THE RELEVANCE OF EUROPEAN LEUKEMIA NET RECOMMENDATIONS IN ACUTE MYELOID LEUKEMIA: A ONE CENTER THREE-YEAR EXPERIENCE

I. Antohe[1,3], Angela Dăscălescu[1,3], C. Dănăilă[1,3], Amalia Titieanu[1,3], Elena Dolachi[1,3], Roxana Dumitru[1,3], Mihaela Zlei[4], I. Ivanov[5], Adriana Sireteanu[5], P. Cianga[2]

"Grigore T. Popa" University of Medicine and Pharmacy Iasi

Faculty of Medicine

1. Department of Medical Specialties (III)

2. Department of Morpho-functional Sciences (I)

Regional Institute of Oncology, Iaşi

3. Department of Hematology

4. Department of Immunophenotyping

5. Department of Molecular Diagnostic

[*]Corresponding author. E-mail: ion.d.s.antohe@d.umfiasi.ro

THE RELEVANCE OF EUROPEAN LEUKEMIA NET RECOMMENDATIONS IN ACUTE MYELOID LEUKEMIA: A ONE CENTER THREE-YEAR EXPERIENCE (Abstract). **Aim:** The cytogenetic and molecular features of leukemia cells are chief predictors of survival in acute myeloid leukemia (AML). However, treatment outcomes in AML differ between low-, medium and high-income countries. We thus aimed to investigate the relevance of the European Leukemia Net (ELN) prognostic system in Romanian AML patients. **Material and methods:** We investigated the clinical significance of the ELN recommendations in 130 newly diagnosed AML patients treated at the Iasi Regional Oncology Institute. **Results:** Favorable, intermediate and adverse risk ELN classes had a similar distribution and retained their prognostic relevance (median overall survival of 18, 11 and 2 months respectively at a median follow-up duration of 18 months) when compared with literature data. However, in both the chemotherapy eligible and ineligible patients a higher prevalence of comorbid conditions and treatment related mortality could be identified. **Conclusions:** The management of Romanian AML patients poses significant challenges mainly due to higher incidence of comorbid conditions and infections. However, growing access to the allogeneic stem cell transplant procedure in our country will significantly improve the outcome of these patients. **Keywords:** ACUTE MYELOID LEUKEMIA, EUROPEAN LEUKEMIA NET, CYTOGENETICS, CHEMOTHERAPY.

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm characterized by extensive bone marrow (BM) infiltration with immature myeloid precursors and subsequent BM failure (1). AML diagnosis requires BM aspirate and thorough morphological, immunophenotypic, cytogenetic and molecular analysis of tumor cells (2). Furthermore, AML cases are classified in different subgroups as proposed by the 2016 revision of the World Health Organization classification of myeloid neoplasms

(3). Since AML therapy responses are highly heterogenous, ranging from deep and sustained molecular remissions in acute promyelocytic leukemia (APL) to primary refractory AML, the prognostic evaluation of patients is highly important in order to determine eligibility for more intensive therapeutic interventions, such as allogeneic stem cell transplantation (4-6). When evaluating the prognosis of AML patients, clinicians must take various factors into account, such as the antecedent hematologic disorders, radiation or cytotoxic drug exposure, hyperleukocytosis, therapy response and minimal residual disease, but also patient age, comorbidities and their performance status. However, the cytogenetic and molecular features of AML blasts are currently the strongest predictors for therapy response and long-term survival (7-10). The impact of cytogenetic and molecular anomalies is highlighted by the 2017 update of the European Leukemia Net (ELN) prognostic classification that defines three prognostic subgroups (favorable, intermediate and adverse risk) with different complete remission (CR) rates, progression and disease-free survival (11). Noteworthy, the ELN risk stratification does not take into account patient-related factors, such as age or comorbidities (12). Furthermore, recent studies revealed that prognosis in AML patients differs from high- to middle and low-income countries, probably related to inherent socio-economic issues (13, 14).

The relevance of the ELN classification in AML patients from Romania has not yet been systematically investigated. However, recently, a national program that ensures widespread access to the immunophenotyping, cytogenetic and molecular tests necessary to formulate the ELN prognostic prediction has been successfully implemented. In a recent report from the World Health Organization (15), Romanian patients have been shown to have higher incidences of cardiovascular diseases, chronic obstructive lung disease, asthma, tuberculosis and depression, all of which are known to have a negative impact on AML patient outcome (16). Furthermore, it is to be expected that the other factors reported by the WHO, such as the inequalities in patient education, the occasionally difficult access to public health services, especially in patients from rural areas, the deficiencies in the primary care system and the lowest levels of public health expenditure among the European Union member countries will likely influence the prognosis of AML in these patients (15).

The aim of our study is to evaluate the impact of the 2017 ELN classification on the prognosis of AML patients treated at the Iași Regional Oncology Institute, in North-Eastern Romania.

MATERIAL AND METHODS

A number of 130 patients diagnosed with *de novo* AML between 2017 and 2019 at the Hematology Department of the Iași Regional Oncology Institute, Romania were included in this retrospective study. AML diagnosis was established based on the 2016 WHO classification of myeloid neoplasms and patients were risk stratified according to the 2017 ELN recommendations. The cases with APL were excluded. This study was approved by the local research ethics committee. Fresh BM samples were collected at diagnosis. Immunophenotypic diagnostic of AML was established according to Euro Flow standards (17). Cytogenetic G-banding

analysis was performed using standard methods. We further examined 4 chimeric gene transcripts (BCR-ABL1 p210, PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11) by reverse transcriptase-mediated quantitative PCR. Patients were tested for NPM1 and FLT3-ITD mutations. We screened for chromosome 11 rearrangements using fluorescence *in situ* hybridization techniques. In case of a dry tap, a BM biopsy and immunohistochemistry tests were performed, and cytogenetic tests were done on peripheral blood samples. Patients <65 years were treated according to the national protocol with "7+3" therapy (Cytarabine 150 mg/m^2 for 7 days in continuous i.v. administration and Idarubicine 12 mg/m^2 for 3 days). Patients > 65 years were treated with "7+3" therapy in selected cases (depending largely on the absence of comorbid conditions and an ECOG (Eastern Cooperative Oncology Group) performance status less than 2), Decitabine, low-dose cytarabine (ARA-C) or best supportive care (BSC). Post-induction response was evaluated by BM morphologic examination and flow cytometry on day 21-28 following "7+3" chemotherapy.

The statistical analysis was performed using the *IBM SPSS Statistics 21.0 Software*. The Chi square and Mann Whitney tests were used to analyze the associations between different variables. A p value <0.05 was considered to be statistically significant. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan Meier method. The log rank test was used for comparisons of Kaplan-Meier curves. OS was defined as the time from diagnosis to death from any cause. Early death was considered when occurring in the first 28 days following diagnosis. For the patients who achieved complete remission, DFS was defined as the time from CR to relapse or death by any cause.

RESULTS

We studied 130 patients with newly diagnosed AML. Baseline clinical and laboratory data of the patients with respect to the 2017 ELN risk classification are summarized in table I. The mean age at diagnosis was 60 years. The median follow-up duration was 18 months. 87.8% of patients presented with *de novo* AML, while 9.1% and 3.1% had secondary and, respectively, therapy-related AML. According to the WHO 2016 classification, patient distribution was the following: AML with recurrent genetic abnormalities (21.2%), therapy-related AML (3.1%), AML associated with myelodysplasia-related changes (9.1%) and AML not otherwise specified (NOS) (66.7%). 28.4% of cases presented with an ECOG (Eastern Cooperative Oncology Group) score greater than 2 due to extensive comorbidities or life-threatening complications at diagnosis.

Cytogenetic data was available in 69.2% of patients. A normal karyotype was detected in 46.7% of cases. The distribution of the cytogenetic anomalies is shown in figure 1A. 41.5% of patients had detectable molecular anomalies that are detailed in figure 1B. In 25.4% of cases we found a FLT3-ITD mutation, which co-existed with a NPM1 mutation in 21.5% of cases. An isolated NPM1 mutation was found in 30% of patients.

Following the analysis of cytogenetic and molecular data, cases were classified according to the 2 017 ELN risk classification, as follows: favorable 20.8%, intermediate 51.5%, adverse 27.7% (fig. 1C).

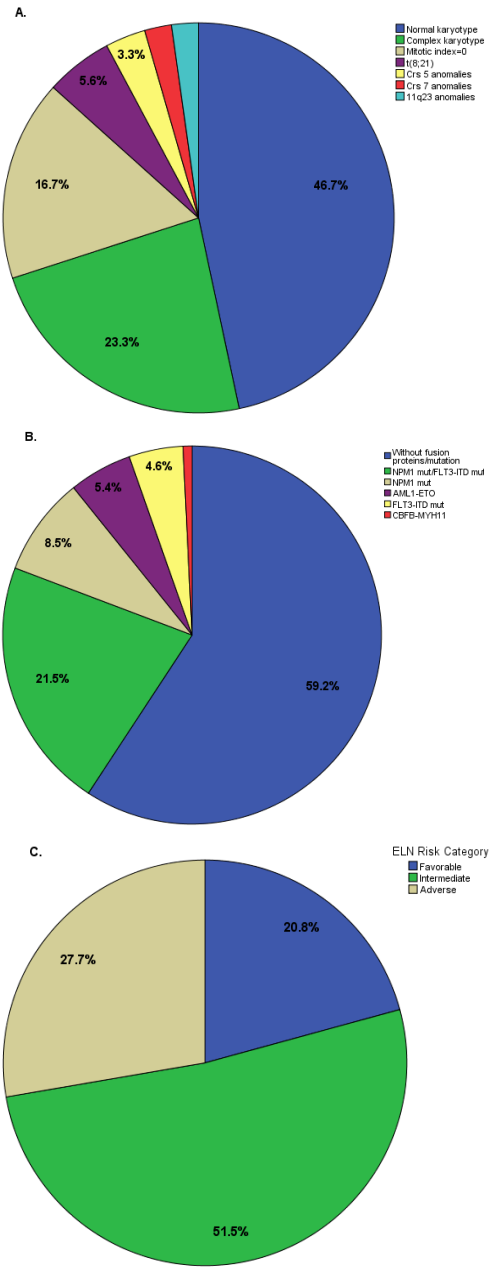

Fig. 1. A. Distribution of cytogenetic characteristics in AML patients
B. Molecular findings in AML patients
C. Distribution of ELN risk subgroups.

66.2% of patients were deemed eligible for intensive ("7+3") chemotherapy. Out of these, 81.4% completed induction chemotherapy and 58.8% achieved CR. The median OS for all patients was 12 months and the median DFS was 9 months. On ELN risk category analysis, the median OS for favorable, intermediate and adverse risk subgroups was 18 (95% CI: 12.5-23.4 months), 11 (95% CI: 7.1-14.8 months) and 2 months (95% CI: 0.8-3.1 months), respectively (fig. 2). Favorable and intermediate risk patients had DFS of 73.4% and 57.2%, respectively, while patients within the adverse category displayed the highest risk of early relapse, with a relapse rate of 75% within 3 months after achieving CR.

41.2% of chemotherapy-eligible patients had refractory disease and a median OS of 4 months. 36% of chemotherapy-eligible patients had an indication for allogeneic bone marrow transplant (ASCT). However, this procedure could be performed in only 8.1% of our patients. All patients who underwent ASCT remained disease-free at 24 months.

Out of the patients deemed ineligible for standard chemotherapy, 27.3% followed Decitabine therapy, 27.3% low dose-cytarabine (LDAC) and 45.4% received exclusively palliative care. 16.7% of the patients receiving Decitabine achieved a complete hematologic response. None of the patients treated with LDAC responded to therapy.

Furthermore, we investigated the presence of correlations between the main clinical and laboratory characteristics of patients and their ELN risk, chemotherapy completion and therapy response.

Extramedullary involvement was more frequent in the *de novo* AML-NOS subtype (p=0.008) and splenomegaly was more

frequent in patients with M7 phenotype (66.6%, p=0.009). An M5 phenotype was more frequently associated with hyperleukocytosis (33%, p=0.005) (tab. I).

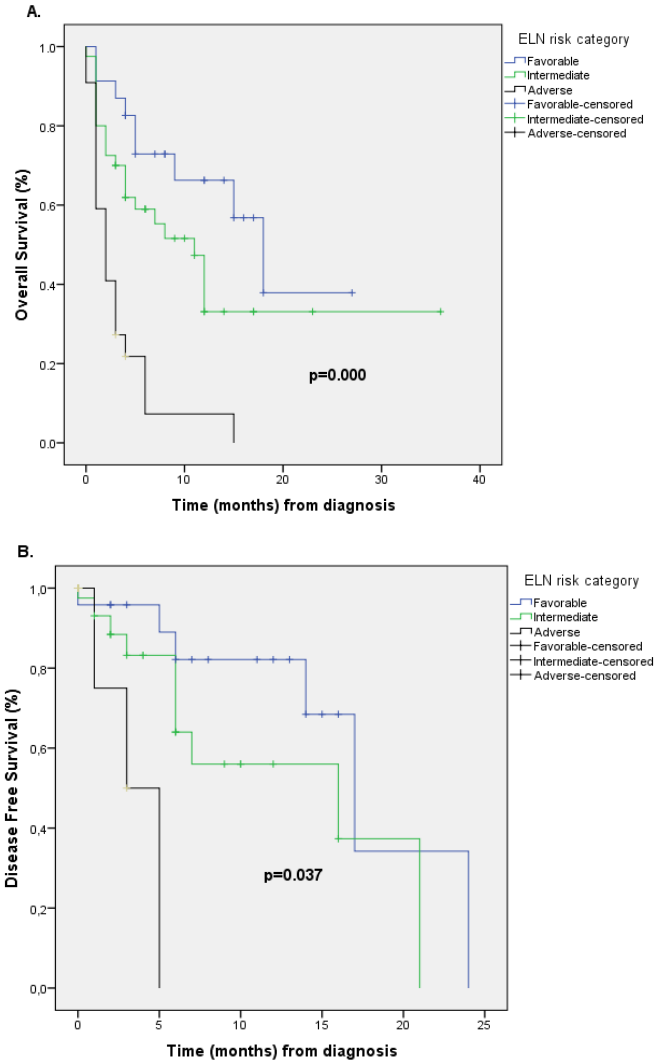

Fig. 2. Probability of overall survival (A) and disease-free survival (B) in AML patients according to the ELN prognostic system.

70.8% of patients had various associated comorbid conditions, of which the majority were represented by cardiovascular (49.2%) and metabolic (13.8%) comorbidities. 28.5% of patients had more than two associated comorbid conditions. The presence of comorbidities had a negative impact on the therapeutic response. Thus, 67.5% of patients achieving CR had comorbidities, while in patients with refractory disease their prevalence was much higher, reaching 82.9%. Moreover, the presence of infection

during or following induction chemotherapy had a detrimental effect on patient outcome. Thus, the presence of infection, irrespectively of the etiology, was associated with a 58.7% probability of refractory disease (p=0.022, Chi-Square Test). Treatment related mortality (TRM) in chemotherapy eligible patients was 16.3%. In older patients treated with Decitabine or LDAC the TRM reached 46.2%. Deaths were mainly due to infection (63.2%) or hemorrhagic complications (13.6%) (tab. I).

TABLE I

Demographic, clinical, biological, cytogenetic, molecular and therapy response data of AML patients

Patient characteristics	
Sex, n (%)	
Male	69 (53.9%)
Female	61 (46.9%)
Age	
Median	63
<65/ >65yrs., n (%)	71 (54.6%)/ 59 (45.4%)
Extramedullary disease n (%)	41 (31.8%)
Splenomegaly	15 (11.4%)
Hepatomegaly	27 (20.8%)
Adenopathies	20 (15.4%)
Gum hypertrophy	8 (6.2%)
CNS involvement	1 (0.8%)
WBC at diagnosis	
Median	9100/µL
<100000/100000, n (%)	19 (14.6%)/ 111 (85.4%)
BM blasts (%)	
Median	42%
Hb level (median/range)	8.3 g/dL/ 3.5-12 g/dL
Platelet level (median/range)	35000/µL/ 1000-1138000/µL
AML type, n (%)	
De novo	110 (84.6%)
Therapy-related	5 (3.8%)
Secondary AML	15 (11.5%)
WHO 2008 classification, n (%)	
AML with recurrent genetic abnormalities	28 (21.5%)
AML with multilineage dysplasia	12 (9.2%)
t-AML	6 (4.6%)
AML-NOS	84 (64.6%)
AML-NOS type, n (%)	
M0	4 (4.5%)
M1	17 (17%)
M2	13(14.8%)
M4	25 (28.4%)
M5	21 (21%)

Patient characteristics	
M7	3 (3.4%)
Acute basophilic leukemia	2 (2.3%)
ELN 2010 cytogenetic risk, n (%)	
Favorable	27 (20.8%)
NPM1 mut/FLT3 WT	11 (8.5%)
t(8;21)	4 (3.1%)
Intermediate	67 (51.5%)
NPM1 mut/FLT3 mut	28 (21.5%)
NPM 1 WT/FLT3 mut	6 (4.6%)
NPM1 WT/FLT3 WT	72 (55.4%)
t(9;11)	1 (0.8%)
Adverse	36 (27.7%)
-7	2 (1.6%)
-5	3 (2.4%)
Complex	21 (16.2%)
Induction therapy, n (%)	
"3+7"	84 (64.6%)
Decitabine	12 (9.2%)
Low dose cytarabine	13 (10%)
Best supportive care	3 (2.3%)
Therapeutic response, n (%)	
CR	40 (58.8%)
RD	28 (41.2%
Death in induction	14 (16.3%)

AML= acute myeloid leukemia, t-AML= therapy related acute myeloid leukemia, BM= bone marrow, CNS= central nervous system, CR= complete response, ELN= European Leukemia Net, FLT3= FMS-like tyrosine kinase, NOS= not otherwise specified, NPM1=nucleophosmin 1, RD= refractory disease, WBC= white blood count, WT= wild type

We found no further statistically significant correlation between classic risk factors such as the total white blood cell count and the WHO AML types or the response to therapy.

The log rank test was used for comparisons of Kaplan-Meier curves.

DISCUSSION

All AML patients who achieve CR are facing the likelihood of relapse (7). Thus, an accurate prognostic assessment is necessary to identify patients with high and intermediate ELN risk, who would benefit immediately from consolidation by ASCT, and patients with favorable risk, for whom high dose consolidation chemotherapy is rather indicated (11).

However, multiple reports indicated that the prognostic value of the cytogenetic and molecular features of AML can be questioned in patients from developing countries such as Romania because of local socio-economic factors (13,14). Large scale national reports regarding the outcome of Romanian patients with AML are missing and a recent WHO analysis has identified multiple issues in Romania's public healthcare system that could influence the outcome of AML patients (15). Thus, we

aimed to investigate the relevance of the ELN risk classification system in the "real-life" setting of patient1s treated at the Iași Regional Oncology Institute.

We analyzed the data of 130 newly diagnosed AML patients and we identified cases with favorable, intermediate and adverse risk with frequencies similar to those reported by other groups (18). In our study group, the ELN risk categories demonstrated survival probabilities comparable to literature data (fig. 2).

TRM in AML is defined as death within four weeks following completion of induction therapy. Literature data reveals that TRM rates vary from 10 to 30% or even more in elderly AML patients (12). Current advances in supportive care in developed countries have reduced TRM rate below 10%, even in the elderly patient population (19, 20), but death rates remain higher in developing countries. In our study group, TRM was 16.3% in chemotherapy eligible patients. However, in elderly patients treated with Decitabine or LDAC, mortality was much higher, reaching 46.2% (data not shown). The high TRM rates in our study group are mainly due to infectious and hemorrhagic complications, but also to associated comorbid conditions. Poor education, inadequate hygiene habits, especially in patients from rural areas, irrational antibiotic use in the general population and colonization with multidrug resistant bacteria all contribute to a higher incidence of infection among our patients when compared to reports from more developed countries (18). Comorbidities, especially cardiovascular ones, which have a higher prevalence among Romanian patients (15), further contribute to even a higher mortality among patients with infectious complications. Moreover, the presence of infection at diagnosis delays the administration of induction therapy and the development of severe, life-threatening sepsis during induction chemotherapy forfeits its completion, which compromises therapeutic response and leads to a dismal prognosis. Thus, the median survival in patients who did not complete induction therapy was 5 months (data not shown). Moreover, the higher incidence of infection also reduces survival in the group of AML patients with favorable ELN risk.

Although the majority of patients were classified as intermediate or adverse risk, allogeneic stem cell transplant was performed only 8.1% of patients. Timely access to this procedure is vital for the survival of AML patients (21). Widespread availability of this procedure remains a major unmet need for Romanian AML patients, even if, in the last years, Romania is cited by reports from the European Bone Marrow Transplantation Society as one of the European countries with a rapid increase in transplanted cases per year (22, 23). Thus, it is expected that the access to this procedure will improve drastically in the near future.

We can conclude that the ELN risk assessment system is relevant for Romanian AML patients, but infections, more frequent comorbidities and the lack of timely access to the bone marrow transplant procedure have a detrimental influence on their short- and long-term prognosis.

CONCLUSION

In our study we examined the relevance of the European Leukemia Net prognostic system in AML patients treated at the Regional Institute of Oncology from North-Eastern Romania. Briefly, we conclude that the ELN risk classes have similar distribution when compared to literature data. Fur-

thermore, despite a higher incidence of comorbidities among Romanian patients and higher treatment related mortality rates in both the chemotherapy eligible and ineligible patients, the ELN prognostic system retains its significance. However, more prevalent comorbid conditions, poor hygiene habits, treatment discontinuation and low availability of the bone marrow transplant procedure can pose major challenges in the management of AML and impact the long-term prognosis of patients.

REFERENCES

1. Dănăilă C, Dăscălescu A, Antohe I. Leucemiile acute mieloblastice. In: *Hematologie Clinică*. Iași: Editura "Gr. T. Popa" U.M.F. Iași: 2017, 254-263.
2. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, *et al*. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med* 2016; 374(23): 2209-2221.
3. Arber DA, Orazi A, Hasserjian R, Borowitz MJ, Beau MM Le, Bloomfield CD, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016; 127(20): 2391-2406.
4. Ommen HB. Monitoring minimal residual disease in acute myeloid leukaemia: a review of the current evolving strategies. *Ther Adv Hematol* 2016; 7(1): 3-16.
5. Deak D, Pop C, Zimta A-A, Jurj A, Ghiaur A, Pasca S, *et al*. Let's Talk About BiTEs and Other Drugs in the Real-Life Setting for B-Cell Acute Lymphoblastic Leukemia. *Front Immunol* 2019; 10: 2856.
6. Larkin K, Blum W. Novel therapies in AML: reason for hope or just hype? *Am Soc Clin Oncol Educ Book* 2014; e341-51.
7. Szer J. The prevalent predicament of relapsed acute myeloid leukemia. *Hematology Am Soc Hematol Educ Program* 2012; 2012: 43-48.
8. Burnett AK, Hills RK, Milligan D, Kjeldsen L, Kell J, Russell NH, *et al*. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *J Clin Oncol* 2011; 29(4): 369-377.
9. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, *et al*. The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial. *Blood* 1998; 92(7): 2322-2333.
10. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. *Blood Reviews* 2004; 18: 115-136.
11. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, *et al*. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* 2017; 129: 178-189.
12. Deschler B, Lübbert M. Acute myeloid leukemia: Epidemiology and etiology. *Cancer* 2006; 107(9): 2099-2107.
13. Datoguia TS, Velloso EDRP, Helman R, Musacchio JG, Salvino MA, Soares RA, *et al*. Overall survival of Brazilian acute myeloid leukemia patients according to the European Leukemia Net prognostic scoring system: a cross-sectional study. *Med Oncol* 2018; 35: 57-68.
14. Benicio MT de L, Ribeiro AFT, Américo AD, Furtado FM, Glória AB, Lima AS, *et al*. Evaluation of the European LeukemiaNet recommendations for predicting outcomes of patients with acute myeloid leukemia treated in low- and middle-income countries (LMIC): A Brazilian experience. *Leuk Res* 2017; 60: 109-114.

15. Vladescu C, Silvia V, Scîntee G, Olsavszky V, Hernández-Quevedo C, Sagan A 18. *Romania: Health system review*. Vol. 18, Health systems in transition, 2016.
16. Storey S, Gray TF, Bryant AL. Comorbidity, Physical Function, and Quality of Life in Older Adults with Acute Myeloid Leukemia. *Curr Geriatr reports* 2017; 6(4): 247-254.
17. Van Dongen JJM, Lhermitte L, Böttcher S, Almeida J, Van Der Velden VHJ, Flores-Montero J, *et al*. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia* 2012; 26(9): 1908-1975.
18. Röllig C, Bornhäuser M, Thiede C, Taube F, Kramer M, Mohr B, *et al*. Long-term prognosis of AML according to the new genetic risk classification of the ELN. *J Clin Oncol* 2011; 29(20): 2758-2765.
19. Pulsoni A, Pagano L, Latagliata R, Casini M, Cerri R, Crugnola M, *et al*. Survival of elderly patients with acute myeloid leukemia. *Haematologica* 2004; 89(3): 296-302.
20. Doubek M, Palasek I, Brychtova Y, Buchtova I, Mayer J. Acute myeloid leukemia treatment in patients over 60 years of age. Comparison of symptomatic, palliative, and aggressive therapy. *Neoplasma* 2005; 52(5): 411-414.
21. Rowe JM, Tallman MS. How I treat acute myeloid leukemia. *Blood* 2010; 116(17): 3147-3156.
22. Passweg JR, Baldomero H, Basak GW, Chabannon C, Corbacioglu S, Duarte R, *et al*. The EBMT activity survey report 2017: a focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. *Bone Marrow Transplant* 2019; 54(10): 1575-1585.
23. Colita A, Colita A, Bumbea H, Croitoru A, Orban C, Lipan LE, *et al*. LEAM vs. BEAM vs. CLV Conditioning Regimen for Autologous Stem Cell Transplantation in Malignant Lymphomas. Retrospective Comparison of Toxicity and Efficacy on 222 Patients in the First 100 Days After Transplant, On Behalf of the Romanian Society for Bone Marrow Transplantation. *Front Oncol* 2019; 9: 892.

NEWS

THE INTERPLAY BETWEEN VAGINAL MICROBIOTA AND INTRAUTERINE ADHESION USING NEXT GENERATION SEQUENCING METHODS

Intrauterine adhesion represent a disease that is a very common cause for female infertility. Since it has an increased recurrence rate after surgical interventions, its pathogenesis and recurrence causes are important to understand better this condition. Liu *et al*. (2019) analysed, using high-throughput sequencing method, the vaginal microbiota of females diagnosed with intrauterine adhesion and healthy patients` vaginal secretion. The results shown that intrauterine adhesion does not bring any important changes for vaginal microbiota, having a slighty effect on the number of vaginal bacterial species. The patients with intrauterine adhesion presented at the phylum level a reduced percentage of Firmicutes and increased percentage of Actinobacteria whereas in the healthy females group the rate was more lower ($P<0.05$). Moreover, in about half of the intrauterine adhesion vaginal samples was identified at the genus level a remarkable decreased number of Lactobacillus and enhanced growth of Prevotella and Gardnerella ($P<0.05$). The interplay between synechiae and vaginal microflora can reveal remarkable changes in the vaginal microbiota diversity in patients with intrauterine adhesion. The increase of Lactobacillus population may bring interesting benefits in the endometrial regeneration process but also may help to decrease de recurrence rate of intrauterine adhesion (Liu Z, Kong Y, Gao Y *et al*. Revealing the intraction between intrauterine adhesion and vaginal microbiota using high-throughput sequencing. *Mol Med Rep*. 2019. 19(5),4167-4174).