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CORRELATIONS BETWEEN DIAGNOSTIC, EVOLUTION, TREATMENT RESPONSE AND COMPLICATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA

SUMMARY OF THE DOCTORAL THESIS

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Content

Acute promyelocytic leukemia (APL) is a rare disease, consisting in a particular subtype of acute myeloid leukemia, this type of neoplasia being described among the most aggressive forms of malignancies. In contrast, one of the characteristics that make this disease unique is the increased rate of achieving complete response and that we can even talk about curability in the case of the patients diagnosed with APL (1,2).

The features that make this type of acute leukemia unique are the impressive hemorrhagic diathesis, the distinct coagulopathy, the risk of thrombotic complications, the variety of genetic and molecular features, the personalized therapy aimed against a molecular target and the theraputic protocols that do not contain chemotherapy in a form of neoplasia, but also certain particular complications secondary to differentiation agents and last but not least, early mortality, which represents a major problem.

The aim of this thesis is to analyze acute promyelocytic leukemia by identifying the detailed profile of patients diagnosed with this disease. Thus, the purpose is to evaluate clinical, morphological, immunophenotypic, genetic and molecular features of APL, correlations between them, analyzing the impact of those features on evolution, response to treatment, complications and survival. Taking into account that the most important challenge in current practice is early mortality, we consider important to evaluate predictive factors for early deaths, from the onset of the disease, in order to apply targeted treatment as fast as possible.Thus, we developed a risk score to predict early death, based on two parameters within reach to any clinician in current practice.

Also, we evaluated the presence at diagnosis of additional somatic mutations in a small group of patients with APL and we analyzed the connections between them and clinical and biological parameters, response to treatment, evolution and complications.

We conducted a prospective, longitudinal, observational and analytical study at the Hematology Department of Emergency University Hospital that include all 48 patients diagnosed with APL over an 18-year period (2006-2024). The small number of patients included in the study is due to low incidence of the disease.

The inclusion criteria based on the FISH exam, cytogenetic or molecular exam results, performed from peripheral blood or bone marrow aspirate, which established the diagnosis, correlating the data with the morphological appearance and immunophenotypic expression of atypical promyelocytes.

The collected data were statistically analyzed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) and Epi Info version 7.2. (Center for Disease Control and Prevention, Atlanta, GA, USA). In order to compare categorical variables we used Chi-Square, Fisher's exact test and mid-p for smaller groups of patients. In order to compare median values we used Independent Samples Median Test and Kruskal-Wallis. To identify associations between continuous variables, we used Spearman's rho correlation. For survival analysis we used Kaplan-Meier curves and Log-rank test. For the elaboration of the score, to identify the threshold value, we used the ROC curve. We calculated relative risk ratios using Odds Ratio and 95% Confidence Intervals. The p value < 0.05 was considered statistically significant.

The results of the first study describes a median age at diagnosis of 51.8 years, noticing a predominance of young patients, most of them being in the 40-59 age group. This aligns with literature data which indicates an incresed age at diagnosis (3,4). Patients with microgranular form had a higher median age, 56.8 years, without significant difference between morphological types. There was no sex predominance, both in the general group and for each morphological subtype. Regarding performance status, approximately two-thirds (60.4%) had ECOG 0-1, with a significant predominance of those with better performance status among patients with hypergranular form.

Eighteen (37.5%) patients associated cardiovascular pathology, followed by obesity (16.7%), a history of breast cancer (8.3%), arrhythmia (6.3%) and a history of acute coronary syndrome (6.3%). Three of the patients with history of breast cancer received chemotherapy and radiotherapy in addition to radical surgery; this is important in terms of treatment, considering the maximum dose of anthracycline that can be administred and the impact on heart function.

Another important aspect, considering the rarity of the association (5), is that two patients were diagnosed during pregnancy, one in the third trimester and the other in the first trimester of pregnancy.

According to Sanz risk score (6), most of the patiets were classified in the intermediate risk group, those with microgranular type being all classified in the high risk group.

The clinical picture was dominated by hemorrhagic complications (72.9%), the most frequent being the skin and soft tissue bleedings, this feature being described with the same frequency in the majority of studies (7,8). Only one patient had cerebral hemorrhage and another one presented hemoperitoneum at diagnosis. Four patients (8.3%) had thrombotic complications at diagnosis: in the cerebral vessels territory, pulmonary infarction, deep venous thrombosis in the lower limbs and pulmonary thromembolism. Twelve patients (25%) had

disseminated intravascular coagulation (DIC) and only two patients associated spontaneous tumor lysis at admission, both with high risk.

The whole group was characterized by pancytopenia and hypofibrinogenemia, which aligns to the biological picture described in the literature (9,10). In contrast, patients with microgranular form had higher white blood cells (WBC) count (p=0.0018) and higher atypical promyelocytes in the peripheral blood (p=0.0045) compared to those with hypregranular form. We observed a significant positive straight-line correlation between atypical promyelocytes in the peripheral blood and LDH level (p=0.000), and a negative one between LDH level and fibrinogen (p=0.001).

Patients with hemorrhagic complications at diagnosis had higer WBC (p=0.0143) and atypical promyelocytes (p=0.0004) in the peripheral blood and lower platelets (p=0.0000), APTT (p=0.0084) and fibrinogen (p=0.0001) levels compared to those without bleeding. This aspect correlates with the theoretical data that support the direct involvment of thrombocytopenia in the pathophysiology of hemorrhages, but also the properties of atypical promyelocytes that play a key role in the hemorrhagic diathesis (4,11).

Patients with thrombosis at diagnosis had five time higher platelets compared to the rest of the patients (p=0.0419).

High-risk patients had significantly higher atypical promyelocytes in the peripheral blood and LDH, respectively lower fibrinogen level compared to those classified as non-high risk.

The bone marrow infiltrate of atypical promyelocytes had a negative straight-line correlation with age, which indicates an increased proliferation rate of atypical promyelocytes in young patients. The immunophenotype of our APL patients showed high frequency of myeloid markers CD33, CD9, CD117, CD13.

Two young patients presented additional chromosomal abnormalities, but it did not have prognostic impact. According to literature, additional chromosomal abnormalities associate an incresed risk in older people (12).

Forty-three patients received induction therapy, the most common was AIDA protocol (53.9%), followed by ATRA monotherapy (16.28%) and ATRA+ATO (9.3%); the rest of the patients (20.93%) received other forms of therapy. Thirty-one patients achieved complete resonse, two – partial response, and eight patients died before the disese status coul be evaluated. LDH was the only predictive factor for obstaining the resonse; neither clinical and other biological features nor immunophenotypic makers influenced the response to induction treatment.

The most frequent complication after induction treatment were cytopenias and infections, followed by differentiation syndrome and hemorrhages. Patients who associated infections at diagnosis had a 5.79 higher risk of developing infections after induction, which emphasizes the compromised immune status of patients with APL. Those who developed differentiation syndrome and hemorrhages after induction were frequently classified as high or intermediate risk. There was a 5.5 increased risk among patients with microgranular form to develop differentiation syndrome compared to those with classical form. We also noticed the expression of CD34, CD13 and CD2 is associated with higher risk of differentiation syndrome.

Regarding the long-term complications, infections were predominant, which indicates the immunodeficiency of APL patients. This is reflected in the increased risk of developing long-term infections in those who associated infections at diagnosis as well as in patients with a history of breast cancer.

Median overall survival was not reached during a median follow-up of 9.98 months. The overall survival of patients with the microgranular form was inferior to those with the hypergranular form. Age and ECOG were predictive factors for survival.

The main cause of death were hemorrhagic complications, followed by infections. The main etiology of early death were also hemorrhages; all the patients had cerebral bleeding, only one of them associating both cerebral and pulmonary hemorrhage. Most studies describe the same main cause for early deaths (13–16). The reason for the increased frequency of cerebral hemorrhages is represented by the expression of annexin II on the surface of the endothelial cells of cerebral microvasculature (17,18).

Among predictive factors of mortality we noticed the increased risk of hemorrhagic death in patients with DIC at diagnosis. Patients who died due to differentiation syndrome were classified as high risk. Age and ECOG were predictive factors for death due to infections.

We developed a risk score for early death, based on the performance status and the LDH level at diagnosis. Thus, we assigned one point for ECOG with values between 2 and 4 and one point for LDH higher than 330 U/L. The score had values from 0 to 2. Patients with 2 points had a median survival of 0.167 months, while patients with a score of 0 and 1 point did not reached the median overall survival for the follow-up period (p=0.000). High risk patients (2 points) had a 19.25 times higher risk of early death compared to low-risk patients (0-1 point).

The second study analyzes the profile of additional somatic mutations in the case of 18 patients diagnosed with APL with PML::RARA fusion, in whom DNA samples obtained at diagnosis were tested by next-generation sequencing.

Following targeted-NGS testing, additional somatic mutations were detected in 11 patients, five patients (27.8%) presented a single mutation, two patients (11.1%) two mutations each, three patients (16.7%) three mutations each and only one patient (5.6%) four mutations. The most frequent mutations were those of the RAS signaling pathway (*NRAS*, *KRAS*, *PTPN11*) found in seven patients (38.9%) and *FLT3*-ITD mutation in seven patients (38.9%) as well, followed by mutations of epigenetic control factors (*DNMT3A*, *ASXL1*) found in two cases (11.1%), and mutations of ARN splicing factor *SRSF2* and transcription factor *RUNX1* were observed in only one patient (5.6%). Two patients (11.1%) had multiple *FLT3*-ITD mutations (two and three respectively), and three patients (16.7%) with *FLT3*-ITD associated other mutations as well.

The median age of the patients who associated additional mutations was 56 years, with a predomiance of the male sex. Patients with FLT3-ITD mutation had older age.

There is a predominance of patients who associated mutations in the high and intermediate risk group and a more frequent association of FLT3-ITD (single or associated with other mutations – NRAS, RUNX1, SRSF2, KRAS, PTPN11) with high risk (p=0.065). This aspects correlate with other studies, patients with associated mutations being classified as high risk (19,20).

Among patients with additional mutations, two had thrombosis at diagnosis, namely one patient with FLT3-ITD and one patient with PTPN11 mutation. The frequency of hemorrhagic complicatins is 53% higher for those who had FLT3-ITD mutation (p=0.011).

Regarding biological features, patients with FLT3-ITD mutation presented with higher WBC counts, higher atypical promyelocytes in the peripheral blood and higher LDH levels. Same results are described in the literature (20,21).

The presence of FLT3-ITD increases the risk for the expression of CD34, CD56 and CD2, all being associated with bad prognosis (22).

Patients with microgranular form had a risk with 72.22% higher of associating FLT3-ITD than hipergranular form (p=0.0552).

All patients received induction treatment, only 14 had complete response. The four patients who died early associated additional somatic mutations.

The most ferquent complication after induction was differentiation syndrome, followed by infections. Most of the patients who developed differentiation syndrome belong to the group of additional somatic mutations.

Patient with FLT3-ITD had a median overall survival (OS) of five months compared to 10 months, the OS for those who din not presented this mutation, for a follow-up period of 9.32 months), but the difference is not significant (p=0.3219).

We recorded six death (33.3%), and most of them (66.7%) were early. The causes of death were thrombosis, differentiation syndrome, multiple organ failure associated with tumoral lysis syndrome and disease progression. All patients who died had additional somatic mutations. We noticed a trend of association of deaths in patients with multiple mutations, respectively in those with FLT3-ITD and RAS mutations, which aligns with some studies in literature (20,21,23–25).

In addition to somatic mutations, germline variants with possible pathogenic significance were detected, as it follows: a heterozygous variant *KMT2A* (*MLL1*), c.10118T>A, p.L3373H in two patients; a heterozygous variant *SH2B3* (*LNK*), c.17T>C, p.L6P lin two patients; a heterozygous variant *ATM* c.5890A>G, p.K1964E in one patient; a heterozygous variant *ATM* c.4973C>T, p.A1658V in one patient; a heterozygous variant *EZH2* c.553G>C, p.D185H, in three patients.

This thesis achieves its objectives, namely to study the complete profile of patients with LAP, to analyze their features and possible associations between them and treatment response, evolution and mortality. Also, the most important objective, we identified in our group risk factors for early mortality, developing a risk score.

The second study brings a note of novelty regarding the completion of the diagnostic panel of patients with APL, namely the detection of additional somatic mutations. Although we had a small number of patients, a relatively large number of them associated additional somatic mutations, respectively germline variants with possible pathogenic significance. This aspect comes as an argument for the proposal to introduce the NGS method in the diagnostic protocol of patients with APL.

Additional studies, on larger groups of patients, are required to analyze the impact of therapy, especially of arsenic trioxide, on the evolution of patients with somatic mutations.

Although during the last 67 years, since the first description of the pathology until now, significant progress has been achieved in terms of the response to treatment and the cure rate of patients, there are still many unsolved problems, such as the high rate of early deaths and especially fatal hemorrhagic complications.

It can be considered that now we open a new chapter in the diagnosis of APL, marked by the identification of additional somatic mutations and the analysis of new therapeutic agents

with a molecular target that could increase the curability rate and reduce the incidence of early death.

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List of published scientific papers

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