSF flow cytometry greatly improves diagnostic accuracy in CNS hematologic malignancies. Neurology. 2007;68(20):1674-1679.
8. Kaplan K, DeSouza TG, Farkash A, et al. Leptomeningeal metastases: comparison of
clinical features and laboratory data of solid tumors, lymphomas and leukemias. J Neurol Neurosurg Psychiatry. 1990;53:225-229.

9. Craig F, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood. 2008;111(8):3941-3967.

10. Quijano S, Lopez A, Manuel Sancho J, et al. Spanish Group for the Study of CNS disease in NHL: Identification of leptomeningeal disease in aggressive B-cell non-Hodgkin’s lymphoma: improved sensitivity of flow cytometry. J Clin Oncol. 2009;27(9):1462-1469.

11. de Graaf MT, de Jongste AH, Kraan J, et al. Role of multidimensional flow cytometry in the diagnosis of leptomeningeal disease in newly diagnosed aggressive B-cell lymphomas. Leuk Res. 2008;32(8):1196-1199.

12. Zeiser R, Burger JA, Bley TA, Windfuhr-Klaus T, et al. Clinical follow-up indicates different accuracy of magnetic resonance imaging and immunocytochemistry of the cerebral spinal fluid for the diagnosis of neoplastic meningitis - a single centre experience. Br J Haematol. 2004;124(6):762-768.

13. Di Noto R, Scalia G, Abate G, et al. Critical role of multidimensional flow cytometry in detecting occult leptomeningeal disease in newly diagnosed aggressive B-cell lymphomas. Leuk Res. 2008;32(8):1196-1199.

14. Annino L, Vignetti M, Paoloni FP, et al. Detection and outcome of occult leptomeningeal disease in adult patients with acute myeloid leukemia: incidence and impact on outcome. Semin Hematol. 2018;55(4):209-214.

15. Mahmoud HH, Rivera GK, Hancock ML, et al. Low leukocyte counts with blast cells in cerebrospinal fluid of children with newly diagnosed acute lymphoblastic leukemia. N Engl J Med. 1999;340(9):314-319.

16. Roma A, Garcia A, Avagnina A, Rescia C, Elini B. Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. Diagn Cytopathol. 2002;27(5):271-275.

17. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebral spinal flow cytometry for the diagnosis of leukaemic meningitis in acute lymphoblastic leukaemia. Leuk Lymphoma. 2014;55(7):1498-1500.

18. Wilson W, Bromberg J, Sterler-Stevenson M, et al. Detection and outcome of occult leptomeningeal disease in diffuse large B-cell lymphoma. Cytometry B Clin Cytom. 2011;80(5):271-281.

19. Blum M, Schulte-Monting J, Behringer DM, Savidou I, Duhrsen U. Detection of malignant lymphoma and Burkitt lymphoma. Cytom. 2011;80(5):271-281.

20. Roma A, Garcia A, Avagnina A, Rescia C, Elini B. Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. Diagn Cytopathol. 2002;27(5):271-275.

21. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebral spinal flow cytometry for the diagnosis of leukaemic meningitis in acute lymphoblastic leukaemia. Leuk Lymphoma. 2014;55(7):1498-1500.

22. Roma A, Garcia A, Avagnina A, Rescia C, Elini B. Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. Diagn Cytopathol. 2002;27(5):271-275.

23. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebral spinal flow cytometry for the diagnosis of leukaemic meningitis in acute lymphoblastic leukaemia. Leuk Lymphoma. 2014;55(7):1498-1500.

24. Roma A, Garcia A, Avagnina A, Rescia C, Elini B. Lymphoid and myeloid neoplasms involving cerebrospinal fluid: comparison of morphologic examination and immunophenotyping by flow cytometry. Diagn Cytopathol. 2002;27(5):271-275.

25. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebral spinal flow cytometry for the diagnosis of leukaemic meningitis in acute lymphoblastic leukaemia. Leuk Lymphoma. 2014;55(7):1498-1500.

26. Wilson W, Bromberg J, Sterler-Stevenson M, et al. Detection and outcome of occult leptomeningeal disease in diffuse large B-cell lymphoma. Cytometry B Clin Cytom. 2011;80(5):271-281.

27. Mitri Z, Siddiqui MT, El Rassi F, et al. Sensitivity and specificity of cerebral spinal flow cytometry for the diagnosis of leukaemic meningitis in acute lymphoblastic leukaemia. Leuk Lymphoma. 2014;55(7):1498-1500.

28. Ranta S, Nilsson F, Harila-Saari A, et al. Detection of central nervous system involvement in childhood acute lymphoblastic leukemia by cytomorphology and flow cytometry of the cerebrospinal fluid. Pediatr Blood Cancer. 2015;62(6):951-956.

29. Martinez-Laperche C, Gomez-Garcia AM, Lassaletta A, et al. Detection of occult cerebrospinal fluid involvement during maintenance therapy identifies a group of children with acute lymphoblastic leukemia at high risk for relapse. Am J Hematol. 2013;88(5):360-365.

30. Benveolo G, Stacchini A, Spina M, et al. Final results of a multicenter trial addressing role of CSF flow cytomtery in NHL patients at high risk for CNS dissemination. Blood. 2012;120(16):3222-3228.

31. Subura D, Castanon S, Roman A, et al. Flow cytometry and the study of central nervous disease in patients with acute leukemia. Br J Haematol. 2001;112(2):381-384.

32. Salvanas JC, Brown PA, Aoun P, et al. Acute lymphoblastic leukemia, version 2.2015. J Natl Compr Canc Netw. 2015;13(10):1240-1279.

33. SArber DA, Borowitz MJ, Cessna M, et al. Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology. Arch Pathol Lab Med. 2017;141(10):1342-1395.

34. SAKEN SM, O’Leary HA, Minnear FL, et al. VE-cadherin and FECAM-1 enhance ALL migration across brain microvascular endothelial cell monolayers. Exp Hematol. 2010;38(9):735-743.

35. SAKEN SM, Rellick SL, Fortney JG, Gibson LE. Cellular elements of the subarachnoid space promote ALL survival during chemotherapy. Leuk Res. 2011;35(7):705-711.

36. Svan der Velden VH, de Launay DJ, de Vries JR, et al. New cellular markers at diagnosis are associated with isolated central nervous system relapse in pediatric B-cell precursor acute lymphoblastic leukemia. Br J Haematol. 2016;172(5):769-781.

37. SFishman-Levy I, Izraeli S. Advances in understanding the pathogenesis of CNS acute lymphoblastic leukaemia and potential for therapy. Br J Haematol. 2017;176(2):157-167.

38. SFua CH, Howard SC. Current management and challenges of malignant disease in the CN in paediatric leukaemia. Lancet Oncol. 2006;9(5):257-268.

CNS involvement in adult ALL patients