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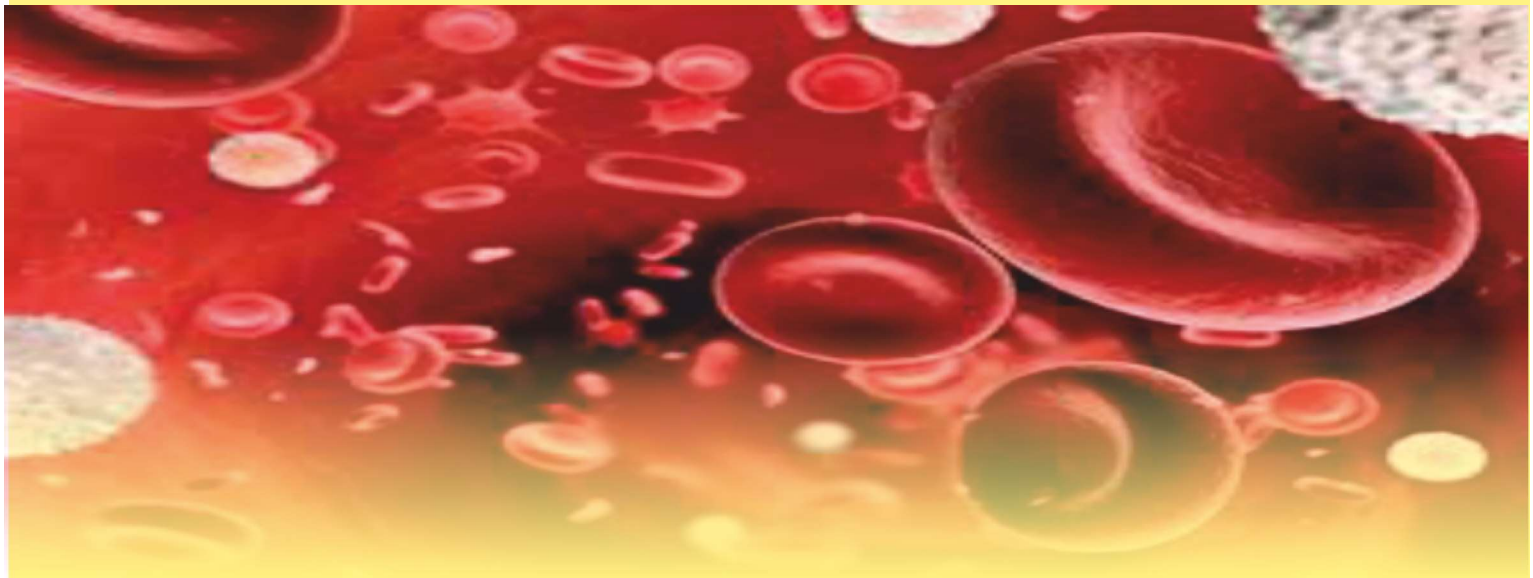

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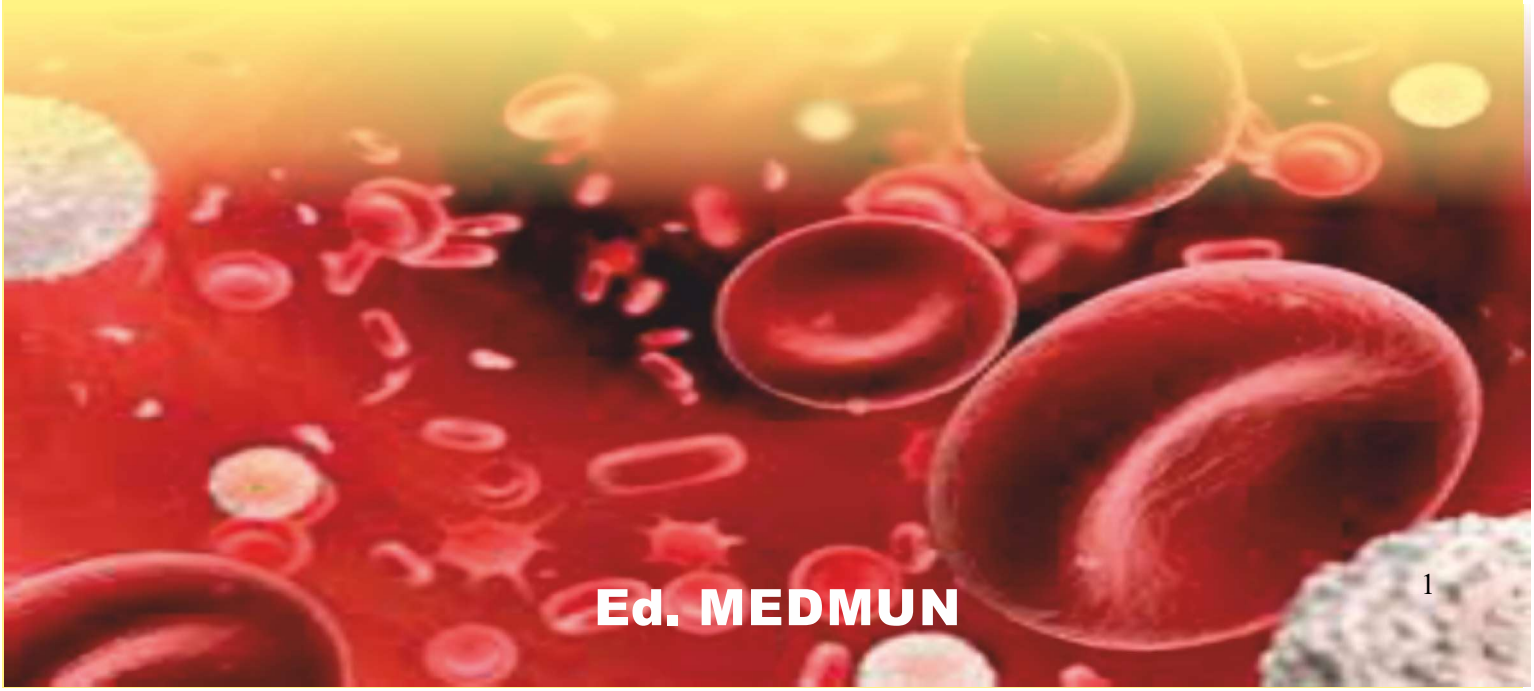

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CONTENTS

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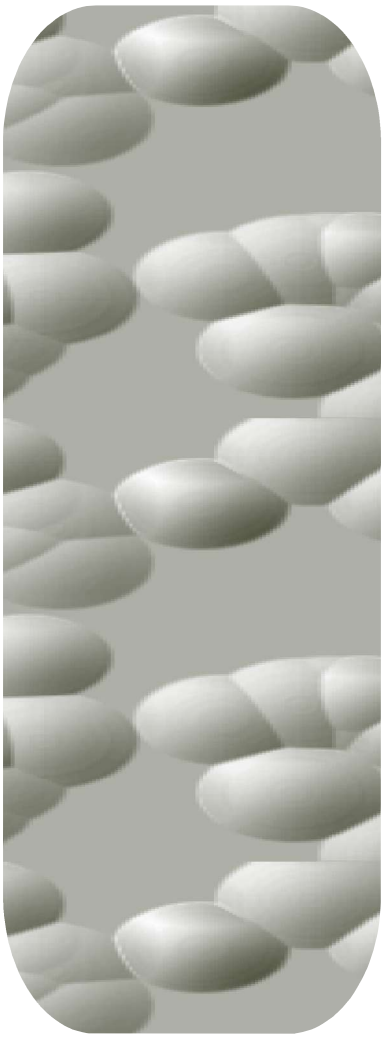

NATIONAL SOCIETY OF BLOOD TRANSFUSION FROM ROMANIA

Diagnosis of Acquired Aplastic Anemia

Anca Maria Popp
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Summary
Aplasic anemia remains a diagnosis of exclusion. Acquired or inherited, aplastic anemia is a rare, but severe disease.It has to be considered a medical emergency.
Recently, steps were done for more understanding the mechanisms of this form of bone marrow failure.
It is important to better characterize the etio-pathogeny of aplastic anemia for: 1. to exclude other bone marrow failures; 2. to decide the most suitable treatment; 3. to evaluate the prognosis of each case of aplastic anemia.
This article is a brief review of the literature concerning some aspects of etio-pathology and diagnosis of aplastic anemia.

Key words*: aplastic anemia, hematopoietic stem cell, paroxistic nocturnal hemoglobinuria.*

Aplastic anemia is characterized **by peripheral pancytopenia** and **marrow hypoplasia.**
In almost 80% of cases it is an **acquired** disease. The other are **inherited** aplastic anemia cases.

It is a rare disease. The incidence rate of aplastic anemia in Europe and USA amounts to 2-3 millions/year. The age distribution of the disease reveals two peaks: one between 10 and 25 years old and the other at and over 60 years old.

To confirm the suspicion of aplastic anemia and exclude other bone marrow failure, also to decide the most suitable treatment and evaluate the prognosis, is necessary (even critical) to understand the etio-pathology of this disease.

Pathophysiology

Today, the development of progenitor assays permits to emphasize that various factors could be responsable for **failure to form colonies** in tissue cultures.

Due to technical advances in cell biology (flow cytometry, molecular biology, immunology) now it is possible to estimate number and function of a very low number of cells. [1,2]

In acquired aplastic anemia there are clinical and laboratory findings suggesting an **immune mechanism** of hematopoietic cell destruction. [3,4]

The morphologic bone marrow evaluation shows that less than 25% of the cells are hematopoietic elements, and the marrow is replaced with fat cells. Stem cells and the early committed hematopoietic progenitors (CD34 cell population by flow cytometry) are extremely reduced in aplastic anemia samples. There is a severe functional loss of the hematopoietic progenitors as it is evidenced by in vitro colony-culture assays. [5,6]

The question is: aplastic anemia is due to: 1. an intrinsic defect of hematopoietic cells, 2. an external injury of hematopoietic cells or/and 3. deffective stroma that has a critical impact on the normal proliferation and differentiation of the hematopoietic cells?

There are many in vitro stem cells culture experiments using stem cells from aplastic anemia patients and normal stroma and other ones with normal stem cells cultured with aplastic anemia patients stroma. Conclusion: the principal mechanism of the disease in the majority of cases is the stem cell defect. Normal stromal function, including growth factors production provided by the host stromal elements, allows the engraftment of the hematopoietic alogenic cells that are transplantated. [6]

Immune mechanisms that might be envolved are unknown. However, it is accepted that human leukocyte antigen HLA-DR2 is overrepresented among aplastic anemia patients and its presence is considered to be predictive for a good response to immunosuppessive therapy (cyclosporine).

In the blood and marrow of the aplastic anemia patients there is an expanded population of CD8+ HLA-DR+, cytotoxic T lymphocytes. These cells produce inhibitory cytokines (gamma IFN,TNF) which suppress stem cells growth, by affecting the mitotic cycle and and cell killing (Fas-mediated apoptosis).

Regulatory T cells (Treg) play a role in autoimmunity. In almost all aplastic anemia patients Tregs are decreased. Also, there is evidence that FOXP3 and NFAT1 protein levels are decreased or absent in this condition. [7,8]

There have been reported variations in telomere length in aplastic anemia patients. A recent publication showed that telomere shortening in these patients was associated with chromosomial abnormalities. Data suggest that patients with shorter telomeres (by means of the Flow-FISH method) are at higher risk of malignant transformation. The measurement of telomere length seems to be an interesting prognostic

tool in acquired aplastic anemia.(9,10,11,12,13)

Cytogenetic abnormalities can be present in 12% of aplastic anemia patients. Abnormal cytogenetic clones (often small) may arise during the course, or may disappear spontaneously or after immunosuppressive treatment. It is important to emphasize that the presence of an abnormal cytogenetic clone does not necessarily exclude the diagnosis of aplastic anemia. In marrow failure syndromes, cytogenetic evaluation is difficult because of the lak of metaphases. Chromosomial abnormalities evidenced in some aplastic anemia cases are: trisomy 8,trisomy 6, 5q-, anomalies of chromosome 7 and 13. [9,10,11,17]

Etiology

Here are the most frequent causes of acquired aplastic anemia: [14,15,16]

- Infections: hepatitis viruses,parvovirus, Epstein Barr virus, human immunodeficiency virus.
- Exposure to radiation and myelotoxic chemicals (benzene)
- Pregnancy
- Severe nutritional deficiencies (B12, folate)
- Paroxysmal nocturnal hemoglobinuria. The disease is caused by an acquired genetic defect affecting the PIGA gene and limited to the stem cell compartment. One third of patients with aplastic anemia patients have evidevce of HPN at presentation. Also, patients who recover after immunosuppressive therapy, may have a HPN clonal hematopoiesis.
- Drugs and elements (chloramphenicol,phenil-butazone, gold). In these cases there is a toxic effect against the hematopoietic cells, not an immune mechanism.

Interestingly, some consider that bone marrow failures due to exposure of ionizing radiation or myelotoxic substances are not referred to as aplastic anemia.

There are rare situations when aplasia appears as a posttransfusional event , like a graft versus host reaction.

In most cases there is no documented etiology of the bone marrow disfunction. Aplasic anemia is **idiopathic.**

Diagnosis of aplastic anemia is based on: history, clinical features, laboratory findings. All of the three are important to establish the etiology, the grade of severity of the disease, the most suitable treatment, the prognosis. [18,19]

History

At the first presentation, one may have symptoms due to anemia (pallor, fatigue, headache, palpitations), due to neutropenia (fever, infection , sometimes recurrent infections) or/and due to thrombocytopenia (petechias, mucosal bleeding).

It is important to identify an event in the recent history that is a potential cause of the disease. Often, the search of an etiologic agent does not find any cause (>80% of cases).

Clinical features

Physical examination may show pallor, tachycardia (low hemoglobin level), purpura, ecchymoses (small number of thrombocytes). A few patients are presenting with fever, but most of them are afebrile, even if they are infected (neutropenia). One must carefully look for physical signs of infection. No enlarged lymph nodes, no hepato or splenomegaly are found in aplastic anemia patients.

Laboratory findings

Complete blood cell count and peripheral blood film examination:

- anemia, when present, is normo or macrocytic, normochromic; reticulocyte count is low.
- leucocytopenia resulting from granulocytopenia and monocytopenia may be present. Lymphocyte count is usually preserved. No immature precursor cells.
- thrombocytopenia is often present, in various degrees, with the absence of giant platelets in blood smears.

The degree of cytopenia is usefull in assessing the degrees of severity of aplastic anemia. (see below).

Bone marrow examination:

- aspiration alone may provide hypocellular samples because of technical reasons.It allows a better evaluation of cellular morphology (assessment of dysplasia)
- bone marrow biopsy has the advantage to assess the cellularity both in a qualitative and quantitative manner. It is considered a hypocellular specimen if it is less then 20-30% cellular.No infiltration of foreign cells has to be found. Without fibrosis. Stromal cells (plasma cells, lymphocytes) are often increased in aplastic anemia bone marrow samples and these cells have to be excluded in the global evaluation of the marrow cellularity.

Sometimes is difficult **to distinguish** between **aplastic anemia** and **hypoplastic MDS**. This two entities are marrow failure syndromes characterized by hypocellularity with increased fat cells in the bone marrow. Absence of dysplasia and of blast cells supports the diagnosis of aplastic anemia.

Other periferic blood tests:

- biochemical profile to asses the renal and hepatic function that may reveal renal or liver disfunction.

- viral screening for hepatitis and for other viral entities are mandatory (EBV, CMV, HIV,Parvovirus B19).
- evaluation for an autoimmune disease has to be performed.
- vitamin B12, folic acid, iron status, coagulation status.
- hemolysis parameters: LDH, haptoglobin, bilirubin, hemosiderin.
- tests to confirm/exclude PNH: the Ham test, the sucrose hemolysis test, and the more recent and accurate CD55 and CD59.
- blood group, direct antiglobulin test.
- tests of histocompatibility has to be done close to the diagnostic time, in order to early identify potential related donors.
- additional tests: chest X-ray, abdominal echography, echocardiogram.
- additional tests: microbiology

It is important to notice that recent studies demonstrated that a part of presumably acquired aplastic anemia patients had a late onset form of congenital disease. This is the reason to recommend screening tests like chromosome breakage and telemetric length determination and targeted molecular tests for young adults. [17]

Staging (20)

The severity of the disease is based on the data of the peripheral blood and bone marrow findings.

According to the International Aplastic Anemia Study Group,

staging criteria for blood are:

- Neutrophyls less than 0.5 x 10 9/L
- Platelets less than 20 x 10 9/L
- Reticulocytes less than 1% corrected

Staging criteria for marrow are:

- Severe hypocellularity
- Moderate hypocellularity with hematopoietic cells representing less than 30% of residual cells

Severe aplastic anemia is defined: any 2 or 3 periferal blood criteria and either marrow criterion. Patients with neutrophil counts less than 0.2 x 10 9/L are considered as very severe aplastic anemia patients.

Once the diagnosis of aplastic anemia and the staging are established, the most appropriate treatment has immediately to be perform.

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The impact of epidemiologic factors, hematologic factors and various regimens on the survival of a group of Myelodysplastic patients diagnosed according to FAB criteria.

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Abstract

Myelodysplastic syndromes (MDS) are a heterogeneous group of acquired clonal stem cell disorders characterized by faulty maturation of myeloid precursors, genetic instability, ineffective hematopoiesis, persistent peripheral cytopenias and high risk of progress to acute myelogenous leukemia. Thr first diagnostic criteria were the FAB classification from 1982.

***Materials and methods**: In this study we retrospectively analyzed 392 patients diagnosed with de novo MDS, according to the FAB criteria, between 1982-2002, in the Department of Hematology of Fundeni Clinical Institute. We analyzed the survival and acute myeloid leukemia (AML) transformation according to sex,FAB subtype, number of cytopenias, bone marrow blast percentage and the impact on survival of different therapeutic therapies. We used the Kaplan Meier survival analysis.*

***Results**: The median age in this lot was 62.46 years. There were predominant those over 60 years, 68.3%, males, 55.6%. In this lot, the median survival wasn't statistically influenced by age and sex. There were 230 patients with refractory anemia/ refractory anemia with ringed sideroblasts (RA/RARS), 137 patients with refractory anemia with excess blasts/ refractory anemia with excess blasts in transformation (RAEB/RAEB-t), 25 patients with chronic myelomonocytic leukemia (CMML). The median survival was significantly lowered by the following hematological parameters: the RAEB/RAEB-t/CMML subtypes (**$p<0.0001$**), the rise of the cytopenias number $p<0.05$, severe neutropenia and thrombocytopenia, statistical **$p<0.0001$**. The risk of AML transformation was significantly increased, **$p< 0.05$**, by: RAEB, RAEB-t and CMML subtypes compared to RA, RARS; bone marrow blasts over 5% compared to under 5%; the rise in number of cytopenias compared to no cytopenias. The percentage of those who turned to AML wasn't significantly influenced by age and sex. The transfusion dependence significantly lowered the survival, **$p<0.0001$**. This highly significant statistical difference was maintained in all FAB groups, **$p<0.0001$**. Low doses chemotherapy, high doses chemotherapy and immunosuppressive therapy didn't significantly influence survival, $p>0.05$.*

***Conclusions**: The FAB classification of the myelodisplastic syndromes is the first to set clear criteria for framing this disease, which was used on a large scale, accessible, and still that is still being used today. This study, conducted on a large lot of patients (392) showed the influence of FAB criteria on median survival and AML transformation, the influence of hematological parameters and transfusion dependency.*

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of acquired clonal stem cell disorders characterized by maturation-defect of myeloid precursors, genetic instability, ineffective hematopoiesis, persistent peripheral cytopenias and high risk of progress to acute myelogenous leukemia. The increased susceptibility to medullary cell apoptosis is the central mechanism of ineffective hematopoiesis, and it causes peripheral cytopenias. The evolution may be indolent, in several years or it may rapidly progress to acute leukemia, depending on the MDS subtype. [1-3]

The MDS diagnosis requires a complete blood count, peripheral blood smear showing dysplastic changes and bone marrow examination (bone marrow aspirate and biopsy). The cytogenetic exam points out the clonality and it has an important role in establishing the prognosis. [4,5,6,7]

The most used diagnosis criteria over the time were FAB classification from 1982 and World Health Organization's (WHO) from 2001 and revised in 2008. [6,7,8]

The prognostic score systems used for de novo MDS patients are the International Prognostic Scoring System (IPSS) from 1997, the Revised International Prognostic Scoring System (IPPS-R) from 2012, the WHO Prognostic System Score (WPSS) from 2005 by Malcovati and the revised WPSS from 2011 also proposed by Malcovati. To calculate these scoring systems one uses hematological parameters as well as cytogenetic abnormalities. [9,10,11,12]

Therapeutic strategies for MDS patients must take into account that it's an elderly disease, with multiple non-hematological comorbidities, that can't tolerate aggressive chemotherapy. The AML evolution is associated with chemotherapy resistance, comparatively to de novo AML. The only treatment with curative intent in this case is hematopoietic stem cell allotransplant, limited to a small number of patients due to the age of the patient and the lack of stem cell donor. [13]

Choosing the treatment depends on patient related factors (age, comorbidities, performance status) and on the complete initial evaluation of the patient that implies

calculating the IPSS, IPSS-R or WPSS risk group. Following the patient over several months allows the assessment of the evolution of cytopenias and disease progression. Some of the patients have asymptomatic cytopenias that has a slow evolution and that don't need therapy for a long time, while other patients have a rapid evolution to AML. [13]

MDS therapeutic options are: supportive therapy, erythropoiesis stimulating agents (ESA), demethylating agents and immunomodulatory agents, low intensity chemotherapy, intensive AML chemotherapy, including stem cell allotransplant, enrolling in clinical trials. [13]

Materials and methods

In this study we retrospectively analyzed 392 patients diagnosed with de novo MDS according to FAB criteria between 1982 and 2002. We analyzed clinical and hematological traits at diagnosis, survival, acute leukemia progression and the impact on survival of different therapeutic strategies.

The data base was obtained from The Hematology Clinic MDS Registry of Fundeni Clinical Institute, founded in 2006 under the direct guidance of Dr. Radu Gologan. This registry used a patient registration form based on the FAB classification, given by The USA Myelodisplastic Syndrome Foundation, whose president is Prof. Dr. J.M. Bennett.

Inclusion criteria: "de novo" MDS patients according to FAB. The data was gathered from the patient's observation sheets and the MDS secondary to chemotherapy or other therapies was excluded.

The following parameters were analyzed: epidemiologic data (age, sex), complete blood work, bone marrow. We considered cytopenia the following values: Hemoglobin <10g/dl, Absolute neutrophil count $<1.8x10^9/l$ and platelets count $100x10^9/l$.

The data was processed using Microsoft Office - Excel 2003. For the descriptive analysis of the data we used the central tendency indicators: mean, median, mode and standard deviation. The quantitative data were comparatively analyzed using statistical significance tests like the Student test (t-test), Fischer test (Fischer Exact Test), the "z" test for comparing differences between proportions.

The survival analysis we used was Kaplan Meyer. For comparing categories we used the "long rank test" and we considered significant a value lower than 0.05 (statistical p value) for a 95% confidence interval.

Results

The group consisted of 392 patients diagnosed according to FAB criteria in the Hematology Department of Fundeni Clinical Institute, between 1982-2002. There were 218 men and 174 women. We analyzed the survival and AML transformation depending on age, sex. FAB subtype, number of cytopenias, blasts percentage in bone marrow and the impact on survival of different therapeutic strategies. The characteristics of the group are presented in table no. 1.

Table 1.
Lot characteristics and the FAB classification

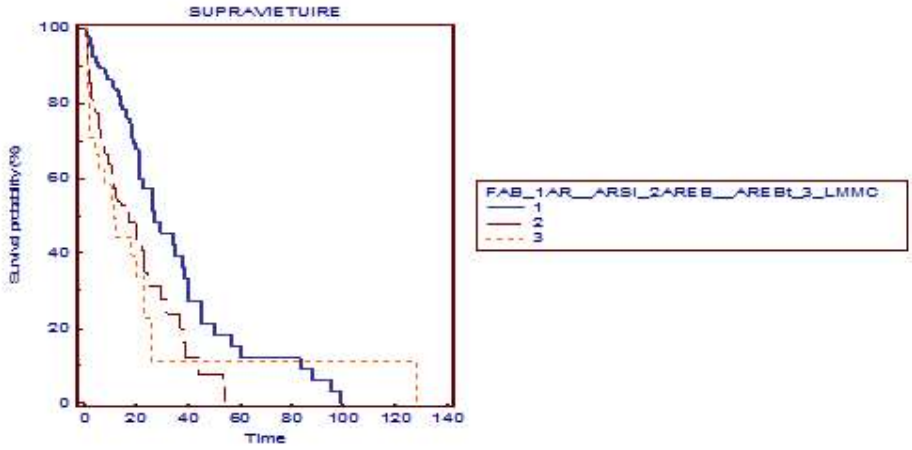
Parameter		No of patients (%)	Log Rank
Age	<60 ani	124 (31,7%)	p>0,05
	>60 ani	268 (68,3%)	
Sex	F	174 (44,4%)	p>0,05
	M	218 (55,6%)	
FAB Subtype	RA/RARS	230 (58,6%)	**p<0,05**
	RAEB/RAEB -t	137 (34,9%)	
	LMMC	25 (6,37%)	
No. of cytopenias	0	193 (49,2%)	**p<0,05**
	1	120 (30,6%)	
	2	58 (14,8%)	
	1	21 (5,3%)	
Hemoglobin	<8g/dl	191(48,7%)	p>0,05
	>8g/dl	201(51.3%)	
Neutrophils	$<0,8 x10^9/l$	70(17,9%)	**p<0,05**
	$>0,8 x10^9/l$	322(82,1%)	
Platelet	$<50 x10^9/l$	101(25,8%)	**p<0,05**
	$>50 x10^9/l$	291(74,2%)	
Bone marrow blasts	<5%	242(61,7%)	**p<0,0001**
	>5%	150(38,3%)	

The median age in this group was 62, 76 years (with limits between 17 and 90 years), of which 124 (31.7%) patients under 60 years and 268 (68.3%) over 60 years. Distribution by sex was: 218 (55.6%) men, 174 (44.4%) women, the men/women ratio was 1.25, whit a slight predominance of the disease in men. In this group, the median survival wasn't statistically significant influenced by age and sex (p statistic over 0.05)

The percentage of patients classified according to

FAB was: RA 171 (43.6%), RARS 59 (15%), RAEB 103 (26.2%), RAEB-t 34 (8.6%) and CMML 25 (6.3%). There were 230 patients with RA/RARS, 137 patients with RAEB/RAEB-t, 25 patients with CMML. Median survival differences in the RA/RARS, RAEB/RAEB-t and CMML groups were statistically significant (p=0.0101). (Fig. 1)

Fig. 1.
Kaplan-Meier survival curve according to FAB classificatioPatient's distribution by the number of

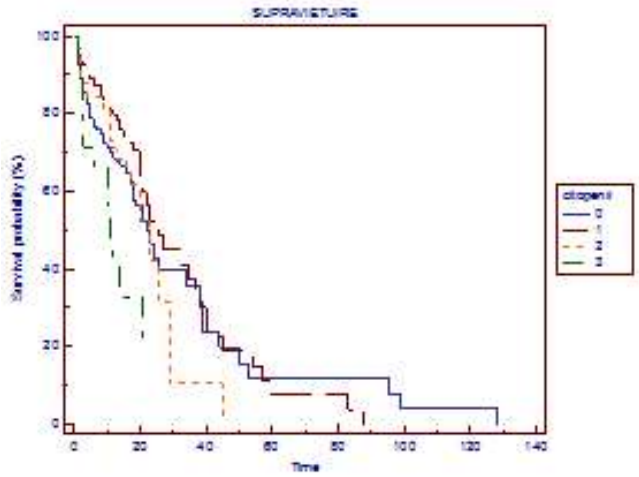


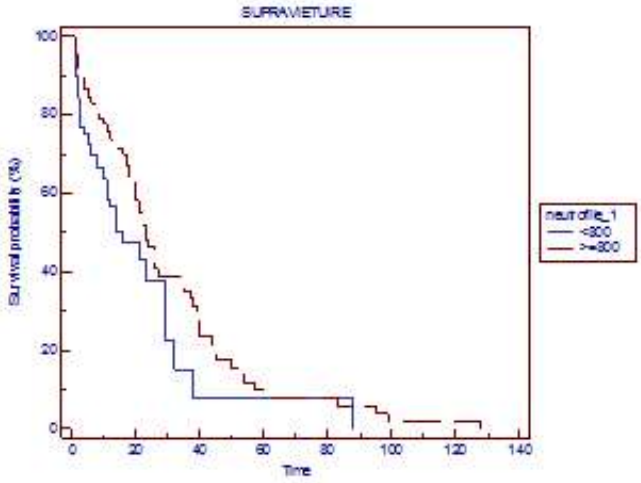
cytopenias was: 193 patients had no cytopenias, 120 had 1 cytopenia, 58 had 2 cytopenias, and 21 had 3 cytopenias. Median survival in these groups of patients was statistically significant in favor of those without cytopenias or with one cytopena (p=0.0101) (Fig. 2)

Fig. 2. Kaplan-Meier curve according to the number of cytopenias.Of the 392 patients, 191 (48.7%) had Hb over 8 g/dl and 201 (51.3%) had Hb under 8 g/dl. The hemoglobin value had no statistical influence over the median survival (p=0.2685).

Regarding absolute neutrophil count, 322 (82.1%) patients had over $0,8x10^9$/l and 70 (17.9%) patients had under $0,8x10^9$/l neutrophils. Median survival for the patients that had under $0,8x10^9$/l netrophils was statistically significant grater in the second group (p = 0.0048). (Fig. 3)

Fig. 3 Kaplan-Meier curve of survival according to absolute neutrophil count.Regarding platelets count, 101 (25.8%) patients had under $50x10^9$/l





platelets and 291 (74.2%) patients had over $50x10^9$/l platelets. Patients that had over $50x10^9$/l platelets had a statisticaly significant grater median survival comparative to those with under $50x10^9$/l platelets (p=0.0338). (Fig. 4)

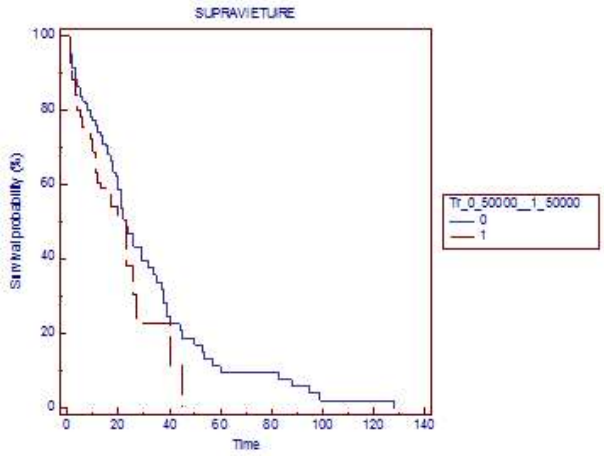


Fig. 4 Kaplan-Meier survival curve according to platelets count.

If we consider the limit values of Hb, platelets and neutrophiles as risk factors that with concurrent action, we find that neutropenia and thrombocytopenia have a significant role in the occurrence of death (for netrophils and platelets p=0.0048 and respectively 0.0338, and for Hb p=0.2685) (Table 2)

Table 2n

	B	S.E.	Wald	df	Sig.	Exp(B)
Hb	**-.098**	**.211**	**.217**	**1**	**0.268**	**.906**
Neutrofile	**-.562**	**.278**	**4.081**	**1**	**0.048**	**.570**
Tr	**.450**	**.211**	**4.535**	**1**	**0.033**	**1.568**
Constant	**.094**	**.311**	**.092**	**1**	**.762**	**1.099**

Regarding the percentage of blasts in the bone marrow, in our group, patients with blasts percentage under 5% prevail, 242 (61.7%) comparative to those with blasts percentage over 5% - 150 (38.3%). The median survival depending on the number of blasts in the bone marrow shows that patients with under 5% blasts were statistically significant higher than those with blasts over 5% (p<0.0001). (Fig 5)

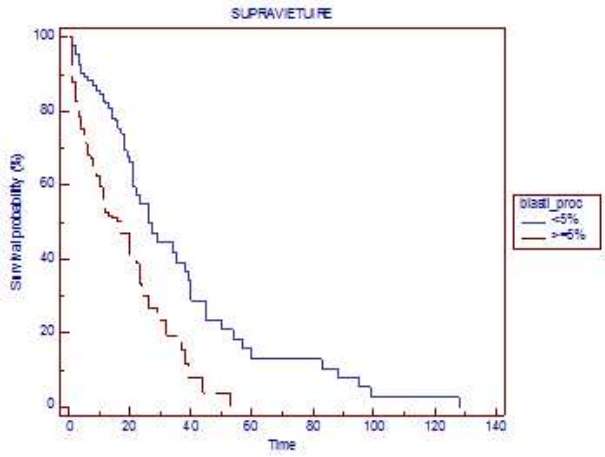


Fig.5. Kaplan-Meier survival curve according to bone marrow blasts percentage

We did the Kaplan Meier curve for the AML transformation according to the FAB classification. From the 230 patients with RA and RASR, 13 (5.6%) turned into AML. The AML transformation risk is 3 times higher for RAEB/RAEB-t/CMML for a 95% CI between 1.5 and 6.1 (Table 3).

Table no. 3. The risk of AL transformation according to FAB subtype.

FAB			LA		Total
			nu	da	
	AR/ARSI	Nr	217_a	13_b	230
		%	61.3%	34.2%	58.7%
	AREB/AREB-t	Nr	137_a	25_b	162
		%	38.7%	65.8%	41.3%
Total		Nr	354	38	392
		%	100.0%	100.0%	100.0%

		CI 95%	
		Min	Max
	3.046	1.507	6.155
Cohorta LA = nu	1.116	1.037	1.200
Cohort LA = da	.366	.193	.694
Nr. cazuri	392		

	LA					
	nu		da		Total	
	Nr.	%	Nr.	%	Nr	%
AR/ARSI	217	61.3%	13	34.2%	230	58.7%
AREB/AREBt	116	32.8%	21	55.3%	137	34.9%
LMMC	21	5.9%	4	10.5%	25	6.4%

We calculated the risk of AML transformation depending on the number of bone marrow blasts. From the 424 patients with under 5% marrow blasts, 16 turned into AML (6.6%). From the 150 patients with over 5% marrow blasts, 22 (14.6%) turned into AML. There is a statistically significant difference between the percentage of those who transformed into AL versus those who didn't go through this change, depending on the number of bone marrow blasts ($p<0.05$). The risk of AML transformation based on the number of blasts is 2.4 (C.I. 95% 1.2-4.7). (Table no. 4)

The percentage of those without cytopenias that turn into AML (2.6%) is statistically significant lower compared to those with one or more cytopenias ($p<0.05$). The percentage of patients that turn to AML dose not significantly differ between age groups.

Table no. 4. The AML transformation risk depending on the number of blasts in the bone marrow**Treatment**

BM blasts			AL		Total
			no	yes	
	<5%	No.	226_a	16_b	242
		%	63.8%	42.1%	61.7%
	>=5%	Nr	128_a	22_b	150
		%	36.2%	57.9%	38.3%
Total		Nr	354	38	392
		%	100.0%	100.0%	100.0%

Estimated risk

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio pentru blaşti în MO (1.00 <5% / 2.00 >=5%)	2.428	1.231	4.790
Cohorta AL = no	1.094	1.016	1.179
Cohorta AL = yes	.451	.245	.830
Valid cases no.	392		

From the 392 patients, 19 (4.8%) didn't receive any treatment, 373 patients (95.2%) received transfusions, immunosuppression (corticosteroids, cyclosporine), vitamin B6 and folic acid, low doses chemotherapy (LDCT) and intensivechemotherapy (ICT).

Low risk patients (RA/RARS) received transfusions (19.9%), vitamin B6 and folic acid (46.7%), corticosteroids (23%) and a small percentage received low doses chemotherapy (7.4%), high doses chemotherapy (0.3%) and cyclosporine (0.5%).

High risk patients (RAEB/RAEB-t and CMML) received transfusions (17.3%), corticosteroids (27.5%), intensive chemotherapy (4.1%), low doses chemotherapy (9.9%), vitamin B6 and folic acid (13.8%).

Patient's distribution depending on the received treatment and the FAB subtype is presented in Table no. 5. 146 patients received transfusions, of which: 78 (19.9%) had RA/RARS, 53 (13.5%) had RAEB/RAEB-t and 15 (3.8%) had CMML.

Immunosuppressive therapy (corticosteroids and cyclosporine) was given to 207 (52.8%) patients: 92 (23.4%) RA/RARS, 94 (23.9%) RAEB/RAEB-t and 21 (5.3%) CMML. Only 9 patients (2.3%) received cyclosporine. Vitamin B6 and folic acid was given to 283 patients: 183 (46.7%) RA/RARS, 85 (21.7%)

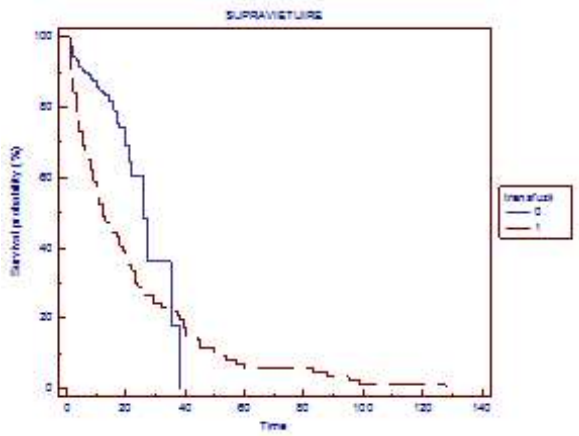
RAEB/RAEB-t and 15 (3.8%) CMML. Low doses chemotherapy was given to 62 patients: 29 (7.4%) had RA/RARS, 19 (4.9%) had RAEB/RAEB-t and 16 (4.1%) CMML. Intensive chemotherapy was given to 17 patients (4.3%): 1 (0.3%) RA/RARS, 14 (3.6%) RAEB/RAEB-t and 2 (0.5%) CMML. Out of the 392 patients, 146 received supportive care.

Table no. 5. Patient's distribution according to the treatment received and FAB subtype. .

Diagnostic FAB	Transfuzii	B6, acid folic	Corticosteroizi	CTDM	CTI	ciclosporina
Nr.(%)	Nr.(%)	Nr.(%)	Nr.(%)	Nr.(%)	Nr.(%)	Nr.(%)
RA 171 (43.6%)	64 (16.3%)	135 (34.4%)	67 (17.1%)	23 (5.9%)	1 (0.3%)	2 (0.5%)
RARS 59 (15.1%)	14 (3.6%)	48 (12.2%)	23 (5.9%)	6 (1.5%)	0	0
RAEB 103 (26.3%)	34 (8.7%)	63 (16.1%)	74 (18.9%)	5 (1.3%)	12 (3.1%)	1 (0.3%)
RAEB-t 34 (8.7%)	18 (4.6%)	21 (5.4%)	19 (4.8%)	14 (3.6%)	2 (0.5%)	0
CMML 25 (6.4%)	16 (4.1%)	16 (4.1%)	15 (3.8%)	14 (3.6%)	2 (0.5%)	6 (1.5%)

Patients who received transfusions had a highly statistically significant lower median survival ($p<0.0001$) than those who didn't receive transfusions. (Fig. 6)

Fig. 6.
Kaplan Meier survival curve of patients who received transfusions (1) and who did not receive transfusions (0)

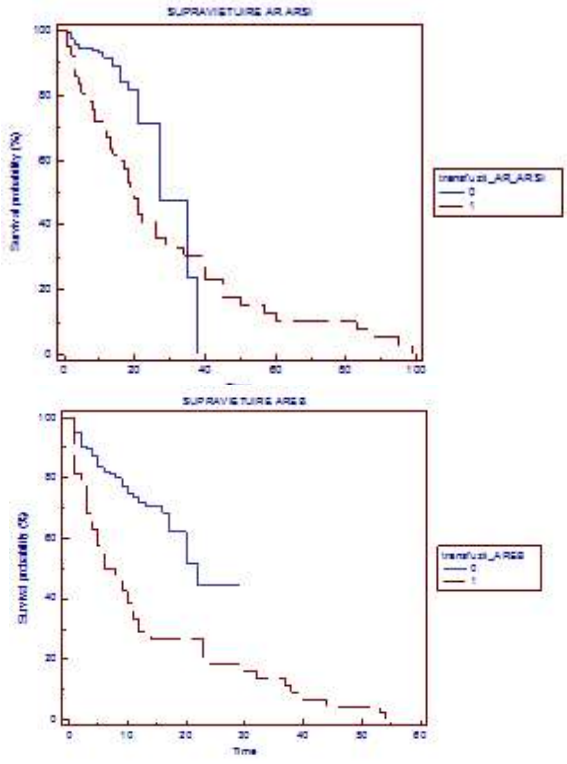


Out of the 137 RAEB/RAEB-t patients, 52 received supportive treatment, The Kaplan Meier curve shows survival differences highly statistically significant in favor of patients who didn't receive substitutive treatment. ($p<0.0001$) (Fig. 8)

Fig. 8
Kaplan Meier survival curve regarding survival of RAEB/RAEB-t patients who received supportive treatment.

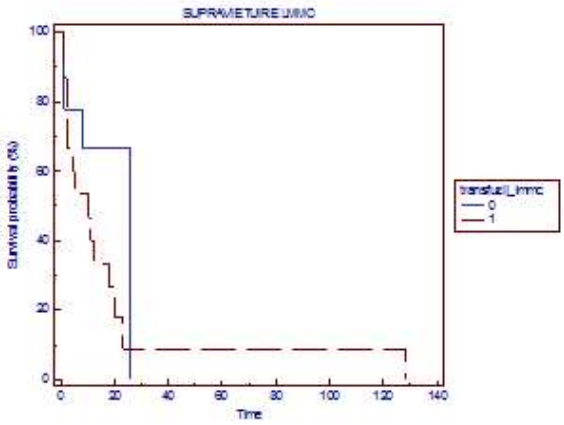
Out of the 230 RA/RARS patients, 78 received supportive care. The median survival estimated for those who received transfusions was highly statistically significant ($p<0.0001$) lower than for those who didn't receive transfusions. (Fig. 7)

Fig. 7
Kaplan Meier curve regarding the survival of RA/RARS patients who received supportive care.)



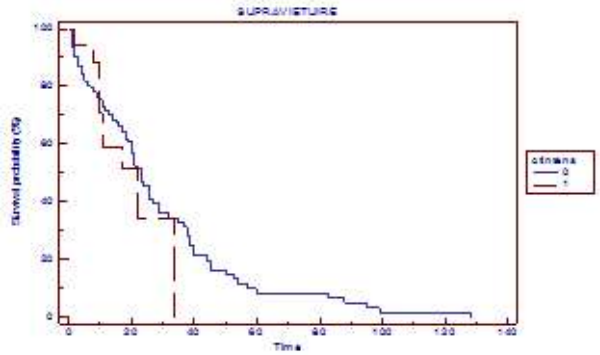
Out of the 25 CMML patients, 16 received supportive treatment, of which 7 died. Estimated median survival for those who received only substitutive treatment was 10 months, whit a CI 95% between 2 and 18 months. Estimated median survival for those who didn't receive substitutive treatment was 26 months. Kaplan Meier curve showed that in this patient category there is no statistically significant difference of survival between those who received transfusions and those who didn't. (p=0.1370) (Fig. 9)

Fig. 9.
Kaplan Meier survival curve for CMML patients who received supportive treatment



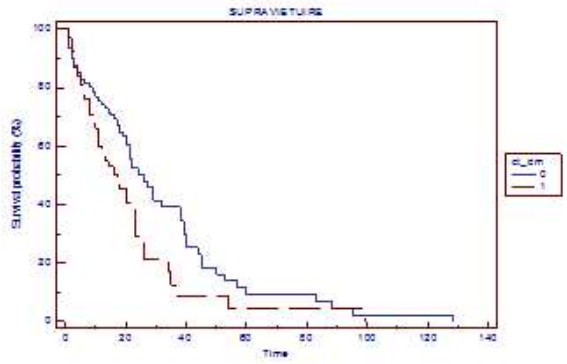
Only 14 of the 137 patients diagnosed with RAEB/RAEB-t and only 2 patients diagnosed with CMML received intensive chemotherapy (ICT) versus non-ICT. The Kaplan Meier survival analysis failed to show any statistically significant difference between the two treatment options (p=0.3705). (Figure 10)

Fig. 10
Kaplan Meier survival curve for RAEB/RAEB-t patients who received intensive chemotherapy.



62 patients received small dose chemotherapy (SDCT) as follows: 29 (7.4%) with RA/RARS, 19 (4.9%) RAEB/RAEB-t and 14 (3.6%) with CMML. The difference between the median survival for LDCT versus non-LDCT is statistically significant (p=0.0043). (Figure 11)

Fig. 11.
Kaplan Meier survival curve for patients who received LDCT.



The difference between median survival for vitamin B6 and folic acid treatment versus non vitamin B6 and folic acid therapy was not statistically significant (p=0.291).

Discussions

We analyzed the median survival and the AML transformation using: hemoglobin, absolute number of neutrophils, platelets, number of cytopenias, bone marrow blasts percentages and the survival impact of the different treatment courses applied.

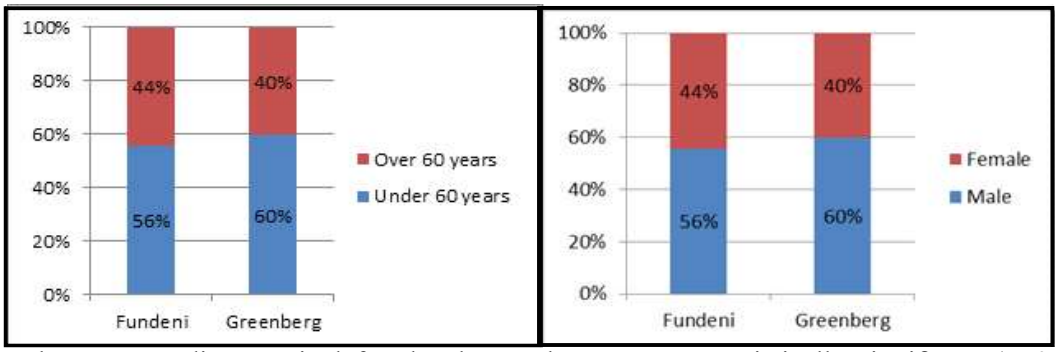
The median age was about 62.5 years (range 17 - 90 years), with 68.3% of patients being over 60. The median age difference was not statistically significant for survival (p> 0.05) and had no impact on AML transformation. In a study conducted on 816 patients by Greenberg et. al. (9) the mean age was about 69 years, with 75% being over 60 years. For this group the age is an important variable for survival, but not for AML transformation. The median survival for the under 60 subgroup was 4,6 years, with a time to 25 % AML transformation of 2,6 years and 2,5 years for the over 60 subgroup, with a time to 25 % AML transformation of 3,2 years. The median survival difference was not statistically significant (p> 0.05).

Regarding the gender distribution, there was a slight predominance in males (55.6%), with a sex ratio (M:F) of 1,25. No statistical significance was found for the median survival difference based on gender (p>0.05). For the study mentioned above, 40% were females and 60 % were males with a M:F= 1,5:1. (9) In figure nr. 12 we presented patients distribution based on

age (A), and gender (B) for the two groups.

Figure nr. 12 .

Patients distribution based on age (A), and gender (B) for the two groups. The FAB classification for this study was as follows in the table 1.



The difference between median survival for the three subgroups was statistically significant ($p<0.001$) the RA/RARS being highest but lower in comparison with other studies (9). Greenberg et. al. describe the following distribution of the FAB classification for their group: RA 25%, RARS 15%, RAEB 35%, RAEB-t 15% and CMML 10%. [9]

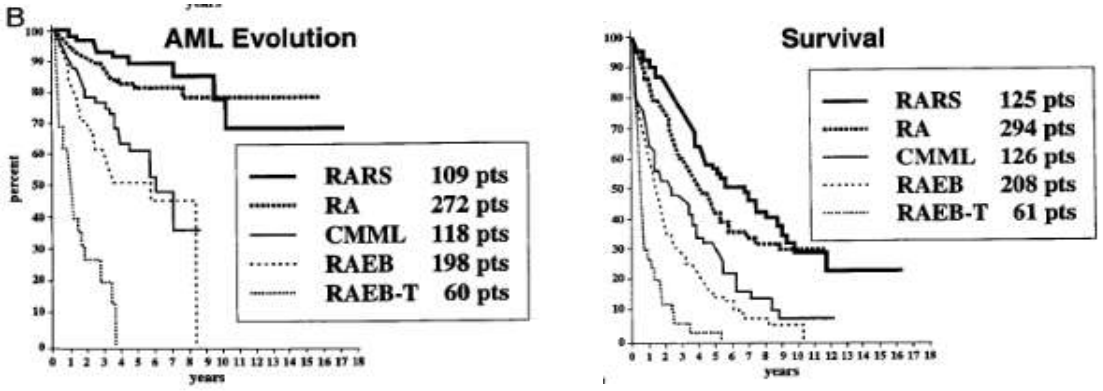
The median survival and the AML transformation rate are shown in table 6. For the RA/RARS the median survival is between 43-73 months, and RAEB/RAEB-t is between 5-12 months. (Table 6, figure 13 A and B).

Table 6

	RA	RARS	RAEB	RAEB-t	CMML
Median survival (months)	43	73	12	5	20
AML transformation-%	15%	5%	40%	50%	35%
FAB classification- %	25%	15%	35%	15%	10%

Patients distribution according to FAB classification, median survival and AML transformation.

Figure nr. 13.



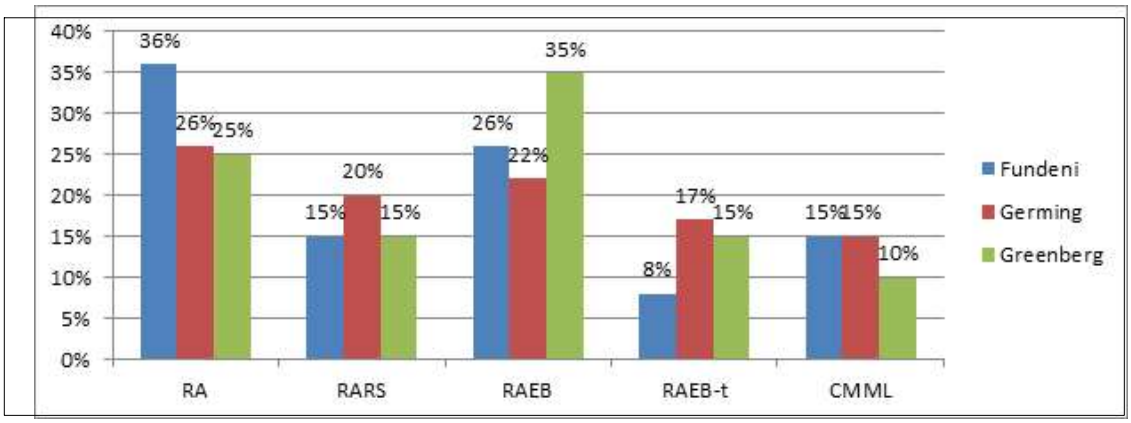
The AML evolution and the Kaplan Meier survival curve according to the FAB classification. [9]

The RARS patients have a less favorable prognosis than RA patients. Several authors describe a higher survival for RA and RARS than for RAEB/RAEB-t. RA has a higher AML transformation rate (14). Aul et. al. describe the following for the SMD registry from Dusseldorf (1600 patients) according to the FAB classification: RA 26%, RARS 20%, RAEB 22%, RAEB-t 17%, CMML 15% (15). AML transformation rate is lower for RA/RARS with unilinear dysplasia then for RA/RARS with multilinear dysplasia and AREB. The median survival for the RA/RARS with unilinear dysplasia was about 69 months and for RA/RARS with multilinear dysplasia was about 33 months. [16]

Figure nr. 14

MDS patient's distribution according to the FAB classification for 3 groups (Fundeni, Greenberg, Germing). [2,9]

Median survival decreased with the increasing number of cytopenias, ($p < 0.05$). Figure no. 15 A and B presents the Kaplan-Meier survival curve depending on the number of cytopenias for Fundeni and Greenberg groups. Greenberg et.



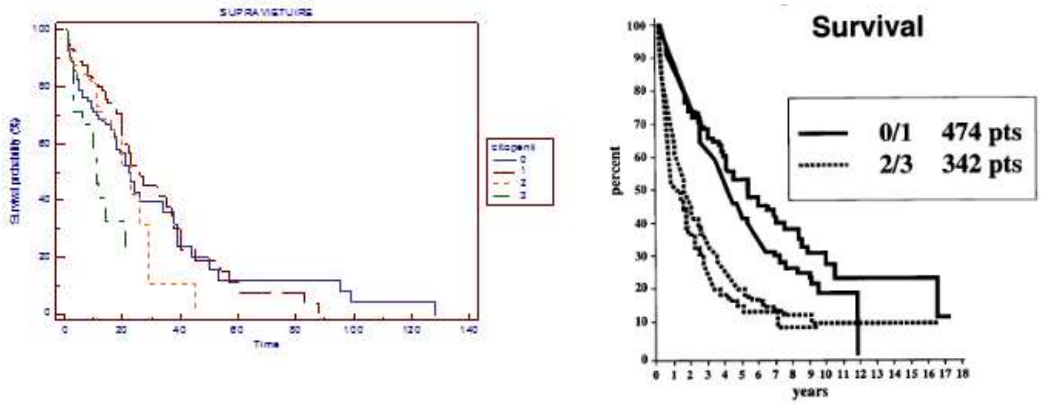
al. also found a survival decreases with the number of cytopenias. (Table nr. 7)

Figure. nr.15.

Kaplan-Meier survival curve according to the number of cytopenias for the Fundeni group (A) compared with the Greenberg group (B).

Table nr. 7.

Median survival and risk of transformation to AML based on the number of cytopenias, according to Greenberg. [9]



In the present study, an increased number of patients with no cytopenias and a decreased number with 3 cytopenias was observed, by comparison with the Greenberg study. Also, median survival was lower for all types of cytopenias

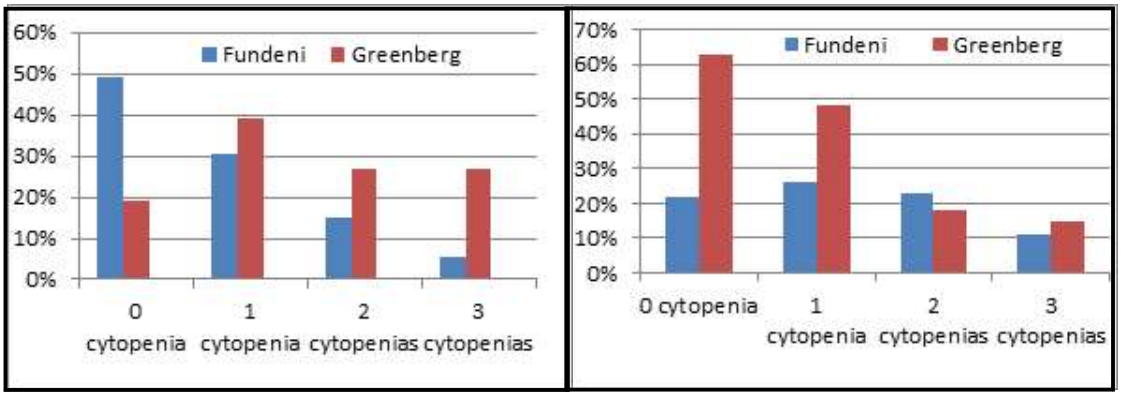
No. of cytopenias	%	Median survival (years)	25% AML (years)
0	19	5,3 (63 months)	7,6
1	39	4 (48 months)	5,6
2	27	1,6 (18 months)	1,6
3	15	1,3 (15 months)	1,3

compared to the above mentioned group (figure nr. 16).

Figure nr.16.

Kaplan Meier survival curve according to the number of cytopenias (left side), and median survival (right side) for the

two study groups. [9]



Of the 392 patients, 51.3% had Hb below 8 g/dl. Hb value did not significantly influence the survival (p> 0.05).

In the present study an increased number of patients with neutrophils count over 0,8 $x10^9$ / l was observed (82.1%) and they had a significantly higher median survival than those with neutrophils counts below 0,8$x10^9$ /l, p <0.05. (Figure nr. 3)

Considering platelet count, patients with a value below 50$x10^9$ /l (25.8%) had a median survival significantly lower than those with platelet counts above 50$x10^9$ /l, p <0.05. (Figure nr. 4)

If Hb, platelet and neutrophile values are taken into consideration as risk factors acting simultaneously, it appears that neutropenia and thrombocytopenia significantly influences the occurrence of death (p = 0.00438 and 0.0338 respectively, while for Hb, p = 0.2685). (Table nr.2)

The percentage of patients with bone marrow (BM) blasts below 5% (61.7%) was greater and they had significantly higher median survival than those with BM blasts over 5% (p<0.0001). (Figure nr.5) Survival analysis based on the percentage of BM blasts and number of cytopenias has shown no statistically significant differences for patients with less and more than 5% BM blasts as the number of cytopenias increases, p > 0.05.

Risk of transformation to AML according to FAB classification is about 5.6% for RA and RARS, and for those with RAEB, RAEB-t and CMML about 12.1%. The latter have a 3 times higher risk of transformation to AML, with a CI 95%, 1.5-6.1 (Table nr. 3).

The risk of transformation to AML for the 242 patients with less than 5% BM blasts was 6.6%, while for those with more than 5% BM blasts was 14.6%. The relative risk of transformation to AL based on the BM blasts was 2.4 (CI 95% 1.2-4.7), p <0.05 (Table nr. 4).

Regarding the no cytopenias patients who transformed to AML, their percentage was significantly lower than for those with one or more cytopenias, p <0.05. The percentage of those with transformation to AML is not significantly different from one age group to another.

Considering the treatment options the majority (95.2%) received treatment: 62.7% transfusions, 52.5% immunosuppressive therapy (corticosteroids, cyclosporine), 72.2% vitamin B6 and folic acid, 15.8% SDCT and 4.3% ICT. Patient's distribution according to received therapy and FAB subtype is presented in table nr.5.

62.7% of patients were transfusion dependent and their median survival was low compared to transfusion independent patients, (p <0.0001). Statistically, this difference is highly significant and maintains for the following FAB groups: RA, RARS, RAEB, RAEB-t (p<0,0001). Several other studies have also shown that transfusion dependency is associated with significantly reduced survival. [17,18]

15.8% received SDCT: 7.4% had RA / RARS, 4.9% had RAEB/ RAEB-t and 3.6% had CMML. Patients received low-dose Cytosar (20 mg /day), Purinthol, Lanvis or Hydreea. Their median survival was lower than the one of those who did not received SDCT, p <0.05. For the Dusseldorf registry the patients who received SDCT (Cytosar 20mg / day), didn't record a statistically significant difference concerning the median survival, by comparison to those who received supportive care (p = 0.05348). [19]

ICT was received by 4.3% as follows: 0.3% RA/RARS, 3.6% RAEB/ RAEB-t and 0.5% CMML. The treatment consisted of at least a course with an anthracycline and Cytosar +/- Etoposide. A significant survival difference was not recorded for these patients, p> 0.05. Concerning the MDS registry from Dusseldorf, 261 patients received intensive chemotherapy, usually 1 or 2 cycles with Cytosar and an anthracycline. The

difference between median survival values was not statistically significant (p = 0.36). The difference in median survival was also not statistically significant in any age group below the age of 60 years, nor over, for SDCT, ICT compared to supportive treatment. (19) The same can be said for the patients who received immunosuppressive therapy or vitamin B6 and folic acid (p>0.05).

Conclusions:

FAB classification of myelodysplastic syndromes is the first classification that established clear criteria for this condition, is accessible, has been widely applied and is still used. This study was conducted on a large group of patients (392). The results showed that median survival values ware significantly decreased because of the following hematological parameters: FAB subtypes (RAEB/ RAEB-t/CMML (p <0.0001), increasing number of cytopenias (p <0.05), neutropenia and severe thrombocytopenia, (p<0.0001).

Survival was not significantly influenced by the following parameters (p> 0.05): gender, age (below and over the age of 60 years) and Hb values. The risk of transformation to AML was significantly increased, p <0.05, by: subtype RAEB, RAEB-t and CMML compared to RA and RARS, more than 5% BM blasts, compared to below 5%, increase number of cytopenias versus no cytopenias.

The percentages of AML transformation were not significantly influenced by age and gender. The transfusion dependence decreased survival significantly (p <0.0001). This difference remained statistically highly significant in all FAB groups (p <0.0001). SDCT, ICT, immunosuppressive treatment (corticosteroids and cyclosporine) had no statistically significant influence over survival p> 0.05. The only treatment with curative intention, for these patients, is stem cell allotransplant.

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Acute Leukemia in adult patients with Down Syndrome : single center experience

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Abstract

Down syndrome (DS) and acute leukemia in the pediatric population is a well documented association. Acute myeloid leukemia in DS patients is related to a mutation in the GATA1 gene, coding a transcriptional factor with role in the erythroid and megakaryocytic development. Acute lymphoblastic leukemia in DS patients is considered as having a poorer outcome compared to non-DS children. Acute lymphoblastic leukemia in DS population involves mutations in the CRLF2 gene (cytokine receptor like factor 2 gene) with cell proliferation through STAT and JAK2 signaling pathways activation.

DS patients tend to have greater chemotherapy related mortality and morbidity. In adult DS population, there are little informations as to what the best course is in those patients. There are no or little data in adult DS population as what is the risk for developing leukemia past the age of twenty or if the biological entities seen in children with DS are still the same in the adult DS patients.

Introduction

Acute leukemia in pediatric DS population is a well studied hematological entity [1,2], most cases occurring in early childhood (predominantly acute myeloid leukemia) till late teenage years (especially acute lymphoblastic leukemia). [2] The pathology of these biological entities is related to the presence of mutations such as JAK2-IL7R-STAT for ALL and GATA1 mutation for AML.[3] In pediatric population, the most common form of leukemia is ALL. In DS population under the age of 4 year, most children develop AML and particularly acute megakaryobastic leukemia (AMkL). A particular form of AMkL is described in this group and is associated with a good prognosis and response rate compared to non DS AML.

Transient abnormal myelopoiesis (TAM) is a unique hematological entity related to DS patients and is characterized by the presence of megakarioblasts in blood or bone marrow or both. TAM patients can present with various degrees of leukocytosis, thrombocytopenia, anemia, hepatomegaly and splenomegaly. It is estimated that TAM occurs in 10% of newborn DS population. TAM`s megakaryoblasts have the same morphology and surface antigen expression as AMkL cells.[4] The defining feature of TAM is the spontaneous clinical regression in a high proportion of cases with supportive care alone without the use of chemotherapy.[4] However, 20% of the DS children with a previous history of TAM will later develop AMkL, usually within the first 4 years of life and it is often preceded by a myelodysplastic (MDS) phase. [4]

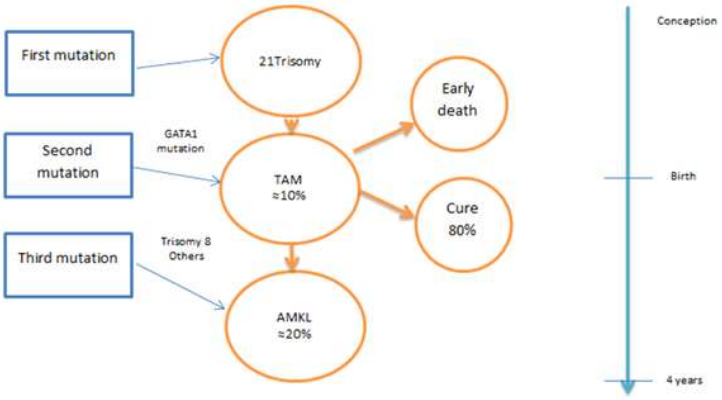


Figure 1. *The proposed pathological events behind appearance of TAM and AMKL in DS is a three hit mutation theory. The first event is the acquiring of the supernumerary 21 chromosome fallowed by GATA1 gene mutation in intrauterine life and early life. At birth up to 10% of newborns with DS have TAM and the majority of these patients will have a spontaneous remission of TAM. Only 20% of TAM cases will undergo transformation to AMKL, a transformation caused by a third mutation mechanism. The hematological events seen after 4 years of life in DS patients usually have other mechanisms and do not involve GATA1 gene.*

In MDS phase preceding the AML in DS population, there is evidence of mielodysplastic changes in the bone marrow: dyserythropoiesis and macrocytosis, dysmegakaryocytopoiesis, myelofibrosis and percentage of bone marrow blasts below 20%. The blood count shows progressive anemia and thrombocytopenia.

The most common form of AML in DS children is AMkL which occurs during the first 4 years of life and prognosis is better than children of the same age group with non DS myeloid neoplasms. After age of 4, prognosis of acute myeloid leukemia in DS patients is worse compared with non-DS AML [1]. Other subtypes of AML are: M0, M1, M2 and M6 [6] but rarely seen. The DS AML cytogenetic abnormalities differ from non DS patients with AML and frequently trisomies 8, 11 and 21, dup(1p), del(6q), del(7p), dup(7q), and del(16q). [9]

In most cases of TAM and AMkL in DS population, there is a somatic mutation involving the GATA1 gene.[5] GATA1 gene product is a transcription factor of the zinc finger family with a role in megakaryocytic differentiation and also erythroid differentiation regulation.

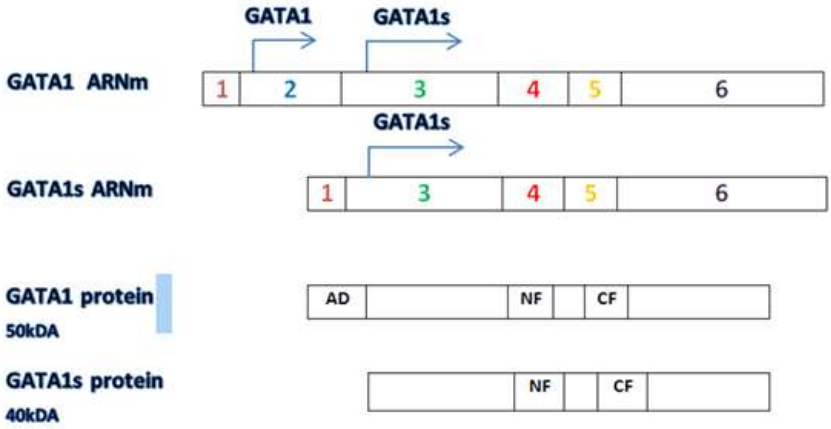


Figure 2. *GATA1 gene consisting of 6 exons numbered from 1 to 6 as represented here in form of messenger RNA. This picture explains the generation of the two GATA1 protein isoforms. The full GATA1 isoform (50kDA) is translated from the full GATA1 mRNA whereas GATA1s (40kDA) can be translated from the full GATA1 mRNA or from the shorter version of mRNA where the exon 2 is excluded. There are two zing finger domains on each protein with roles in protein and DNA binding.*

Its location is on chromosome Xp11.23 and is involved in X-linked genetic disorders with mutations in this region of the X chromosome are associated with thrombocytemia and/ or anemia but not with a higher risk of developing leukemia. [6]

The biological result of the mutation involving GATA1 gene is a shorter protein which through its activation properties contributes to a proliferation advantage of immature megakaryocites. [3,5]

Although a direct link between the presence of constitutional trisomy 21 and the development of GATA1 mutation wasn't established, it is suggested that genes present on chromosome 21, mainly cystathionine-β-syntethase and zinc copper superoxide dismutase, are involved in the DNA damage that happens in DS through an increased oxidative stress. [3,7]

CRLF2 (cytokine receptor-like factor 2) together with the alpha subunit of the interleukin 7 receptor forms a correspondent receptor for the cytokine thymic stromal lymphopoietin. This receptor is found on cells involved in inflammatory and allergic responses, mainly T cell lymphocytes and basophiles. Through mutation involving the CRLF2 receptor, leukemic cells gain a proliferation and survival advantage.

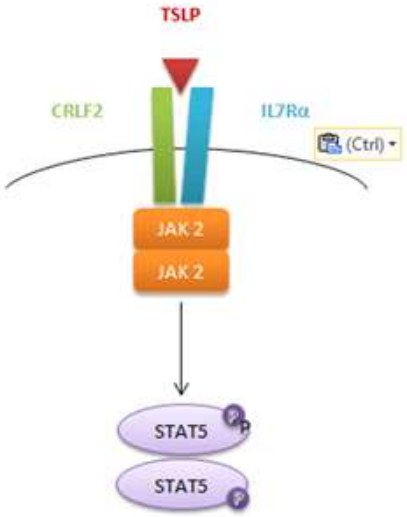


Figure 3. *JAK2/ STAT signaling activation through CRLF2/IL7α receptor. The target for thymic stromal lymphopoietin is the transmembrane receptor CRLF2/IL7α found on many inflammatory cells. Mutation in the CRLF2 or IL7α can result in an activation of the JAK2/STAT pathway independent of TSLP thus leading to uncontrolled proliferation of leukemic cells.*

In pediatric DS ALL population, there are quantitative or qualitative chromosomal abnormalities involving chromosome 21. Hyperdiploidy with multiple copies of chromosome 21 and TEL-AML1 [t(12;21)(p13;q22)] are frequently seen in pediatric ALL. [6,12] Another frequent translocation seen in DS ALL is t(8;14) while other unfavorable cytogenetic aberrations are rarely seen. T cell ALL is rarely seen in DS. [6]
In this article, we present two acute leukemia cases in patients with DS.

Case 1: Adult DS ALL

A 22 years old DS patient was admitted to our ward after been seen in outpatient department for vertebral pain and fever. At clinical exam, the patient presented severe pallor, no enlarged lymph nodes but liver and spleen enlargement were noted.

Lab tests showed: Hb=6.7g/dl, Wbc= 5500/mm3 and Plt=25000/mm3. The differential count revealed blasts= 28%, promyelocytes= 1%, neutrophils=20% , lymphocytes= 49% and monocytes= 2%.

The bone marrow exam (aspirate and trephine) showed 98% bone marrow blasts with a morphological appearance of lymphoblasts.

The peripheral blood immunophenotype exam analyzed 7000 events using FACS Calibur flow-cytometry technique and CellQuest software and showed a blast population which expressed CD19, HLA-DR, CD38, cyCD79a, cyIgM, CD123, CD22, CD10, CD 24 and weak CD58 and CD34.

A fresh bone marrow (BM) sample was obtained and standard cytogenetic technique was performed. The bone marrow sample was processed using overnight and synchronized cultures and conventional cytogenetic procedures with GTG banding. At least 20 metaphases were analyzed and the karyotype was described according to International System for Human Cytogenetic Nomenclature (ISCN) recommendations and showed presence of trisomy 21.

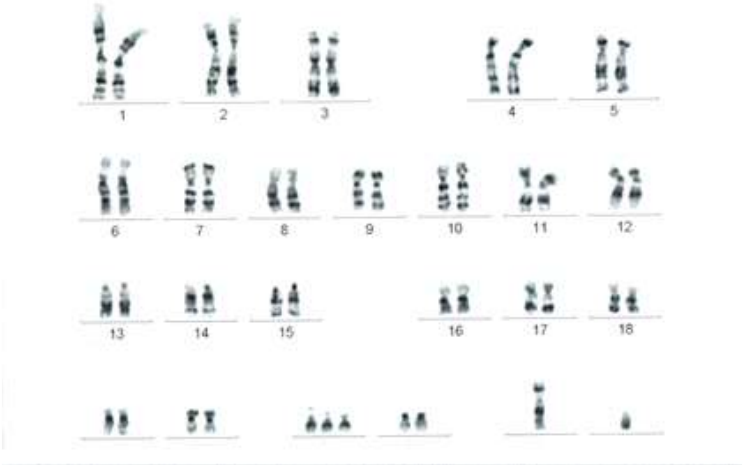


Figure 4.
Conventional cytogenetic exam with normal GTG banding showing trisomy 21

Qualitative Real Time PCR analysis for molecular testing was performed using LightCycler™ platform and did not detect any abnormal transcripts of the following genes: E2A-PBX1[t(1;19)(q23;p13)]; MLL-AF4 [t(4;11)(q21;q23)]; BCR-ABL p190 [t(9;22)(q34;q11)]; BCR-ABL p210 [t(9;22)(q34;q11)]; TEL-AML1[t(12;21)(p13;q22)]; SIL-TAL1 [del(1)(p32;p32)].

The patient was diagnosed with pre B- cell ALL and treated according to GMALL protocol with reduced Methotrexate dose. After first induction course, the patient achieved complete remission. The chemotherapy course was complicated with frequent and prolonged infections. During maintenance therapy, Methotrexate and 6-Mercaptopurine doses were reduced due to infections and liver dysfunction. At the moment, the patient is in complete remission despite reduced intensity chemotherapy and during second year of maintenance treatment.

In the pediatric population, there are numerous studies that account for the increase in toxicity of chemotherapy in DS-ALL subgroup. Infections and mucositis are more frequent and more severe in DS-ALL. [11] Hyperglycemia tends to occur in most of these patients and is directly linked to an increased risk for developing serious infections. Treatment with corticosteroids also is associated with a risk for developing severe infections.

Although the impaired metabolism for certain cytotoxic drugs is well documented, an example being the altered metabolism of Methotrexate leading to severe mucositis, this is not the only causal factor for treatment related mortality and morbidity in those patients. There are other factors contributing to life threatening infections such as congenital heart defects associated with DS, intrinsic immunodeficiency and anomalies in the respiratory airways. [11]

Several studies conducted in children with DS-ALL population have shown that most physicians do not adhere to the specific protocol dosages for cytostatic drugs when it comes to these patients. In general, there are reductions in dosages of Methotrexate either being in the consolidation blocks or during maintenance period. Also 6-Mercaptopurine tends to be administrated at a lower dosage and there are pediatric groups that avoid using anthracyclines. [11,13]

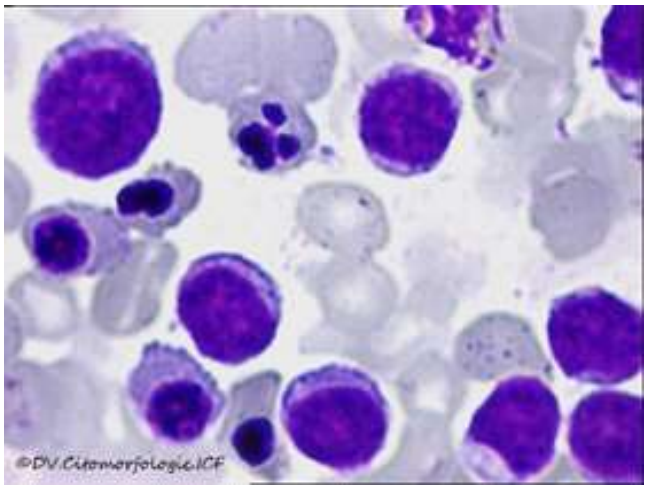
As for the adult DS ALL population, due to rarity of cases, there is little or no consensus as to what is the appropriate way to treat. The insight gained from the pediatric population should be relevant for the adult population.

In our patient, treatment related severe complications and frequent severe infections during neutropenia periods were seen mainly due to Methotrexate toxicity.

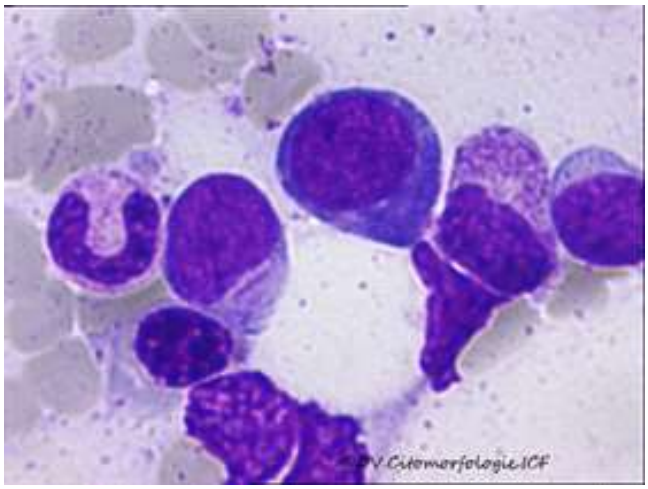
Case 2: Adult DS AML

A 32- years old DS patient was referred to our department for ongoing spontaneous bruising and gingivorrhagia. The clinical examination revealed DS morphological stigmata, medium pallor, disseminated bruises and petechiaes, gingivitis and gingivorrhagia.
The blood count showed Hb= 8.4g/dl, Ht= 28.1%, MCV=109 fl, Wbc= 93400/mm^3 and Plt= 93000/mm3. The differential count revealed blasts= 80%, promyelocytes= 1%, myelocytes= 2%, metamielocytes= 1%, bands= 3%, neutrophils= 10% and lymphocytes= 3%.

The bone marrow exam (aspirate and trephine) showed the presence of 66-67% myeloblasts with secondary dyserythropoiesis.

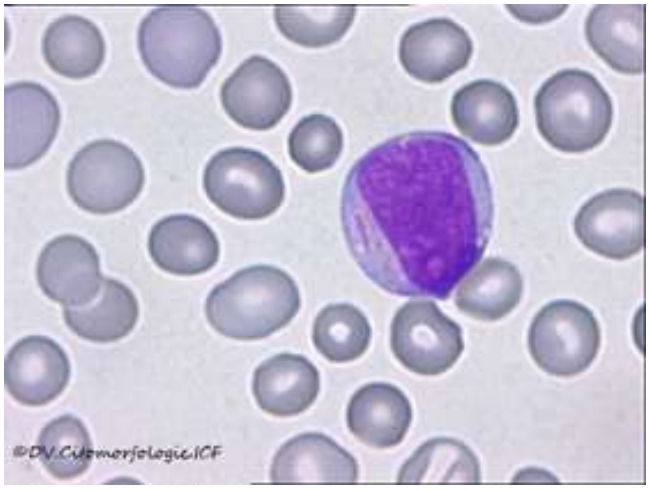


A)

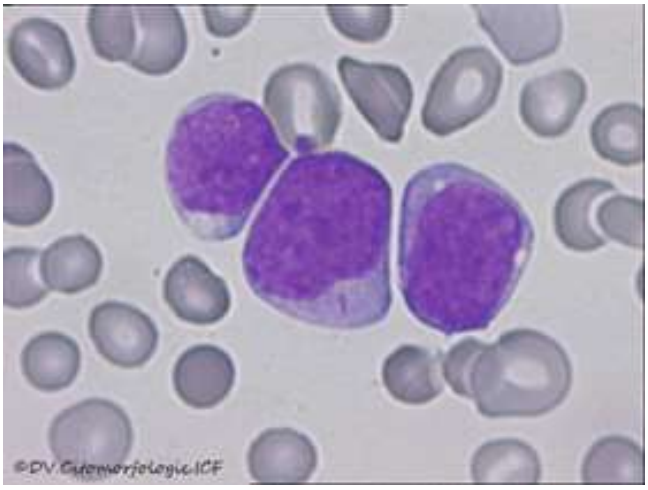


B)

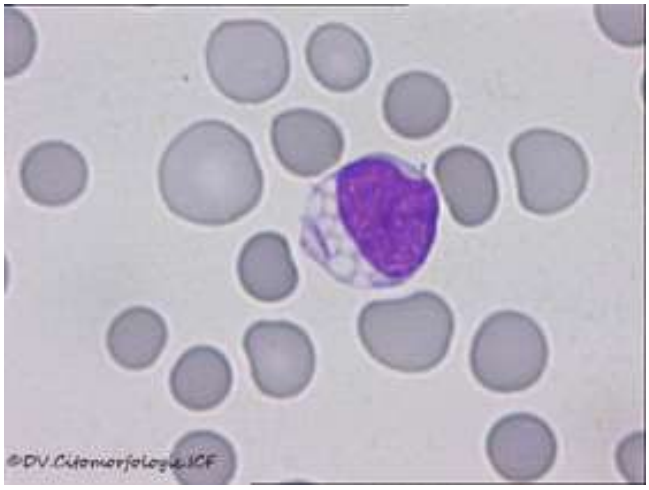
Figure 5.



C)



D)



E)

Figure 5. *A) Bone marrow aspirate smear showing dysplastic erythroblasts. B) Bone marrow aspirate smear: a myeloblast with an Auer body near the center of the field, also showing erythroblasts and neutrophils. C, D) Peripheral blood smear showing several myeloblasts in the center of the field, some with Auer bodies. E) Vacuolated myeloblast with an Auer body in the peripheral blood smear.*

The peripheral blood immunophenotype exam analyzed 7000 events using FACS Calibur flow-cytometry technique and CellQuest software showing a population of immature myeloid cells positive for cyMPO, CD117, HLA-DR, CD34, CD123, CD38 with weak expression of CD13 and Cd33.

A bone marrow (BM) sample was obtained and standard cytogenetic technique was performed. The bone marrow sample was processed using overnight and synchronized cultures and conventional cytogenetic procedures with GTG banding. At least 20 metaphases were analyzed and the karyotype was described according to International System for Human Cytogenetic Nomenclature (ISCN) recommendations and showed presence of trisomy 21.

Qualitative Real Time PCR analysis for molecular testing was performed using LightCyclerTM platform and did not detect any abnormal transcripts of the following genes: E2A-PBX1[t(1;19)(q23;p13)]; MLL-AF4 [t(4;11)(q21;q23)]; BCR-ABL p190 [t(9;22)(q34;q11)]; BCR-ABL p210[t(9;22)(q34;q11)]; TEL-AML1[t(12;21)(p13;q22)]; SIL-TAL1 [del(1)(p32;p32)].

The patient was diagnosed with type M0 of AML according to FAB classification and started on reduced dose intensity induction treatment consisting of Dounorubicin 25mg/m^2 on days 1-2, Cytarabine 100mg/m2 on days 1-7, Etoposide 100mg/ m^2 on days 1-3. The pancytopenia period was without major complications but patient did not achieve complete remission. The patient received another induction treatment with achievement of complete remission followed by consolidation treatment with Cytarabine 1.5g/m^2 on days 1, 3 and 5. The bone marrow exam showed maintenance of complete remission.

There are various studies regarding treatment mortality in children DS AML showing that risk of fatal complications regarding toxicity should be balanced with intensity of chemotherapy treatment. [1,6] There are

reports which suggest that the metabolism pathway for Cytarabine in the AML blasts is altered leading to a higher concentration of intracellular Cytarabine metabolites.

Compared to in vitro sensitivity of AML blasts, the sensitivity to chemotherapy of ALL blasts seems to be identical to ALL blasts from the non-DS population. [15] There are genes located on chromosome 21 that have an impact on chemotherapy sensitivity such as cystathionine-β-synthase gene (21q22.3) that is involved in the folate and nucleoside metabolism. The overexpression of this gene accounts for the altered metabolic pathway of nucleosides leading to increased sensibility to Cytarabine. [16] Other genes found are Bcl-2 and Hsp-70 with roles in cell apoptosis.

There seems to be a correlation between the transcript of GATA1 gene and the increased sensitivity of AML blasts and this is mainly postulated around the cytidinedeaminase which deaminates cytarabine to the inactive metabolite, uridinearabinoside. The transcript of GATA1 gene seems to bind to the promoter region of the cytidinedeaminase gene resulting in decreased expression of the gene and subsequent reduced metabolisation of Cytarabine. [6,17]

As for adult population of DS-AML, there no indication of what is the OS and EFS due to the rarity of these patients. Those patients have a general OS and EFS as to non-DS adult AML population if not worse due to increased treatment toxicities and Down syndrome cardiac and pulmonary morbidities.

Conclusions

There are no clinical trials available regarding AML and ALL in the adult patients with DS due to the fact that this population is very rare. In our two cases either being AML or ALL, reduced dose dose chemotherapy protocols were used and both patients achieved and maintained complete remission.

Acknowledgment: This work was supported by the grant PN 41-087/2007 from the Romanian Ministry of Research and Technology. The authors express the gratitude to European LeukemiaNet for their permanent support.
Disclosures: none

List of abbreviations:

DS- Down syndrome
GATA1- Globin Transcription Factor 1
CRLF2 -Cytokine receptor like factor 2
STAT- Signal Transducer and Activator of Transcription
JAK2- Janus kinase 2
IL7R- Interleukin 7 receptor
ALL- Acute lymphoblastic leukemia
AML- Acute myeloid leukemia
MDS- Myelodysplastic syndrome
AMkL- Acute megakaryobastic leukemia
TAM - Transient abnormal myelopoiesis
AD- activation domain
NF- N terminal zinc finger domain
CF- C terminal zinc finger domain
mRNA- messenger RNA
TSLP- Thymic stromal lymphopoietin
CRLF2- Cytokine receptor-like factor 2
IL7α- Interleukin-7 receptor alpha

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The Council of Europe recommendations

Alina Mirella Dobrotă

MS Expert
MS Reprezentant in CD-P-TS and GTS

Founded in 1949, the Council of Europe is an European institution gathering 47 Member States. Incresing cooperation between its members to improve the quality of life is one of its founding principles. Romania is an active member of the Council of Europe.

In this respect, health is one of the main field for action. Ethical issues like comercialisation of human substances, ie blood, organs and tissues are in the core of its policy and strategic actions.

The work of the CoE in the blood transfusion field has started in 1950". Two committees are working on technical aspects related to blood transfusion, clinical use of blood and plasma derivatives, ethical aspects of blood donation, protecion of both blood donors and patients:

1. the European Committee on Blood Transfusion (Steering Committee) (CD-P-TS)
2. the Committee on Quality Assurance in Blood Transfusion Services (Expert Committee) (GTS)

Since 1950, CoE has elaborated a serie of agreements and recommendations covering ethical, scientific and training aspects of common interest and /or concern for all its members. Recommendations are policy statements to governments proposing a common agreed direction of action to be followed in a particular health area.

We introduce to the lecturer the last two recommendations adopted,focused on haemophilia treatment and the use of imunoglobulin.

Aditional information on the whole package of the recommendations adopted since 1950 may be found to te following link:

http://www.edqm.eu/en/blood-transfusion-recommendations-resolutions-71.html

COMMITTEE OF MINISTERS
COMITÉ DES MINISTRES

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

Resolution CM/Res(2015) 2
on principles concerning human normal immunoglobulin therapies for immunodeficiency and other diseases

(Adopted by the Committee of Ministers on 15 April 2015
at the 1225th meeting of the Ministers' Deputies)

The Committee of Ministers, in its composition restricted to the representatives of the States Parties to the Convention on the Elaboration of a European Pharmacopoeia (ETS No. 50);[1]
Considering that the aim of the Council of Europe is to achieve greater unity between its member States and that this aim may be pursued, *inter alia*, by the adoption of common action in the health field;
Having regard to the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETS No.164), and in particular to Article 3, Chapter I – General provisions – of the Convention;
Recalling Recommendations Rec(90)9 on plasma products and European self-sufficiency and Rec(93)4 concerning clinical trials involving the use of components and fractionated products derived from human blood or plasma;
Having regard to Recommendation Rec(95)15 on the preparation, use and quality assurance of blood components and its Appendix, the Guide to preparation, use and quality assurance of blood components (17th Edition 2013);
Having regard to Recommendation Rec(2002)11 on the hospital's and clinician's role in the optimal use of blood and blood products;
Having regard to Recommendation Rec(96)11 on documentation and record-keeping to guarantee the traceability of blood and blood products, especially in hospitals;
Taking into account the recommendations of the European symposium on optimal use of clotting factors and immunoglobulins organised under the auspices of the European Committee on Blood Transfusion (CD-P-TS) of the Council of Europe (26-27 April 2013, Wildbad Kreuth, Germany);[2,3]
Considering that the demand for plasma-derived medicinal products has continuously increased in the past 20 years; however, the consumption per capita varies greatly from country to country;
Considering that the demand for human normal immunoglobulin preparations will keep increasing mainly due to new indications and emerging markets;
Considering that availability of human normal immunoglobulin therapies (and in some cases adequate doses of immunoglobulin) are not equitable across Europe, and that some patients are experiencing significant harm and reduced life expectancy because of this;
Taking into account the fact that, in light of the experience acquired in the implementation of its recommendations set out in the appendix to the present resolution, that appendix may be updated by the European Committee on Blood Transfusion (Partial Agreement) (CD-P-TS) five years after its adoption or sooner if new developments, insights or data so require,
Recommends that governments of States Parties to the Convention take appropriate measures to step up the promotion of the principles contained in the appendix to this resolution.

[1]Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, "the former Yugoslav Republic of Macedonia", Turkey, Ukraine and United Kingdom.
[2]Optimal use of clotting factors and immunoglobulins, European symposium proceedings, 26-27 April 2013, Wildbad Kreuth Germany, available on http://www.edqm.eu/en/proceedings-international-conference-83.html
[3]Sewell WC, Kerr J, Behr-Gross ME, Peter HH: European consensus proposal for immunoglobulin therapies, *Eur J Immunol.2014 Jun* 28; doi: 10.1002/eji.201444700.
Internet : http://www.coe.int/cm

Appendix to Resolution CM/Res(2015)2

Principles

1. To acknowledge the status of "essential medicine" granted to human normal immunoglobulin by the World Health Organisation (WHO) and to ensure that all patients in need have access to this medicine in quantities sufficient to be clinically effective;
2. To adopt a suitable process, e.g. evidence-based human normal immunoglobulin demand management, in European countries to ensure adequate supplies for all patients in need, and to implement a strategy to assure supplies for obligate users4 for times of immunoglobulin shortages;
3. To make available to patients all recognised routes of human normal immunoglobulin administration;
4. To take into account that human normal immunoglobulin therapeutic products differ from one another in terms of production processes, which might have an impact on specifications and clinical performance;
5. To expand the basis of Health Technology Assessment (HTA) of human normal immunoglobulin therapies (e.g. to evaluate general and brand-specific efficacy of different immunoglobulin preparations for off-label uses) by considering disease-specific patient registries;
6. To promote research on the use of human normal immunoglobulin in the treatment of secondary immunodeficiencies;

[4]See "Guideline on core Summary of Product Characteristics (SmPC) for human normal immunoglobulin for intravenous administration (IVIg)", Committee for Medicinal Products for Human Use (CHMP), EMA/CHMP/BPWP/94038/2007, revision currently ongoing.

COMMITTEE OF MINISTERS
COMITÉ DES MINISTRES

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

Resolution CM/Res(2015)3
on principles concerning haemophilia therapies

(Adopted by the Committee of Ministers on 15 April 2015
at the 1225th meeting of the Ministers' Deputies)

The Committee of Ministers, in its composition restricted to the representatives of the States Parties to the Convention on the Elaboration of a European Pharmacopoeia (ETS No. 50); [1]
Considering that the aim of the Council of Europe is to achieve greater unity between its member States and that this aim may be pursued, *inter alia*, through common action in the health field;
Having regard to the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETS No. 164), and in particular to Article 3, Chapter I – General provisions – of the Convention;
Recalling Recommendations Rec(80)5 concerning blood products for the treatment of haemophiliacs, Rec(86)6 on guidelines for the preparation, quality control and use of fresh frozen plasma (FFP), Rec(90)9 on plasma products and European self-sufficiency and Rec(93)4 concerning clinical trials involving the use of components and fractionated products derived from human blood or plasma;
Having regard to Recommendation Rec(95)15 on the preparation, use and quality assurance of blood components and its appendix, the "Guide to the preparation, use and quality assurance of blood components" (17th Edition 2013);
Having regard to Recommendation Rec(2002)11 on the hospital's and clinician's role in the optimal use of blood and blood products;
Taking into account the recommendations of the European symposium on optimal use of clotting factors and immunoglobulins, organised under the auspices of the European Committee on Blood Transfusion (CD-P-TS) of the Council of Europe (26-27 April 2013, Wildbad Kreuth, Germany);[2,3]
Considering that great variability in patient care and availability of the different coagulation factor concentrates persists across member States and that the differences in per capita use of coagulation factor VIII are particularly striking;
Considering that, in addition to available plasma-derived and recombinant coagulation factors, several new and innovative products are in different stages of development;
Considering that haemophilia therapies (and in some cases adequate doses of coagulation factors) are not equally accessible across Europe, and that, as a result, some patients are experiencing significant harm and reduced life expectancy;
Taking into account the fact that, in light of the experience acquired in the implementation of its recommendations set out in the appendix to the present resolution, that appendix may be updated by the European Committee on Blood Transfusion (Partial Agreement) (CD-P-TS) of the Council of Europe five years after its adoption, or sooner if new developments, insights or data so require,
Recommends that the governments of States Parties to the Convention take appropriate measures to step up the promotion of the principles contained in the appendix to this resolution.

[1]Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Montenegro, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, "the former Yugoslav Republic of Macedonia", Turkey, Ukraine and United Kingdom.
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Internet : http://www.coe.int/cm

Appendix to Resolution CM/Res(2015)3

Principles

1. To optimise the organisation of haemophilia care, a system should be established in each member State to allow the implementation of a multidisciplinary approach for the treatment and care of patients (for example by setting up an advisory body including representatives of the relevant clinicians, national haemophilia bodies, patients' organisations, the health ministry, the paying authority, blood establishments and the regulatory authorities or by setting up centres of excellence);
2. In each member State, the coagulation factor VIII utilisation level should be at least 3 International Units (I.U.) per capita;
3. Decisions on whether to use a new or an alternative product should be based on evidence of safety and effectiveness and not solely on cost;
4. The evidence of the effectiveness of different treatment regimes should be strengthened. Prophylaxis is currently recognised as the optimum therapy for children with severe haemophilia. Ongoing prophylaxis for adults should be provided, when required based on a clinical decision by the clinician in consultation with the patient;
5. Prophylactic treatment with bypassing agents should be offered to haemophiliac children who have developed inhibitors and in whom immune tolerance induction therapy has failed or was unsuitable;
6. Single coagulation factor concentrates should be used as therapy wherever possible in patients with rare bleeding disorders.

IN MEMORIAM

Conferențiar Doctor

Andrei Cucuianu

Destinul a făcut să ne despărțim în 11 feb. 2015 de mentorul, susținătorul și prietenul nostru, Conferențiar Doctor Andrei Cucuianu. Noi, colegii și prietenii, hematologia, studenții, pacienții, toți îi datorăm foarte mult.

Ne amintim de energia lui deosebită, dăruirea și profesionalismul cu care aborda fiecare caz în parte, bucuria de a învinge boala și tristețea în caz de nereușită.

A fost un medic de excepție acordând bolnavilor săi maxim de atenție, omenie și, de multe ori prietenie. Aceasta relație specială se regăsește în bloggurile create de pacienți, precum și în zecile de mesaje de adio și regret trimise de aceștia sau familiile lor.

Cadru didactic remarcabil, cu un simț pedagogic deosebit, a transmis cu generozitate cunoștințe medicale studenților și medicilor rezidenți, care au avut șansa să se pregătească sub îndrumarea lui și care au devenit specialiști hematologi apreciați chiar și în clinici prestigioase din străintate. Și-a pus definitiv amprenta științifică și umană asupra noastră.

Va rămâne veșnic punct de reper prin conferințele susținute, zecile de articole și cărți publicate. Il regăsim în "Cartea de Hematologie Clinică", "Manualul de hematologie" pentru studenții de limba engleză, "Diagnozer hematologie" pentru studenți și rezidenții de hematologie, primul ghid de "Protocoale terapeutice" în hematologia clinică.

A fost apreciat și în plan internațional fiind investigator principal la numeroase studii clinice internaționale, multicentrice, și câștigătorul unui Grant International "Nanotechnology approach in acute myeloid leukemia chemotherapy – NanoLAM".

A fost apreciat și iubit de întreg personalul Clinicii Hematologie Cluj-Napoca. Găseai oricând sfaturi competente medicale sau organizatorice, soluții la numeroasele probleme care, de multe ori, păreau fără rezolvare. Față de colegi a dovedit întotdeauna corectitudine, echitate și susținere.

Valoarea lui profesională era recunoscută de hematologii din țară și străinatate.

Trebuie să ne amintim și de Andrei omul și, mai ales prietenul. Generos, corect, disponibil, un sprijin de nădejde. E doar o mică parte din ceea ce-l caracteriza ca om.

În timpul liber, a citit și, mai ales a scris (vezi: www. Youtube.com: "Merita citit cu Andrei Cucuianu"). Mintea lui strălucită, umorul și inspirația se regăsesc în istorioare în limba română sau într-o engleză perfectă, online, pe site-ul de cultură www.artactmagazine.ro.

Ne-a arătat că simțul datoriei este dovadă luminoasă a sănătății unui suflet.

Ne rămâne să încercăm să-i urmăm exemplul de profesionalism și umanitate.

"Nu e de ajuns ca o țară să dea talente, ea trebuie să știe să le țină vii" (Nicolae Iorga).

Colegii