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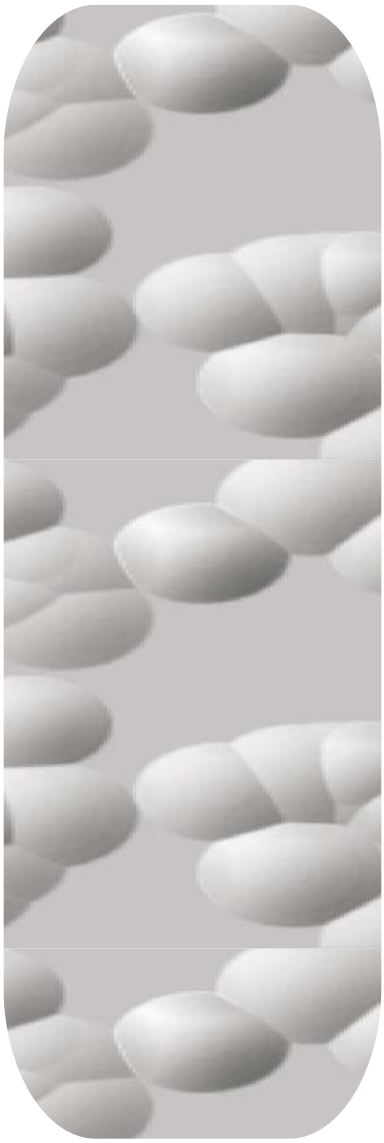

NATIONAL SOCIETY OF BLOOD TRANSFUSION FROM ROMANIA

Chronic Myelomonocytic Leukemia: classification and diagnosis

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Summary
Chronic myelomonocytic leukemia(CMML) is a heterogeneous group of disorders with features both of myelodysplasia and of myeloproliferation. The World Health Organization(WHO) classification places CMML into a new category called myelodysplastic/myeloproliferative disorders.CMML has been divided into two types, CMML type 1 and CMML type 2,based on the percentage of blast in the peripheral blood and bone marrow.
Key words :myelodysplastic syndrome,myeloproliferative syndrome,chronic myelomonocytic leukemia,WHO classification.

Introduction

Chronic myelomonocytic leukemia (CMML) represents a heterogeneous group of diseases with both dysplastic and myeloproliferative characteristics.

Classification

In 1976, French-American-British (FAB) introduced the terminology of "dismyelopoetic syndromes". There were two main types that were recognized: refractory anemia with blasts in excess and chronic myelomonocytic leukemia (CMML), that were distinguished based on the percentage of blast in the peripheral blood and bone marrow.

In 1982, the same group proposed new criteria for myelodysplastic disorders classification, by defining five types: refractory anemia (RA), refractory anemia with sidero blasts as rings, refractory anemia with blasts in excess, refractory anemia with blasts in excess in transformation and chronic myelomonocytic leukemia (CMML). FAB classification for myeloproliferative disorders, proposed in 1982, continues to be largely used in both clinical and research practice.

CMML is included in MPD and is distinguished through absolute monocitosis more than 1000/mmc, increase of the myelo monocitary marrow precursors and uni/multiliniar dysplasia. Blood blasts have not to be over 5% together with less than 20% marrow blasts. The controversy refers to the inclusion of CMML in MPD because of myeloproliferation appearance that indicates a relationship with chronic myeloid leukemia.

In 1994, FAB group proposed the division of CMML in two types according with leucocytes level: proliferative CMML (L>13ooo/mmc) and dysplastic CMML (L<13000/mmc). Several clinical studies that divide the patients according with group FAB criteria concluded there are no cytogenetic or biological differences in survival or progression towards acute myeloid leukemia between these two entities; actually, dysplastic CMML can develop proliferative CMML. Nevertheless, patients suffering from proliferative CMML have spleenomegalia.These are the reasons that WHO Committee decided not to divide CMML in these two subtypes.

WHO Group (1999) creates a new diagnostic category: myelodysplastic disorders/ myeloproliferative disorders with both dysplastic and proliferative characteristics that includes CMML, atypical chronic myeloid leukemia, juvenile myelomonocitary leukemia and unclassified MPD's. WHO classification does not bring important modifications in the diagnostic criteria for CMML comparing with criteria proposed by FAB group (see table I).

As a result, WHO divides CMML in three prognostic categories, according to the peripheral and marrow blasts number and associated increased number of eosinofilis (table II): CMML -1 associated with marrow blasts under 10% and under 5% in peripheral blood, CMML -2 with marrow blasts 10-19% and /or 5-19% peripheral blasts and CMML -1 or CMML-2 with increased number of eosinofiles.

A new classification system for pediatric MPD's (2003) according to the morphological alterations and according to the WHO classification for adults recognizes three groups: MDD/MPD, Down syndrome and MDD. The group MDD/MPD includes JMML, CMML (only secondary) and negative CML BCR ABL that is extremely rare.

The adjustments presented by Prof. Dr. Bennett in WHO classification (2008) of MDD/MPD refers to the inclusion of CMML with eosinophilia with rearrangement of PDGFRB gene in a separate chapter. This entity includes translocation t(5,12).

General Data

Clinical data

CMML is not a frequent disease. Lot of patients suffering from this disease are more than 50 years old. The disease's debut is generally insidious: fatigue, infection or bleeding brings the patient to the doctor. Hepatomegalia and spleenomegalia are usual present when diagnostic is identified.

Laboratory data

This disease is characterized by anemia and blood monocitosis over 1000/mmc. Anemia is usually normocitary, but it also can be macro citary or with dismorphic population. Monocites can be normal as morphology or having atypical alterations and increase of basophiles or cytoplasmic granulation. Leucocites number can be normal, moderate increased or easily decreased. The granulocytes' precursors can be present through blood, generally less than 5%. Thrombocites' number can be normal or decreased.

Generally, the marrow is hyper cellular as a result of granule monocitary hyperplasia with a variable degree of triliniar dysplasia. Marrow blasts do not overpass 205 from nucleates bone marrow cells.

From the cytochemical point of view, naphtol-ASD-acetat esterase coloration is useful for the identification of the monocitary precursors. Perls coloration can identify abnormal sideroblasts or increased iron deposits. MPO and Sudan black colorations can be applied for the cases with increased blasts number for confirming the line and for the exclusion of Auer corps. Polyclonal hypergamaglobulinemia and increase levels of blood and urinary lisosim are frequent.

In vitro studies demonstrated various increase models of CFU- GM colonies, starting with decrease of CFU-GM colonies with lots/colonies rapport increased typical to marrow to the spontaneous forming of the lots.

Molecular studies distinguish CMML particularities that make it comparable with CML. Ras gene anomalies have been founded in CMML and in CML Ph(-). Ras proteins are involved in increasing signals from outside cell to nucleus. There disturbances of signals' transmitting can be determined by mutations of Ras genes or through alterations of the activating proteins of Ras gene.

Several data analyzed by Lacronique and collaborators showed that phenotypical aberrations of the patients suffering from myelodisplazia, including CMML are CD 36 and CD 117 in granulocytes and CD 56 in monocytes.

Conclusion

Analysis of these hematological and clinical parameters altogether with information gathered from patients can help in clarify the position of these rare diseases.

Table I. Diagnosis criteria for CMML according to FAB (1982-1985):

1	blood and marrow monocitosis over 1000/mmc
2	erythrocytes" and/or granulocytes" and/or megakaryocytic' dysplasia
3	blood blasts under 5%
4	marrow blasts under 20% (initial under 30%)
5	absence of Auer corps in myeloid cells

Table II. Diagnosis criteria for CMML according WHO (1999 2000):

1	persistent blood monocitosis (over 1000/mmc)
2	absence of Ph chromosome or gene BCR - ABL
3	blood or marrow blasts less than 20%
4	dysplasia on one or more cell lines, or, if dysplasia is absent, CMML can be diagnosed together with one of the following: - presence of clonally cytogenetic anomaly - persistent monocitosis for the last three months and all monocitosis causes have been excluded (cancer, inflammation)

Table III. Subgroups of CMML:

1	CMML -1: peripheral blood blasts fewer than 5% and fewer than 10% in bone marrow
2	CMML -2: peripheral blood blasts 5 19%, 10-19% in bone marrow or presence of Auer corps and blasts fewer than 20% in peripheral blood and bone marrow.
3	CMML-1 or CMML-2 with eosinophilia: criteria CMML-1 or CMML-2 present with eosinophiles number at peripheral blood level over 1500/mmc CMML myelo-monocitary chronic leukemia - blasts include myeloblasts, monoblasts and promonocites.

Abbreviations:

CMML-chronic myelomonocytic leukemia
FAB- French American British group
WHO-World Health Organization
MDD- Myelodisplazic Disorder
MPD Myoproliferative Disorder
RA- Refractory Anemia
MAL Myeloid Acute Leukemia
ACML- Atypical Chronic Myeloid Leukemia
JMML Juvenile Myelo Monocitar leukemia
MPO- mieloperoxidasis

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Results of Splenectomy in Hodgkin's Lymphoma

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Summary
The place and the role of the splenectomy in Hodgkin's lymphoma was very well defined by the hematologists which have improved diagnostic methods and have standardised the indications for the splenectomy, preventing the results of spleen removal in some hematological diseases. The study refers to 65 patients with Hodgkin lymphoma in which we performed exploratory laparatomy with splenectomy, hepatic and lymph node biopsy. In 21 patients we performed splenectomy before therapy for diagnosis, staging or therapeutical purpose and in 44 patients we performed the splenectomy after therapy, in relapse, for restaging or for complications. Post splenectomy 25% of our patients had a different stage after splenectomy than before this procedure. The splenectomy in hematological diseases has an important role in diagnosis, staging and therapy.
Key words: splenectomy, Hodgkin's lymphoma

Introduction

Starting with strengthen of knowledge regarding spleen physiology and it' s functional role in human body, splenectomy's indications enlarged a lot, especially regarding hematological diseases. Hematologists' work improved and enlarged the spectrum of diagnosis methods, enlarged and standardized splenectomy's indications and can accurately anticipate the results of splenectomy in some hematological diseases.

Experience shows that, excepting some risks and contraindications pretty well defined, splenectomy has a very low rate of mortality.

The risks of the patients which due to lesions or spleen affection need surgical intervention depend on the individual particularities and cannot be precisely established based on existing statistics.

Mortality decrease after splenectomy is linked with knowledge improvement regarding the spleen disorders, establishing clear operatory indications and improvement of preoperative investigation methods combined with improvements of the pre- and post-operatory principles, good quality anesthesia, altogether with good surgical thinking and technique.

Purpose

We proposed to analyze indications and results of splenectomy in malign lymphomas, altogether with presentation of our conception regarding the place and role of splenectomy in Hodgkin's Lymphoma (HL).

Materials and method

We analyzed 120 patients with splenectomy during the last 20 years (1984-2004), splenectomies proceeded in Oncological and General Surgery Department of Municipal Hospital Timisoara and General Surgery and Emergency Surgery of County Hospital Timisoara. From the total 120 cases, 65 were diagnosed with Hodgkin's Lymphoma (HL) and 55 cases diagnosed with NonHodgkin's Lymphoma (NHL) located mainly at the spleen level.

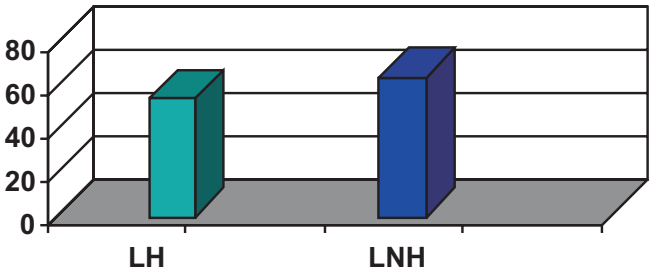

Table 1. ***Repartition according to diagnose in the studied arm***

Regarding gender repartition, we noticed predominance for female gender (72% female and 58% male).

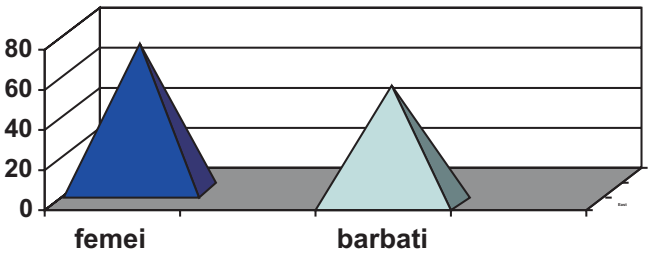

Table 2. ***Repartition according to gender in the studied arm***

Repartition according to age indicates a higher frequency for the ages between 20 and 35 years for malign lymphomas.

Acknowledging important limits that clinical and paraclinical investigations do have in determining precise expanding of the Hodgkin disease, Gladstein (1979) proposed as investigation method exploratory abdominal laparatomy associated with splenectomy as final intervention of an adequate pre therapeutic evaluation.

Laparatomy associated with splenectomy advantages in Hodgkin's Lymphoma (HL)

1. Determines a precise survey of disease's stage
2. Allows a better orientation for the radiation fascicle
3. Splenectomy eliminates the necessity for spleen irradiation
4. Patients' tolerance to radiotherapy and polichemotherapy is ameliorated
5. Early splenectomy prevents liver dissemination
6. Allows studying of the natural disease's history
7. Removes a real or future tumor.

Our study refers to 65 patients suffering from HL that were treated by exploratory and exploratory laparotomia with splenectomy, liver and ganglions' biopsy (according to specific case). The arm consists of 37 female and 23 males, aged between 17 and 45 years old; 21 have been treated by splenectomy before starting any treatment and 44 after receiving one or more chemotherapy administrations. The average duration between clinical debut and diagnostic, and implicitly splenectomy for the patients that were not previously treated with chemotherapy was 7, 4months (with maximum 3 years). (Table 3)

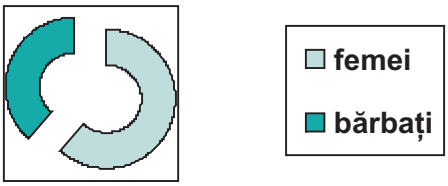

Table 3. *Repartition according to gender of case with HL*

In most cases the diagnostic was elaborated according to biopsy examination of ganglions obtained through surgical interventions in specialty clinics:

- 39 peripheral ganglions biopsies (6 from them are large later cervical extraction)
- 9 thoracotomies (for types with mediastinal debut)
- 17 abdominal laparotomies with splenectomy (preoperatory diagnostic spleen malign lymphoma) (table 4).

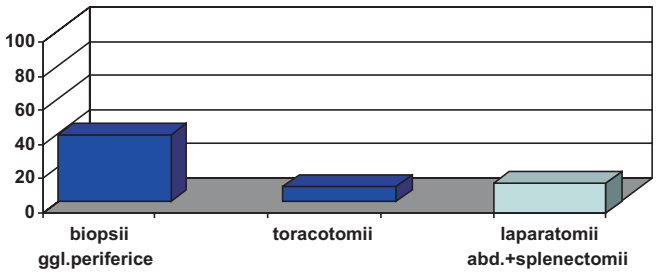

Table 4. *Surgical confirmation of diagnosis*

Determination of histological type (table 5) was made according to recommendations Rye conference:

- Predominant lymphocytes - 4 cases
- Nodular Sclerosis - 10 cases
- Mixed cellular - 43 cases
- Depletion of the lymphocytes - 8 cases

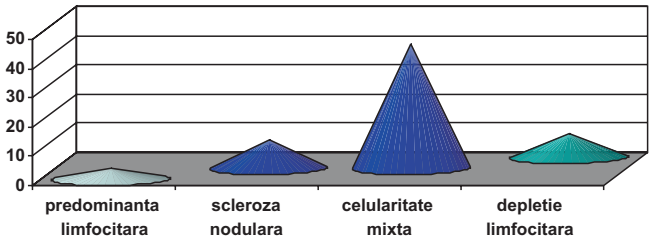

Table 5. *Repartition according to the histological type of the arm*

Pre operatory evaluation of the patients was followed through a clinical, biological, immunological and radiological survey.

Pre operatory evaluation of the patients and tumor extension

Clinical survey	Anamnesis + complete physical examination (examination of the ganglions, spleen and liver)
Biological survey	Complete blood tests Complete exploration of blood coagulation (tests) Investigation of liver and kidney function Bone marrow tests Immunological tests VSH, fibrinogen, LDH, ceruleoplasmina (±)
Radiological Survey	Kidney Rx (±) Pulmonary Rx, Gastric, Bone Rx
Other Investigations	Abdominal echo tomography exam, RMN ±
Surgical survey	exploratory laparotomy with splenectomy and biopsy of the ganglions and liver

Analyzing these data, we can observe:

1. General signs of Hodgkin sufferance were present at 45 of total patients (70%) and absent in 20 cases (30%); frequency of general signs is mo0re frequent to the operated cases, to the advanced disease cases (III and IV) and it is caused by para neoplastic signs and also to the clinical impact of alteration of extra lymphatic organs.

2. Decision for exploratory laparotomy with splenectomy, liver and ganglions biopsy was taken in the next three cases:

- Pre therapeutically splenectomy, for correct stadialization was decided and applied for 17 patients :
- clinical stage I 3 patients
- clinical stage II 7 patients
- clinical stage III 6 patients

- clinical stage IV 1 patients
- post therapeutic splenectomy, for reevaluation of the disease's stage or on the occasion of the relapses, in absence of identifiable ganglions, was done to 48 patients:
- clinical stage I 4 patients
- clinical stage II 12 patients
- clinical stage III 26 patients
- clinical stage IV 6 patients
- post or pretherapeutic splenectomy of necessity for patients with clinical phenomenon of:
- hyper spleen (5 cases)
- mechanic jaundice (2 cases)
- autoimmune hemolytic anemia (3 cases)

2. After the exploratory laparotomy with splenectomy, liver and ganglions' biopsy (surgical survey) 25 patients (38%) modified the disease's stage established pre operatory, as following:
- 5 patients moved from an localized stage of the disease to an advanced stage of the disease
- In 20 cases post operatory evaluations evaluated bigger determinations than the reality, these cases being redistributed to less advanced stages.

The percent of disagreement founded in cases evaluated is similar with literature data (Caplan 1992).

The most frequent modifications attributed to the untreated cases were the modifications between initial stages to advance staged and vice versa from generalized stages to loco regional stages for the cases receiving treatment.

Discussions

Data obtained from the study based on the analyze of the cases belonging to the last 20 years (1984-2004)let us consider that laparotomy and splenectomy were indicated to the majority of patients with HL, except some general contraindications. Regarding the patients in initial stages (I, II) these data contributed to correct evaluation of stages that will be followed by adequate treatment. In advanced stages (III, IV), operatory intervention permits identification of unknown centers of disease, extirpation of the affected organs and solution of the complications (hyper spleens, compressions, hemolysis, etc).

Per- and post- operatory mortality for this arm of patients was zero. In the last years, splenectomy's place in defining the stages of Hodgkin lymphoma is taken by modern investigations as: computerized tomography and nuclear magnetic resonance. These investigations can also offer some percent of false + or false results.

By analyzing the results obtained after laparotomy with splenectomy, liver and ganglions' biopsy resulted:

- in all cases of clinical increase of spleen dimensions, anatomic pathological examination of the spleen showed a lymphoma tic invasion; still, in 6 cases with lack of clinical increase of spleen dimensions, the histopathology examination of the extracted operatory organ we can find specific Hodgkin lesions.
- abdominal ultrasound examination determined for 65 patients was positive in 49 cases, totally in accordance with spleen and ganglions' lesions located intra abdominal, being false negative to 8 patients.
- it could not be established any connection between the liver tests and the alteration of the organ in the lymphoma tic process, especially to the patients with previous specific treatments.
- there were found specific lesions in 18 cases when analyzing the ganglions extracted from the abdominal cavity.
- we didn't noticed the lymphomatic liver alteration by itself, it always coexisted with neoplastic invasion of the spleen. These findings confirm the classical notion admitted that there is not liver invasion without spleen invasion.
- another classical notion refers to the situation when spleen weight is higher or equal with 400g and exists a lymfomatic invasion of the spleen. The arm of studied patients confirms these findings with 2 cases as exceptions when post therapeutically splenectomy was not found as invaded with the occasion of histopathological examination.

Conclusions

- Increase of spleen dimensions is frequent in most of the malignant and non malignant hematological diseases.
- In hematological diseases, splenectomy has proved its benefits for identification of the stages of the hematological disease, diagnosis and treatment.
- For the hematological patients splenectomy necessitates a strong teamwork and a very good collaboration between surgeon, anesthetist, hematologists and laboratory specialist.

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An analysis of the causes of death in Romanian haemophiliacs in the last 35 years

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Summary
A well-grounded realistic estimation of the treatment resources for haemophilia patients requires a thorough research into hospital records to document bleeding diatheses, home treatment and causes of death in patients with haemophilia type A, haemophilia type B, or von Willebrand's disease for an established period of time. A previous study in 345 haemophilia patients shows that severe forms of disease are more common in young patients under 30 of age, whereas the number of severe cases is quite low in patients over 60. Considering these facts, we aim at a deeper analysis of the causes of death and we performed a retrospective study on 85 complete clinical records of patients with haemophilia type A, haemophilia type B or von Willebrand's disease registered and treated at our Institute, and who died in the last 35 years. Bleeding is still the main cause of death in the Romanian haemophilia population. This article is a report and an alarm signal to draw the attention on the causes of death of haemophilia patients registered at INHT Bucharest and to demonstrate the need for a pilot programme aimed at recording the frequency and type of patients' bleeding episodes, and estimating the need for medicinal products to improve the quality of life of these patients.
Key words: *Haemophilia, causes of death.*

Introduction

Haemophiliacs demand a proper therapeutic management, as they start in life with special challenges and need proper medical and social care. At the same time, nature compensates by developing their mind and spirit, endowing them with qualities which can be gainful for their family or social environment, making them useful for themselves, their families, and, last but not least, for the society they live in. Haemophilia is a challenge in the context of emergency care during the bleeding episodes, motor disability research, prophylactic measures, and professional orientation of the medical staff. Psychology of the haemophilia patient is a key issue, and should provide an optimistic outlook. Haemophilia patients face a range of social problems. Before modern treatment was available, they could not go to normal schools, barely found employment, and were treated like outcasts of society. Currently there are still a lot of incompletely resolved issues concerning their social inclusion.

Moreover, the progress of disease is influenced not only by individual genetic profiles, but also by external factors with indirect, but significant action, such as the social-economic conditions, the geographic location and the family and social environment of the patient. Concentrates were not available in Romania until the 1990s, when they came as donations, and after 1998 purchased or obtained by industrial fractioning of plasma, but they were still scarce. This lead to the fact that the greater number of haemophilia patients were treated with labile blood products, with a lot of undesired consequences on their life.

Haematoma and haemarthrosis are by far the most common and significant clinical events in haemophilia. Progression of these complications may lead to motor disabilities, with psychological and social consequences for the patient. Death events usually followed massive unmanageable bleeding, as a result of post-surgery or infection-related complications.

Life expectancy for haemophilia patients

The survival analysis of 345 haemophilia patients (haemophilia type A, n=300, and haemophilia type B, n=45) registered and followed-up at INHT in a local study over 10 years (1996-2006) revealed a rather gloomy outlook on the life expectancy of these patients. We came to the conclusion that certain factors (the high demand of medical services, the low accessibility to medical care and treatment, the lack of a proper education and information) prevent many haemophiliacs to survive into old age18.
(Fig. 1).

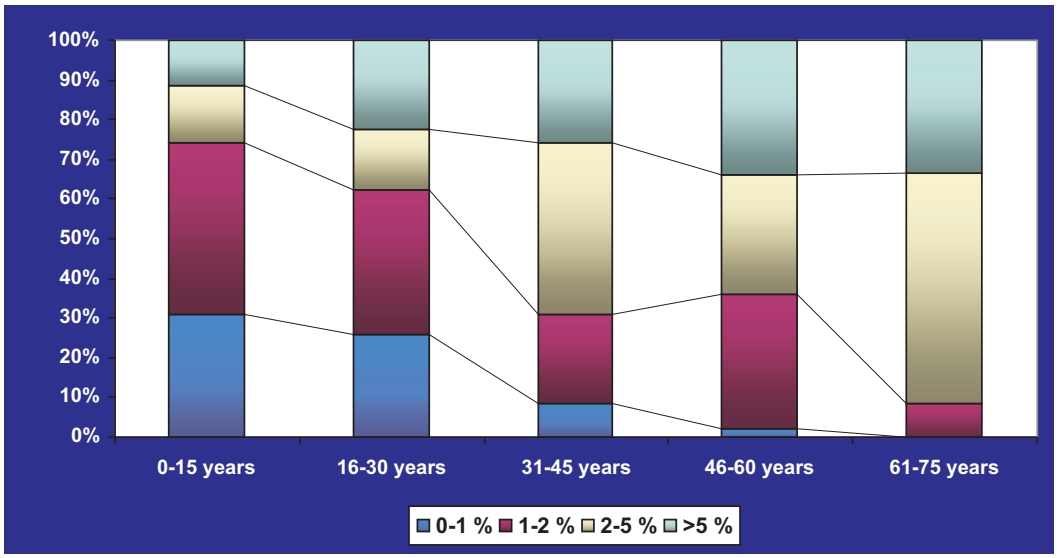

Fig. 1. *Life expectancy for the studied group of patients*

In the studied group, 73.5% of patients aged 0-15 had factor levels under 2%, whereas a factor level higher than 2 percent occurred in a smaller percent of patients, probably due to screening gaps in the general population. In the next age group, 16 to 30 years, cases are distributed over the 4 severity categories, with a slight decrease of cases with factor levels under 2% due to patients' death, and an increase of the percent of patients diagnosed with mild forms of disease.

The 31-45 age group revealed a two-fold reduction of cases with severe forms of disease, while the mild and moderate forms were prevalent. In the age group over 46 we found only 14 patients with factor levels over 1% and significant bone and joint changes, whereas in the last age group 2 patients had a factor level between 1% and 2%, and the rest were cases of mild and moderate forms of disease.

These numbers can relate to the care of parents who come with their children for diagnosis, and to the new treatment options, whereas aged people with severe forms of disease could not survive to bleeding episodes due to improper care. Cases with factor levels higher than 2% are equally spread over all the age categories considered in the study. If cases with factor levels lower than 2% are predominantly children and young patients, the higher factor level cases included only 27 children, after screening patients who have undergone tonsillectomy/adenoidectomy or appendicectomy. We came to the conclusion that a screening to detect mild forms of disease and minor deficits can be of great benefit to the population. The screening of all family members would also be useful to detect all cases in the family as early as possible.

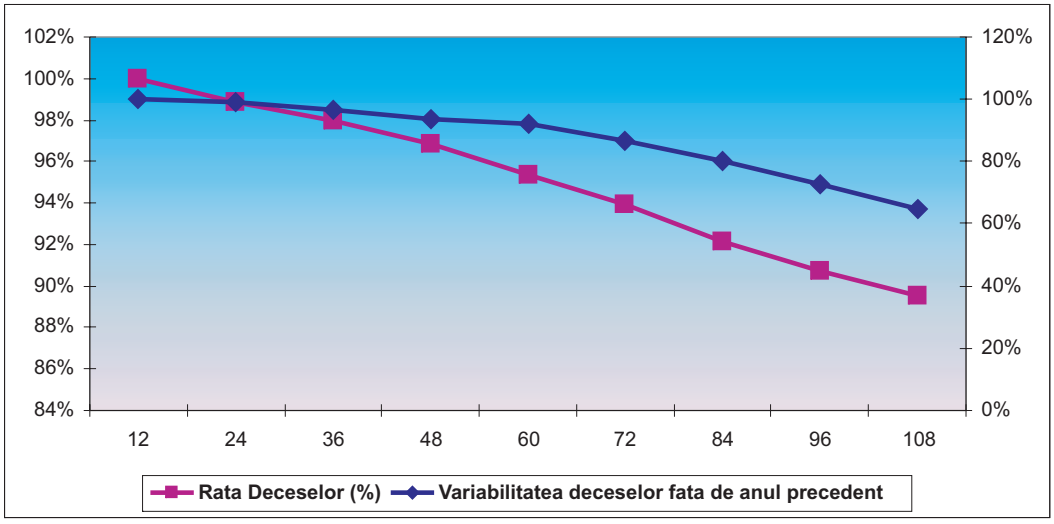

Fig. 2. *Survival in the studied group of haemophiliacs. Death rates (%). Change in death rates as compared to the preceding year.*

In this patient group, 36 (10.5%) patients died of various causes over the 10 years of follow-up, bleeding being the first cause (24%), and chronic liver diseases the second (19%). The number of death events was 4 during the first year and 3, 4, 5, 5, 6, 5, 4 in the following years, respectively, while the patients' age ranged between 7 and 54 years.

Materials and method

Considering these facts, we aim at a deeper analysis of the causes of death and we performed a retrospective study on 85 complete clinical records of patients with haemophilia type A, haemophilia type B or von Willebrand's disease registered and treated at INHT, and who died in the last 35 years (1972-2007). Our study included 78 haemophilia patients, of whom 72 had haemophilia type A, the rest, haemophilia type B, and 7 patients were diagnosed with von Willebrand's disease. We should stress the fact that this was not the total number of patients deceased in the considered time frame, but the rest of 25 records could not be used due to insufficient data. All patients were treated on-demand, fresh-frozen plasma and cryoprecipitate were used for bleeding; some of the patients deceased between 2000 and 2007 had received double virus-inactivated factor VIII concentrate. Death of patients between 2000 and 2007 occurred either in hospital or at home.

Results and discussions

For a better comparison of results, we chose to analyse a relatively well-balanced number of records for patients deceased over 3 distinct periods of time, corresponding to different treatment methods and different medical insurance policies, as can be seen in the picture below. Treatment options between 1972 and 1989 were exclusively fresh-frozen plasma and cryoprecipitate only from Romanian donors. The 1990-1999 therapeutic approach brought a reform in the often-overstretched blood transfusion services, and the first factor VIII concentrates began to be available in Romania either by donation or purchase. During the last analysed time period, the common treatment option was factor VIII concentrate.

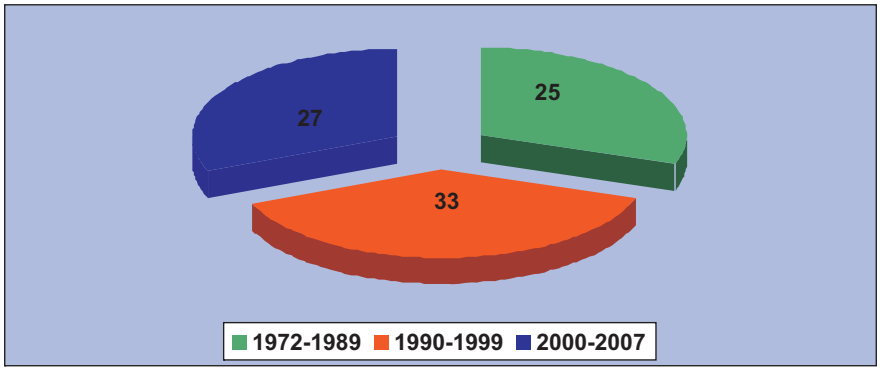

Fig. 3. *Death events in haemophilia patients per time frames*

The analysis of haemophilia patients' age on time of death revealed the fact that 75.3% (64) of patients died before the age of 46, whereas the mean life expectancy of the Romanian general population was around 68 in the 1970s and rose to 70 by 2007.

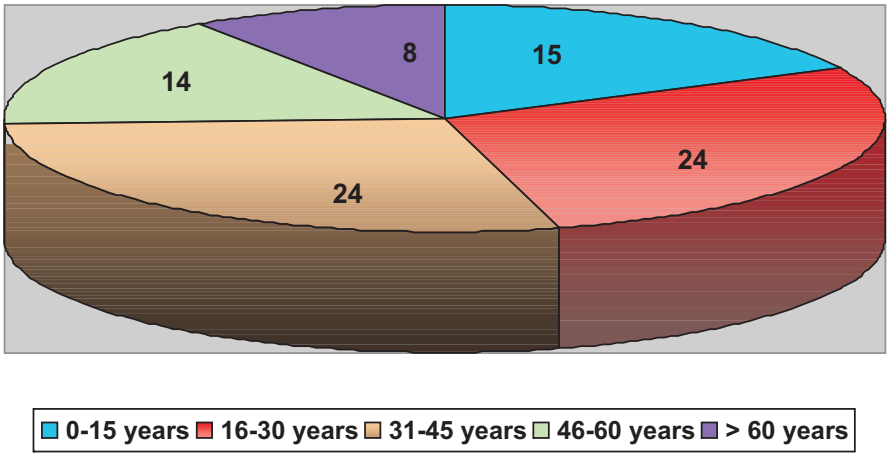

Fig. 4. *Death events in haemophilia patients per age groups*

The following table reflects the time frame and age distribution of deceased patients from INHT records.

Age / Period	0-15 years	16-30 years	31-45 years	46-60 years	> 61 years	**TOTAL**
1972-1989	7	10	4	2	2	**25 - 30 %**
1990-1999	4	9	8	8	4	**33 – 39 %**
2000-2007	4	5	12	4	2	**27 – 31 %**
Total	**15 – 18 %**	**24 – 28 %**	**24 – 28 %**	**14 – 16 %**	**8 – 10 %**	**85– 100%**

The distribution of patients with haemophilia type A according to clinical severity shows that 36 patients suffered from a severe form of disease, 31 had moderate forms, and only 5 patients were diagnosed with a mild form of disease.

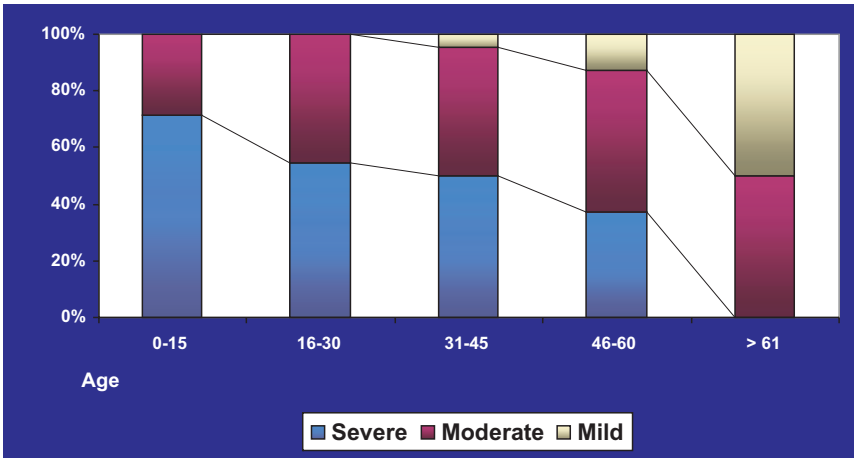

Fig. 5. *Distribution of death events per form of disease and age group*

The diagram above shows that death events with severe disease are more common with young patients, whereas persons with moderate disease died at older ages.

Causes of death

In a study on mortality among haemophiliacs in the UK, Darby concluded that AIDS was the commonest cause of death, while liver infection complications ranked on the third place. According to the same study, mortality is 16.7-fold higher in HCV-infected patients and 5.6-fold higher in liver cancer haemophiliacs than in the general population.6, 7, 12. An American study performed on 700 HCV-infected patients noted a decrease in their quality of life, which was close to insulin-dependant diabetes mellitus and chronic arthritis QoL scores. Re-evaluation of these patients after 24 weeks of burdening IFN-treatment showed a significant improvement of the considered parameters.2, 5

Table. Causes of death in the studied group of Romanian haemophilia patients and literature cases

CAUSE OF DEATH	**RO PATIENTS**	**RIZZA STATISTICS – UK**
AIDS	**11 (13%)**	**558 (43%)**
INTRACRANIAL BLEEDING	**12 (14%)**	**148 (11.5%)**
CANCER	**7 (8%)**	**109 (8.5%**
CARDIORESPIRATORY DISEASES	**9 (10%)**	**77 (6%)**
LIVER DISEASES	**16 (19%)**	**75 (5.8%)**
BLEEDING	**20 (24%)**	**40 (3%)**
INJURIES	**5 (6%)**	**21 (1.7%)**
OTHER RARE CAUSES	**5 (6%)**	**270 (20.9%)**
TOTAL	**85 (100%)**	**1298 (100%)**

The table data are compared with data published by Rizza from a study on the UK population of haemophiliacs, analysing the causes of death over 15 years (1981-1996)14, 19. From the table above we conclude that in Romania the HIV infection / AIDS is not a common cause of death as compared to the UK haemophilia group, where it is the first cause of death (43% of patients), followed by intracranial bleeding and by cancer. The obvious gap between the two statistics shows that only a small number of Romanian haemophilia patients have been HIV-contaminated, despite the fact that plasma began to be tested for HIV only much later.

Paradoxically, acute bleeding is the commonest cause of death in the Romanian patient group (30%), due on the one hand to belated request for specialized care from the patients or their families, and on the other hand (in the past) due to the lack of concentrates which could have stopped bleeding rapidly. Death by various bleeding types included gastrointestinal and intracranial bleeding, haemoptysis, and also intra- and post-surgery massive bleeding, unmanageable with plasma products alone. Bleeding episodes may become unmanageable also due to the inhibitor presence, or to other unthoroughly investigated coagulation factor deficits, associated with haemophilia. The next cause of death remains liver disease caused by chronic hepatitis virus infection, often combined with alcoholism.

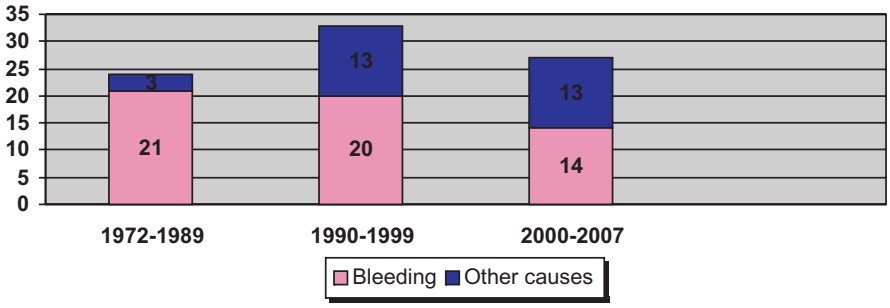

Fig. 6. *Number of death events per various bleeding sites as compared to other causes of death in patients with haemophilia type A.*

As shown in this figure, even if the time frames are not equal, the ratio between the two large groups of causes is surprisingly high, including the 2000-2007 interval, when the factor VIII concentrate became more widely available in Romania.

Costs

A USA report shows that haemophilia patients are a high resource-consuming group, disproportionately higher than other chronic patients.4, 8, 20

The availability of proper treatment with concentrates is highly dependable on the GDP of each country. All countries have their resource limitations, and try to avoid wasting them on approaches which are set for failure from the start. Each state should decide on the treatment adequate for its population, based on a proper economical analysis and on the cost-benefit ratio.11

If we compare hospital treatment costs per patient with outpatient or home treatment costs, we observe a reduction by 27.5% for outpatient and by 32.8% for home treatment costs.

There are different stages of haemophilia treatment, as assessed by Schramm and shown in Figure 7. Prophylactic treatment, although effective for improvement of the quality of life of haemophilia patients in other countries, is difficult to apply in Romania due to its high costs.16 More realistically, the support for implementation of a home treatment programme is much more likely to succeed. Patients' eligibility should be determined responsibly.

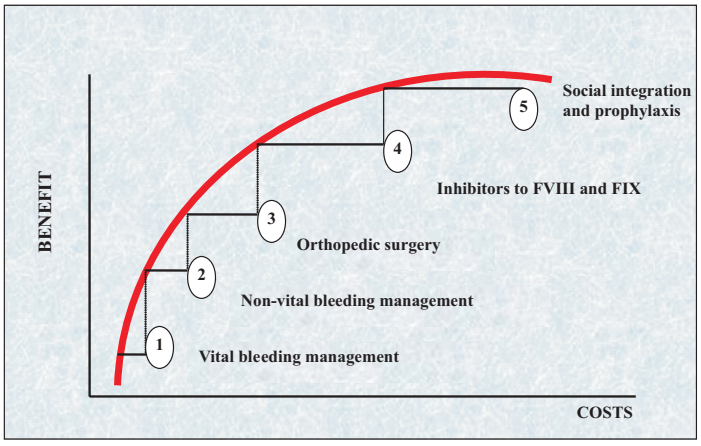

Fig. 7. *Curve of costs for haemophilia treatment 16*

HCV-related economic issues raised discussions and proposals for the reduction of costs even since the introduction of IFN-therapy. A British study admits the infection of haemophiliacs via the administered products and estimates that their treatment requires approximately 10,000 GBP per patient, taking also into account the bleeding risk needing replacement therapy, particularly in cases with liver biopsy.10

Quality of life

A whole range of studies are trying to find a method to quantify and assess the quality of life of persons with haemophilia.1, 9, 13, 17 A recent European study published data on quality of life differences between prophylactic and on-demand factor replacement therapy in European haemophilia patients on a group of 1,033 haemophilia patients in 16 centres. A number of subjective and objective criteria were considered. Physical status, role of physic limitations, bodily pain, general health, vitality, social functioning, role of emotional disability and mental health were evaluated. Authors report differences between the two patient groups referring to physical status, bodily pain and general health index, with a $p<0.001$. Patients receiving prophylactic treatment have an increased quality of life as compared to those treated on-demand, and they reported reduced bodily pain, better physical status and increased general health. Some physical criteria as bodily pain and physical function are significant to haemophiliacs.3, 15

Conclusions

Bleeding is still the main cause of death in the haemophilia population of Romania. Life expectancy shows a slight increase, in spite of morbidity related to hepatitis C virus (HCV).

Management of bleeding episodes and prevention of massive bleeding can be achieved by factor VIII concentrates administered as soon as possible after onset of bleeding.

The main objective of this study is to demonstrate the need for a pilot programme aimed at recording the frequency and type of patients' bleeding episodes, and estimating the need for medicinal products to improve the quality of life of these patients. It is imperative to dedicate sufficient resources for the purchase of factor VIII concentrate and recombinant, vital for the proper management of haemophilia patients, so as we can at least equal the mean factor use per capita from the neighbouring countries.

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Erythrophagocytosis extensive in bone marrow, hemophagocytosis from a patient with severe anemia who developed acute myeloid leukemia M4/M5b (Case report and patogenesis)

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Summary

By using May Grünwald - Giemsa stain for conventional light microscopy, the marrow smear showed the presence of macrophage with 4-7 red cells - erythrophagocytosis, from a patient with severe anemie. The hemoglobin level was 3.7g per deciliter. The patient developed an acute myeloid leukemia M4/M5b and hemophagocytosis. The fagocytozed myeloid blood cells are peroxidase positive.
Key words: erythrophagocytosis, bone marrow, acute myeloid leukemia M4/M5b

Introduction

Hemophagocytic Macrophages

Phagocytosis of the hematopoietic cells by macrophages has been observed in a variety of neoplastic and non neoplastic conditions [1-10]. The most prominent phagocytozed cells are erythrocytes. However, erythrophagocytosis is often associated with phagocytosis of other cellular elements, such as platelets and neutrophils. In a number of viral infections, such as herpes simplex virus, cytomegalovirus, EBV, adenovirus, parainfluenza and measles, a significant number of reactive macrophages may display erythrophagocytosis [11-17]. The erythrophagocytic cells show abundant vacuolated cytoplasm, with one or several red blood cells or remnants of red blood cells, and contain round, oval or kidney-shaped nuclei with lacy chromatin and inconspicuous nucleoli. In malignant histiocytosis, unlike reactive processes, phagocytic histiocytes are atypical and pleomorphic, and may show hyperchromatism, prominent nucleoli and multinucleation [18, 19].

Cancer-associated hemophagocytosis.

Reactive histiocytosis with hemophagocytosis has been reported in association with a variety of malignant neoplasms, particularly lymphoid malignancies [20-24], but also acute myeloblastic leukemia [25-28, 19] and acute monocytic leukemia [29,19]. Most of the lymphoid malignancies are of the T-cell type, may show evidence of EBV infection and may simulate malignant histiocytosis [30, 31]. Most of the cases originally diagnosed as malignant histiocytosis later proved to be a reactive histiocytosis with hemophagocytosis
associated either with infection or with lymphoid malignancies.
The syndrome can occur with lymphomas as a result of cytokines released by lymphoma cells that stimulate histiocyte proliferation and phagocytosis [32-35].
The histiocytic cells are considered reactive and may appear as a result of the release by the tumor cells of cytokines [36, 37] that recruit monocyte.
Acute mixed lineage leukemia M4/M5 with an inv(8) (p11q13) resulting in fusion of the genes for MOZ (monocytic leukemia zinc finger protein) and TIF2 (transcriptional intermediary factor 2) have been associated with prominent erythrophagocytosis [38, 39,

29].

The MOZ-TIF2 fusion is one of a new family of chromosomal rearrangement, that associate HAT domain (hystone acetyltransferase), transcriptional coactivation, and acute leukemia. Two distinct clinical syndromes have been associated with 8p11: a chronic myeloproliferative disorder complicated by T-cell lymphoblastic leukemia/lymphoma and peripheral blood eosinophilia and M4/M5 acute monocytic leukemia with prominent erythrophagocytosis.

The involvement of MOZ-TIF2 in acute leukemia suggests that yet another class of cellular processes can be deranged by gene fusion events. Each of these proteins possess or recruits HATs to chromatin. The ability of HATs to affect chromatin structure and regulate gene expression is well appreciated [40].

Acute myeloid leukemia with translocation t (8;16) demonstrates specific cytomorphological, cytogenetic and gene expression characteristics and can clearly be discriminated from other AML with balanced translocations.

Balanced chromosomal rearrangements leading to fusion genes on the molecular level define distinct biological subsets in AML. The four balanced rearrangements t(15;17), t(8;21), inv(16), and 11q23/MLL) show a close correlation to cytomorphology and gene expression patterns.

Translocation t(8;16)(p11;p13) is rare. It is more frequently found in therapy-related AML than in de novo AML. Citomorphologically, AML with t(8;16) is characterized by striking features: the positivity for myeloperoxidaze on bone marrow smears >70% and intriguingly, in parallel >80% of blast cells stained strongly positive for non-specific esterase (NSE) and **erythrophagocytosis.**

These cases can not be classified according to FAB categories. These data suggest that AML t(8;16) arise from a very early stem cell with both myeloid and monoblastic potential.

The translocation t(8;16) were intercalating with FAB-M4 and M5b. Thus, monocytic characteristics influence the gene expression pattern stronger than myeloid.

Due to this unique features, it is a candidate for inclusion into the WHO classifications as a specific entity.

Patogenesis.

Conditions of hemophagocytic lymphohistiocytosis (HLH)

Genetic HLH

Familial HLH (Farquhar disease)
Known gene defets (perforin, munc 13-4, syntaxin 11)
Unknown gene defects

Imune deficiency syndromes

Chédiak-Higashi syndrome (CHS)
Grisceli syndrome (GS)
X-linked lymphoproliferative syndrome (XLP)

Acquired HLH

Exogenous agents (infectious organisms, toxins)
Infection-associated hemophagocytic syndrome (IAHS)

Endogenous products (tissue damage, metabolic products)

Rheumatic diseases

Macrophage activation syndrome (MAS)

Malignant diseases, leukemias

Familial HLH

Genetic HLH (Hemophagocytic Limpho Histiocytosis) is inherited in an autosomal recessive fashion aspect and can be divided into 2 subgroups: familial HLH (FHLH) and the immune deficiencies Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS) and X-linked lymphoproliferative syndrome

(XLP). Both genetic subgroups are associated with impaired NK cell function. In FHLH, originally described by Farquhar and Claireaux in 1952 [41], the clinical syndrome of HLH is the primary and only manifestation.

CHS, GS and XLP are immune deficiencies with distinctive clinical features in which the development of HLH is sporadic. HLH is often the presenting symptom, but may also occur later, during the course of disease.

Patients with CHS show albinism and frequent pyogenic infections. Their white blood cells exhibit decreased chemotaxis and characteristic giant inclusion bodies (lysosomes). Patients with GS also have hypopigmentation and various degrees of neutrophil disfunction but lack the giant granules. XLP is mainly characterized by a predisposition for Epstein-Barr virus (EBV) associated - HLH. XLP patients may develop lymphomas and dysgammaglobulinemia.

The hallmark of HLH is impaired or absent function of natural killer (NK) cells and cytotoxic T cells (CTL) [42].

Genetic defect in FHLH elucidate the pathophysiology of the spectrum of HLH.

The cytotoxic activity of NK cells and CTLs (cytotoxic T-cells) is mediated by the release of cytolytic granules (containing large amounts of perforin, granzymes and other serine like proteases) via the immunological synapse to the target cell. Several independent genetic loci related to this activity have been implicated in the pathophysiology of genetic HLH. In1999 mutations in the perforin gene at locus 10q24 were described in 8 patients with FHLH [43].

The overall frequency of perforin mutations in FHLH is between 15% and 50% and depends on the geographical and ethnic origin of the patients [44].

UNC13D at locus 17q25, was the second gene associated with FHLH. The encoded protein (Munc 13-4) is important for cytolytic granule exocytosis [45, 46]. The protein is strongly associated with intracellular membrane fractions and is detectable only in monocites and not in lymphocytes.

Defective Cytotoxic Function is Crucial in the Pathophysiology of HLH.

The clinical picture of HLH is due to an increased inflammatory response caused by hypersecretion of pro-inflammatory cytokines, such as interferon (IFN), tumor necrosis factor (TNF), interleukin IL-6, IL-10 and macrophage-colony-stimulating factor (M-CSF) [47]. These mediators are secreted by activated T lymphocytes and histiocytes that infiltrate all tissues, and lead to tissue necrosis and organ failure. Inflammatory cytokines are responsable for the characteristic disease markers such as cytopenias, coagulopathy and high triglicerides.

Patients with HLH have severe impairment of the cytotoxic function of NK cells and CTLs. Impaired NK cell activity is seen in FHLH, GS, CHS and XPL as well as in acquired HLH.

NK cells and cytotoxic T lymohocytes kill their targets through cytolytic granules containing perforin and granzyme. Upon contact between the effector killer cell and the target cell, an immunological synapse is formed and cytolytic granules have to traffic to the contact site, dock and fuse with the plasma membrane and release their contents [48]. All known genetic defects in HLH seem to be involved in the process.

Viruses may interfere with CTL function by specific proteins [49] and high levels of cytokins may have the same effect [50]. HLH cases associated with genetic defects in the granule exocytosis pathway demonstrate a critical role of the granule-dependent cytotoxic activity in lymphocyte homeostasis [46].

The acquired HLH may be more common than previously believed. Acquired HLH has:

Exogenous agents (infectious organisms, toxins)

Infection associated hemophagocytic syndrome (IAHS)

Endogenous products (tissue damage)

Rheumatic diseases

Macrophage activation syndrome (MAS)

Malignant diseases, leukemias

Pathogenesis of acquired HLH

The clinical picture of HLH can be induced by a variety of infectious organisms, mostly viruses (especially EBV), but also bacteria, protozoa and fungi.
The syndrome can occur with lymphomas as a result of cytokines released by lymphoma cells that stimulate histiocyte proliferation and phagocytosis.
HLH in association with malignant diseases, especially lymphomas (lymphoma-associated hemophagocytic syndrome, LAHS) is a well known entity in adults but is rare in children.

The histiocytic cells are considered reactive and may appear as a result of the release by the tumor cells of cytokines [36, 37] that recruit monocyte.
The manifestations of the disorder are thought to be mediated by inflammatory cytokines, including interferon-, TNF-, soluble IL-2 receptor, IL-6, IL10, FAS-ligand, granulocyte-monocyte colony- stimulating factor (GM-CSF) and monocyte colony - stimulating factor (MCSF), and perhaps other. Agents like Epstein-Barr virus activate T lymphocytes, resulting in cytokine release. Elevated levels of TNF-, soluble IL-2 receptors, IL-1, and FAS-ligand are associated with the severity of the manifestations. Soluble IL-2 receptor is thought to contribute to immune impairment by negating the effect of IL-2 [3, 47].

Cytopenias may result from the effects of elevated concentrations of interferon-, TNF-, or transforming growth factor β (TGF-β)-mediated suppression of the marrow and M-CSF-mediated accelerated clearance of platelets by histocytes.

A similar syndrome can occur in patients with a variety of malignancies, perhaps as a result of the enhanced susceptibility to infection of the imunosuppressed state associated with cancer, chemotherapy and radiotherapy.

Macrophage Activation Syndrome is closely related to HLH.

The macrophage activation syndrome (MAS) occurs in children and adults with autoimmune diseases. It is most commonly seen in association with systemic onset juvenile arthritis (sJRA) or adult-onset Still's disease, but also occurs rarely with systemic lupus erythematosus or other entities [51, 52]. Patients with MAS have the defective NK cell function common to other patients with HLH [53]. They may also have decreased expression of perforin, mimicking the defects associated with FHLH.

Viruses have been identified as triggering factors. It has been suggested by some rheumatologists that MAS be classified as a form of secondary HLH [54, 55].

Matherials and methods

Bone marrow smear stained with May Grünwald Giemsa for conventional microscopy, from a patient with severe anemia.
Record number of patient 3064/2002, on 31 October.
Bone marrow smear from 1 November 2002.
The patient developed an acute myeloid leukemia M4/M5b and hemophagocytosis. The cytochemical test peroxidase by using Graham Knoll's method.

Results

Marrow aspirate smear: moderate hypocellularity
Differential cell count: M/E-ratio = 4-5
Myeloid cells are normal
Erythroid cells: 60% with iron deficiency aspect. Erythroid precursors, particularly intermediate and late normoblasts, show scanty, ragged rims of poorly hemoglobinized cytoplasm.
Megakaryocitic cellularity is normal
Cell count: moderate hypocellularity
Promyelocyte: 4
Myelocyte: Neutrophilic: 10
Metamyelocyte: 10
Band :12
Segmented: 20
Eosinophil: 1
Lymphocyte: 19
Monocyte: 1
Plasma cells: 5
Proerythroblast: 1
Erythroblast:
Basophilic: 3
Polychromatophilic: 6

Ortochromatic: 3
Megakaryocyte: 1
Macrophage: 4
M/E ratio: 4-5

The marrow smear from particle preparate showed moderate hipocellularity

The marrow showed the presence of macrophages with 4-7 red cells included into their cytoplasm - erythrophagocytosis.

The erythrophagocytic cells show abundant cytoplasm, with 4-7 red blood cells and contain round or oval nuclei with lacy chromatin an inconspicuous nucleoli.

The microscopic images with erythrophagocytosis are showed in Figures 1 to 6.

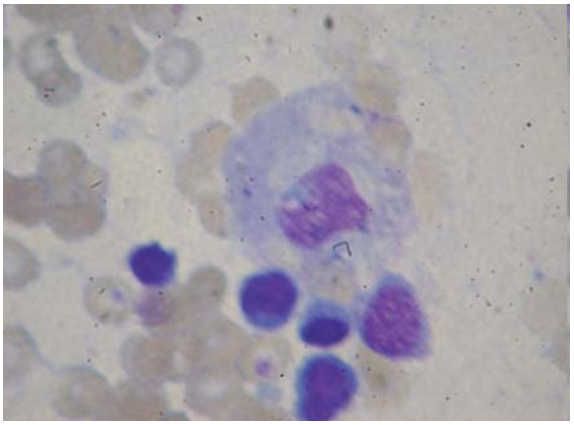

Figure 1. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 1*

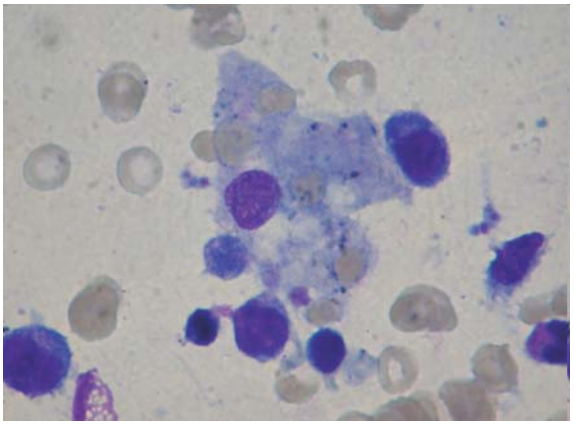

Figure 2. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 2*

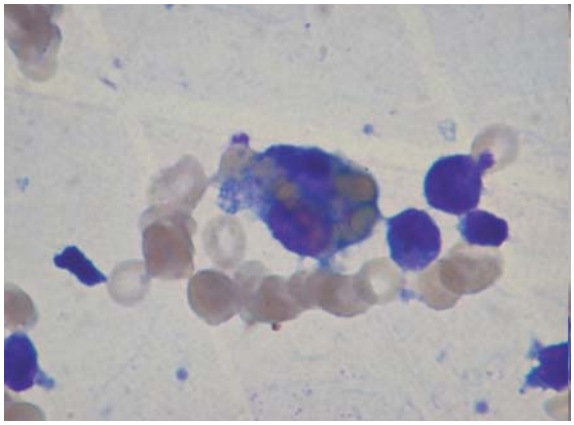

Figure 3. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 3*

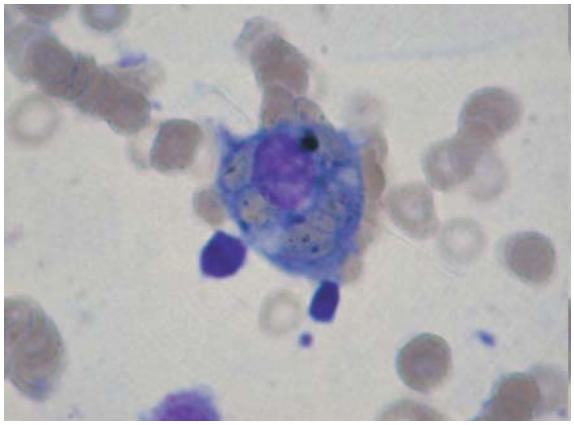

Figure 4. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 4*

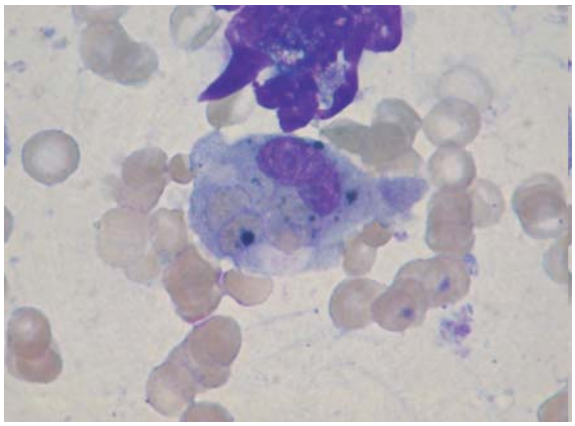

Figure 5. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 5*

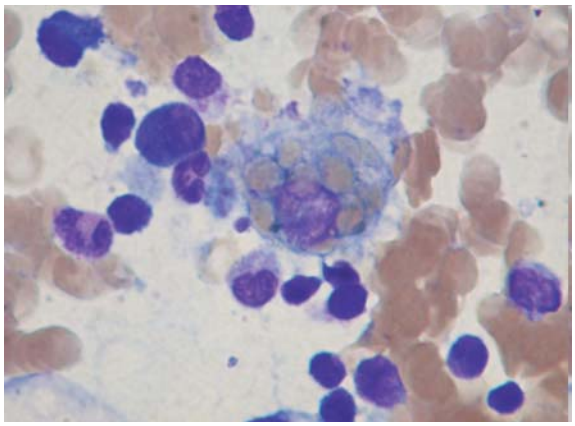

Figure 6. *Erythrophagocytosis. Bone marrow smear, microscopic image (May Grünwald Giemsa stain, 1000 x) 6*

Bone marrow stained with May Grünwald Giemsa for conventional microscopy, from a patient with severe anemia.

Case report

The patient C.G., a 57-year-old man, was admitted to Medical Clinic 1 (Hematology Department) Targu-Mures on 31st October 2002 (record number 3064/2002) for severe anemia that started 3-4 months before the hospitalization. Symptoms at that time included asthenia, weakness, dyspnea, weight loss, symmetrical paresthesias in his legs and fingers. He has no infectious or hemorrhagic symptoms. The patient's physical examination reveals nonspecific pallor of the skin and of the mucous membranes, hepatomegaly with a diameter of 14 cm; he has no palpable lymph nodes or splenomegaly.

Lab studies: blood hemoglobin concentration 3.7g/dl, leucocytes 2400/μL, platelets 88000/ μL, reticulocytes 0.1%. Peripheral blood smear:normocytic and normochromic red cells, mature polymorphonuclear granulocytes 67%, lymphocytes 29%, monocytes 4%.

Bone marrow aspirate: moderate hypocellularity, 60% red cells with iron deficiency aspect (erythroid precursors, particularly intermediate and late normoblasts, show scanty, ragged rims of poorly hemoglobinized cytoplasm), lymphocytes 19%, erythrophagocytosis (macrophages contain 4-7 red cells included into their cytoplasm), without shift to left or pathologic cells.

Blood chemistry: serum iron 38.78 μmol/L, total bilirubin 1.07 mg/dl, LDH 187 U/L, total proteins 65 g/L, albumin 51.4%, α1-globulin 5.3%, α2-globulin 12.2%, β-globulin 15.5%, γ-globulin 15.6%, protein C positive, hepatitis markers absents for hepatitis B or C.

Chest radiography and computed tomography, gastroscopy and urology examination were negative.
Abdominal echography: hepatomegaly 14 cm diameter with homogeny and denser echostructure; no other pathologic signs were detected.

A diagnosis of hemophagocytic syndrome was established and the patient was treated with erythrocytes concentrates, folic acid and hepatoprotectors drugs.
The patient returns to hematology department for other three hospitalizations in the next months. He presented symptoms of anemia and had the following results of hematological examinations:

December 2002 (3498/2002): hemoglobin 4.1 g/dl, leucocytes 2400/μL, platelets 34000/μL; peripheral blood smear: mature polymorphonuclear granulocytes 46%, lymphocytes 49%, monocytes 5%, normocytic and hypochromic red cells.

Bone marrow aspirate: moderate hypocellularity, normal aspect and maturation of white cells, decreased percent of red cells with normal morphology, G/E ratio = 6-7; small number of megakariocytes, the majority of them of reduced size; reticular cells 6%, macrophages 3% with the presence of erythrophagocytosis; lymphocytes 15%. Transfusions with erythrocytes concentrates, folic acid, lithium carbonic, and hepatoprotectors drugs were administered. A favorable outcome was recorded.

January 2003 (150/2003): hemoglobin 4.8 g/dl, leucocytes 3100/μL, platelets 33000/μL; peripheral smear: mature polymorphonuclear granulocytes 63%, lymphocytes 26%, monocytes 8%, eosinophils 2%, normocytic and hypochromic red cells. The bone marrow aspirate aspect was similar to that of previous hospitalizations, with 3% sideroblasts and Pearls stain reaction positive for hemosiderin in macrophages. Serum iron 40.7μmol/L. Erythrocytes concentrates, folic acid, corticotherapy (60mg/day) were administered. The patient's condition improved.
March 2003 (681/2003): The patient is hospitalized with severe anemia, without hemorrhagic signs or fever. Hematological examinations: hemoglobin 6.2 g/dl, leucocytes 7000/μL, platelets 66000/μL, reticulocytes 0.2%. The aspects of peripheral smear and of bone marrow aspirate were similar to that of previous hospitalizations; erythrophagocytosis was present in bone marrow. The treatment is resumed with erythrocytes concentrates, folic acid, corticosteroids, with a favorable clinical and hematological outcome.
The patient did not return to our clinic. During the summer 2003 the patient developed an acute myeloid leukemia M4/M5b and was hospitalized and treated in Hematology Department in Cluj-Napoca (Romania).

Data obtained from Oncological Institute "Ion Chiricuță", Laboratory of Hematology Department, Cluj-Napoca, Romania are shown in Figures 7-11 (bone marrow smear from 20 March 2003 - Figures 7, 8 and bone marrow smear from 7 October 2003, Figures 9 to 11).

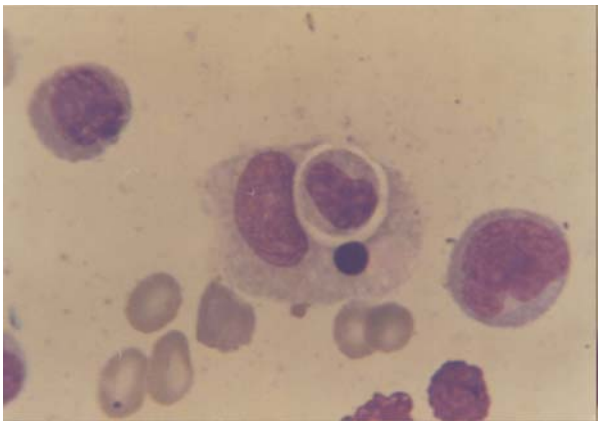

Figure 7. ***Bone marrow smear, hemophagocytosis (May Grünwald Giemsa stain, 1000 x)***

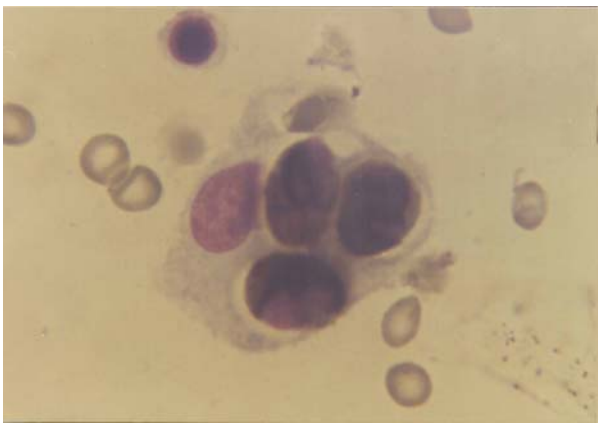

Figure 8. *Bone marrow smear, peroxidase reaction. The phagocytozed cells are peroxydase positive* (1000 x)

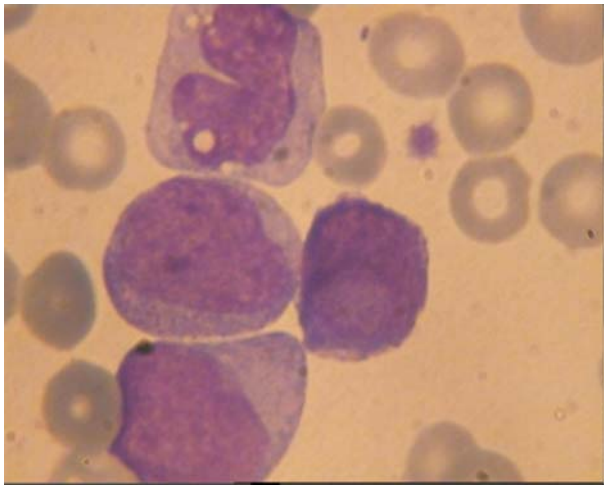

Figure 9. Bone marrow smear, acute myeloid leukemia M4/M5b (May Grünwald Giemsa stain, 1000x) 1

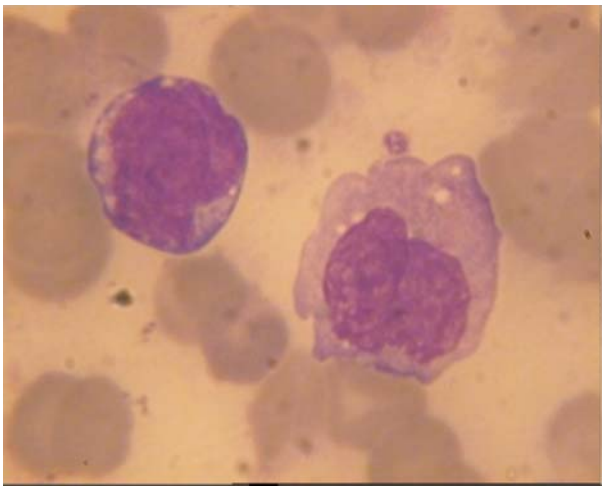

Figure 10. Bone marrow smear, acute myeloid leukemia M4/M5b (May Grünwald Giemsa stain, 1000x) 2

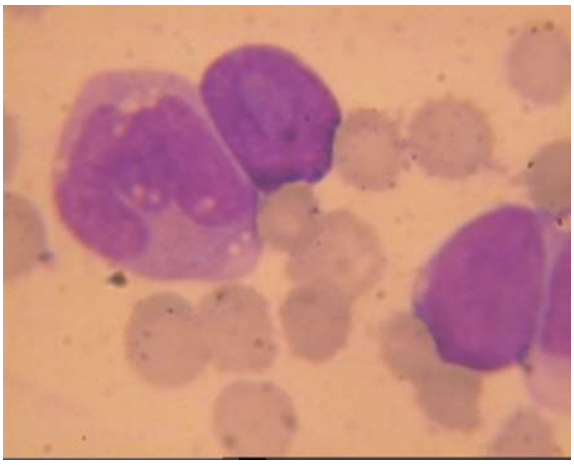

Figure 11. *Bone marrow smear, acute myeloid leukemia M4/M5b (May Grünwald Giemsa stain, 1000x) 3*

Discussions

It is a case report of a patient with severe anemia and extensive erythrofagocytosis in bone marrow, who developed acute myeloid leukemia M4/M5b with hemophagocytosis.

The phagocytozed myeloid blood cells are peroxidase pozitive.

Acute mixed lineage leukemia M4/M5 with an inv(8) (p11q13) resulting in fusion of the genes for MOZ (monocytic leukemia zinc finger protein) and TIF2 (transcriptional intermediary factor 2) have been associated with prominent erythrophagocytosis.

The reactive hemophagocytic syndromes are distinguished from malignant histiocytosis based on the cytologic characteristics of the proliferating histiocytes. In the former hematophagocytosis is often prominent and histiocytes appear mature and bland while in the latter there is evidence of immaturity, marked nuclear pleomorphism and atypical mitotic figures. Evidence of

a familial history, infection or a nonhistiocytic malignancy distinguishes the three major subtypes of reactive hemophagocytic syndrome from each other (familial hemophagocytic histiocytosis, infectious hemophagocytic histiocytosis and tumor-associated hemophagocytic histiocytosis).
The syndrome can occur with lymphomas as a result of cytokines released by lymphoma cells that stimulate hystiocyte proliferation and phagocytosis.
The histiocytic cells are considered reactive and may appear as a result of the release by the tumor cells of cytokines that recruit monocyte.
Laboratory investigations are a complete blood count, liver enzymes, bilirubin, triglycerides, ferritin and a coagulation profile including fibrinogen.
Some data of patogenesis are also presented.

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The Role of Laboratory Graft Quality Indicators in Autologous Stem Cell Transplantation

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Summary
Introduction: Peripheral blood as source of stem cells is the main method in autologous stem cell transplantation (Auto-SCT) graft obtaining; a better haematopoietic reconstitution is recorded in this case, when the graft quality is obviously in good order.
Patients and analysis criteria: One hundred patients were studied, summarizing 165 grafts finally. The number of mononuclear cells, and CD34+ cells, the percentage of viable cells obtained by trypan blue exclusion method, and CFU-GM number represented the graft quality indicators related to existing disease, the chemotherapy administrated for CD34+ mobilization and the type of samples (before /after cryopreservation).
Results: Mobilising polychemotherapy method associated with growth factors (G-CSF) results in a better harvest of CD34+ cells and CFU-GM, comparatively with G-CSF values obtained by growth factor mobilization alone. Good values for these two parameters were obtained in Hodgkin Disease and non- Hodgkin Malignant Lymphoma when DHAP protocol mobilization was performed. Cryopreservation scored an important decrease for all these parameters. It was not observed a correlation between number of leukocytes, mononuclear cells, viable cells, and CFU-GM number before/after cryopreservation.
Conclusions: The quality of graft obtained by leukaphaeresis may by estimated by counting CD34+, and mononuclear cells (deducted from blood smear), viable cells (by trypan blue staining method), and the CFU-GM colonies number. Best results of CD34+ cells and CFU-GM harvest were found in patients with a G-CSF associated chemotherapy mobilization.
Key words: haematopoietic stem cell, cytomorphology, flow cytometry, haematopoietic stem cell culture

Introduction

Peripheral blood stem cells (PBSC) are an important source for autologous transplantation in haematological disease cases or in solid tumours. The number of PBSC may be grown by administration of growth factors such as rhG-CSF alone or combined with chemotherapy.
Stem cell harvest is performed by discontinuous MCS Plus (Haemonetics) or continuous flow COBE Spectra (Gambro) by processing of total blood volumes (TBV) as follows: the standard volume leukaphaeresis (SVL), when 2-3 volumes are considered, and the large volume leukaphaeresis (LVL) procedure, with 3-6 volumes treated. The second method is more efficient with an important yield of CD34+ cells x 106/kg and the aphaeresis reducing number.

Stem cell harvest was established to be not lower than 3 x 106 CD34+/kg body weight, but optimal more than 5 x 106/kg. Post-transplantation neutropaenia and thrombocytopaenia might be minimalised by an optimal dose administration.

The quality of aphaeresis product is given by the subsequent laboratory parameters: total mononuclear cells (x 108/kg), cell viability (%), CD34+ cells (x 106/kg), and in vitro colony forming units granulocyte-macrophage (CFU-GM x 104/kg).

Haemogram, blood smears, viable cell number and also flow cytometry, CFU-GM assay, and bacteriological tests were determinated before and after PBSC harvest cryopreservation.

Patients and methods

Patients

Between September 2000 - November 2007, a number of 100 patients (42 male, 58 female) supporting autologous stem cell transplantation (ASCT) were enrolled in Haematology and BMT Fundeni Clinical Institute Centre. Median age at transplantation was 38 years (4 68) and by WHO criteria the following diagnoses were established: multiple myeloma (MM), 37 patients; Hodgkin disease, HD, 36 patients; non-Hodgkin's lymphoma (NHL), 14 patients; acute leukaemia (AL), 7 patients, neuroblastoma (NB), 3 patients and Ewing sarcoma (ES), 3 patients.

Stem cells harvest and freezing

Mobilisation of a sufficient number of HSC into the peripheral blood was achieved in 95 patients by administration of chemotherapy (DXM + Ifosfamide + Cisplatin/ Cyclophosphamide high dose/ DXM + Ifosfamide + Epirubicin/ DXM + Cisplatin + Cytosar/ Mitoxanthron + Cytosar) associated with growth factors (rhG-CSF), and in 5 cases (MM, NB) by growth factor administration only.

Mobilisation of CD34+ cells in peripheral blood was carried out by daily control of leukocytes and CD34+ cells number, when leukocytes value was ≥ 1.5 x 109/L. The large majority of leukaphaeresis procedures started when peripheral blood CD34+ cells number was

≥ 20 x 106/L.

In Fundeni Hospital Transfusion Unit 165 leukapheresis procedures were realised with MCS Plus and/or with Cobe Spectra in the following manner: in the first situation at 69 patients (123 leukaphaeresis procedures) and in the second situation 31cases (42 leukaphaeresis procedures). In 53 cases a single collection of PBSC was sufficient for a good harvest of CD34+ cells (≥ 3 x 106/kg).

Peripheral blood stem cells were mixed with DMSO (10% final concentration) in order to be protected during freezing. This one was achieved in a programable freezer MiniDigit Cool (-1450C temperature), or in liquid nitrogen freezer.

An electric or liquid nitrogen freezer were methods for yield cryopreservation (Espace 330 recipients).

Aliquots of the PBSC grafts were obtained for laboratory tests both from the native product (haemogram, blood smears, cell viability, number of CFU-GM, and CD34+ cells), from the mixture with DMSO solution (cell viability, bacteriological control), and after cryotubes/ HSC product thawing (haemogram, blood smears, cell viability, number of CD34+ cells, CFU-GM and bacteriological control).

Quality assessment of the graft

For this purpose, the samples were controlled before and after HSC product cryopreservation by the following investigations:

Haemogram (Coulter device) + *blood formula*, to obtain a number of leukocytes and percentage of mononuclear cells >> Cytomorphology Lab., Haematology Clinic, Fundeni Hospital;

Flow cytometry, to determinate the number of CD34+ cells x 106/kg; i.e. samples are treated with fluorescent counting beads, after specific staining of CD34+ cells >> Flow Cytometry Lab., idem;

Cell viability, using 0.4% trypan blue stain >> Haematopoiesis Lab., idem. Principle: viable cells do not take up certain dyes, whereas non-viable cells do. Cells are exposed to trypan blue solution (2p cell suspension : 1p trypan blue) for 5 to 15 min. A small amount of mixture is transfered to haemocytometer chamber and a separate count of viable and non-viable (blue stained) cells is carried out. After microscope counting, the following formula is applied:

$$\frac{\text{(total cell number - died cell number) x 100}}{\text{total cell number}}$$

CFU-GM assay, to determinate CFU-GM x 10^4/kg number by plating granulocyte-monocyte progenitors (GM) in soft medium >> Haematopoiesis Lab., idem. Mononuclear cells obtained from the HSC harvest (graft) by density gradient separation (Ficoll-Hypaque, 1.077g/ml density) are suspended in Iscove's Modified Dulbecco's Medium (IMDM, Gibco, BCR) after two successive cell washes. Cells are counted in a haemocytometer chamber before duplicate plating into methylcellulose based medium supplemented with optimal quantities of haematopoietic growth factors (rhStem Cell Factor, rhGM-CSF, rhIL-3, rhIL-6, rhG-CSF). Optimal number of plated cells is established by each laboratory, after a number of trials. Cells are incubated for 12 days in humidified incubator at 370C and 5% CO_2. Colonies (CFU-GM) are scored using an inverted microscope (clusters more than 40 granular and translucent or monocyte/macrophage large elements).
In TMO, CFU-GM number/kg is calculated by the following formula, applied to a harvest bag:

$$\frac{axbxcxd}{100000xg}$$

a: total leukocytes number; b: harvest volume; c. mononuclear cells percent; d: colonies number/ 10^5 mononuclear cells plated.

Bacteriological investigations were performed on aerobic and non-aerobic media, and results will be noted in another paper.

Results and discussion

Quality of the stem cell graft

Between September 2000 November 2007, at Fundeni Hospital Transfusion Unit, 165 PBSC harvest procedures in 100 patients were carried out. Approximate value of the following results is due to the fact that not all the leukaphaeresis procedures were tested before and after cryopreservation regarding cell viability, number of CD34+ cells, and CFU-GM.

Pattern and number of the investigations in the 165 PBSC products were as follows: cell viability % (CV) 143; CV % + DMSO 133; CD34+ cells x 10^6/kg 96; CFU-GM x 10^4/kg - 94; CV% thawed (thw) samples 94; CD34+ thw x 10^6/kg 48; CFU-GM x 10^4/kg thw 83. In 81/ 100 studied patients a good harvest (CD34+ > 3 x 10^6/kg/pacient and in 60/100 pacients an optimal harvest (CD34+ > 5 x 10^6/kg/pacient) were obtained.

The mean per patient of the CD34+ cells was 8.7 x 10^6/ kg (0.2 33) and the mean of the CD34+ collected cells was 5.5 x 10^6/ kg (0.04 33) per procedure, whereas the mean of PBSC product volume was 184.4ml (50 353).

Cell viability, just after harvesting or after keeping PBSC product at +40C till the next day, was 96.6% average (80 - 100%), whereas after the mixture with DMSO cryopreservation solution was 83% average (32 100%). (Table 1.)

In vitro testing of progenitor cell clonogenic features indicated a mean value of 231 x 10^4 CFU-GM/kg/sample, with a wide spread values (0.2 - 1704). A good correlation between the number of CD34+ icient

harvest cells/procedure and the number of CFU-GM/procedure (correlation coefficient of 0.64 - figure 1.) was observed.

Table 1. Mean values by procedure of the studied parameters in assessment of graft quality prior cryopreservation

No.LK/ pacient	CD34+ periphx10^6/ L	BVml	CD34+ harvestx10^6/k gc	Lex10^9/L	CV %	CV%+ DMSO	CFU-GM x10^4/kg	Mo%	Total Mo/ bag
1.65	69	184.4	5.5	201	96.6	83	231	83	251
(1-6)	(1.9-498)	(50-53)	(0.04-33)	(12.5-496)	(80-100)	(32-100)	(0.2-1704)	(42-100)	

Abbreviations
LK=leukaphaeresis;
BV=bag volume of HSC product;
CV=cell viability;
DMSO=dimethylsulfoxide;
CFU-GM=colony forming unit granulocyte-macrophage;
Mo=mononuclear cells;
Le=leukocytes of HSC product

Leukapheresis products were kept at +4^{0}C until next day. Freezing procedure was accomplished after mixing HSC harvest with DMSO solution at a final concentration of 10% DMSO. Total nucleated cells before cryopreservation indicated a mean value of 201 x 10^9/L (12.5- 496) and the mean value of mononuclear cells was 83% (42-100%). (Table 1.)

The average for haematopoietic stem cell product volume after mixing with DMSO solution was 325ml (106 - 617). The graft quality control after thawing was in most cases determinated from aliquots (cryotubes). The mean time of cryotubes/grafts preservation to thawing (+37^{0}C) was 2.4 month (0.5- 21). In the thawed product the mean value of cell viability (CV thw) was 62% (13-97%) and the average number of colony forming units granulocyte-macrophage (CFU-GM thw) was 65 x 10^4/kg (1 667). (Table 2.).
A positive correlation was found between CV ths and CFU-GM thw (0.30) (Figure 2.). All grafts were clean regarding bacteriological control.
The influence of freezing procedure in liquid nitrogen and cryopreservation on CV, number of total of nuclear cells/bag, CD34+ cells and number of CFU-GM was evaluated. A significant decrease of the above parameters was noticed (an average of 26%, 36%, 45% and 72% for CFU-GM). (Table 2.)

Table 2. Thawed state graft - quality evaluation by mean values of the studied parameters per procedure

Storage time/month	BV	CD34+ x 10^6/kgc	Lex10^9/L	CV%thw	CFU-GM x10^4/kgc	Mo %	Ttotal Mo /bag
2.4	325	3.0	60	62	65	86	161
(0.5-21)	(106-617)	(0.2-8.7)	(4.6-215)	(13-97)	(1.0-667)	(38-99)	

Abbreviations
CVthw=thawing cell viability;
DMSO=dimethylsulfoxide;
BV= product bag volume (DMSO added);
CFU-GM=c olony forming unit granulocyte-macrophage;
Mo=mononuclear cells;
Le=leukocytes

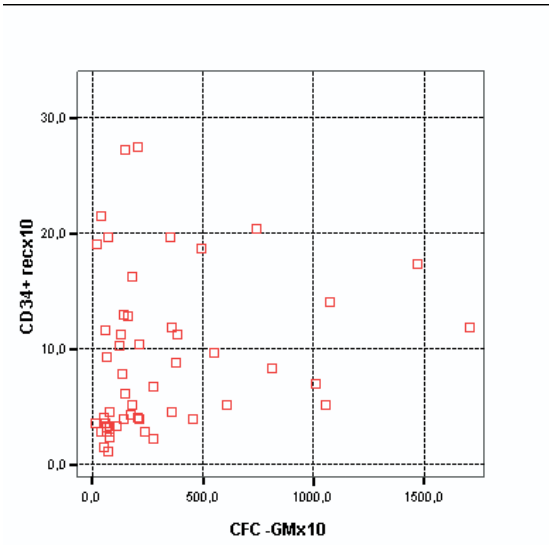

Figure 1. Correlation between CD34+ and CFU-GM yield per procedure

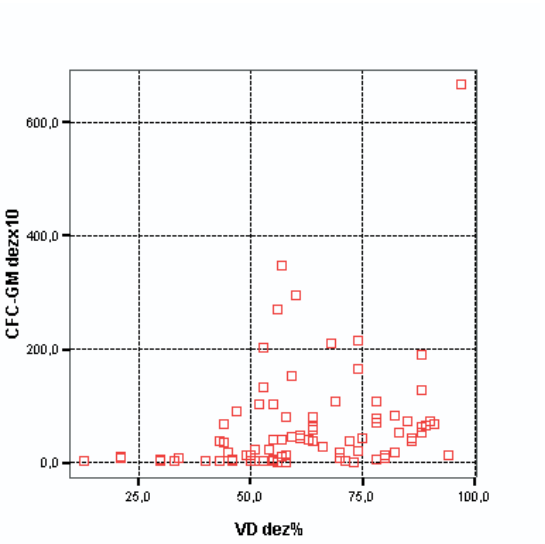

Figure 2. Correlation between CVthw and CFU-GM thw per procedure

Table 3. Studied parameters values per procedure by mobilisation mechanisms applied

Regimens	CD34+ perifx10^6/L	CD34+ yieldx10^6/kg	CV%	CV%+ DMSO	CFU-GMx10^4	CV% thw	CFU-GM x10^4 thw/kg 9.9(1.8-18)	Dgn(no. pac.)
Ne	33	2.1	94.3	85	133	70	only two samples	MM - 3 NB - 2
Cy	64.8	4.8	97.5	78.9	161.6	49	47.2	MM- 22 HD- 2
DHAP	113.7	9.3	96.9	80.8	359	60	83.3	HD - 24 NHL - 12
IFO+VP16	45.3	3.7	97.1	86.7	86.7	77.8	142.5	ES - 3
HAM	29.8	4.8	93.3	78.3	361.3	52.3	5.3	AL - 2
Cy+ETO	13.2	1.1	98.7	94.3		66.7	29	AL - 1
Cisplatin	62.5	3.9	96	87	453			NB - 1
Cytosar	12.1	0.8	97.8	96	35.9			AL - 1
EPI-IFO	33.3	2.1	95.2	88.7	98.3	83.4	76.4	MM - 2

Abbreviations
AL=acute leukemia; CFU-GM=colony forming unit granulocyte-macrophage; CV=cell viability; CVthw=thawing CV; Cy=Cyclophosphamide; Ne=neupogen; Dgn=diagnosis; DHAP=Dexamethasone+Cytosar+Cisplatin; DMSO=dimethylsulfoxide; EPI=Epirubicin; ES=Ewing sarcoma; ETO=Etoposide HAM=Ara-C+Mitoxantrone HD=Hodgkin disease; IFO=Ifosfamide; MM=multiplemyeloma; NHL=non-Hodgkinlymphoma; NB=neuroblastoma; perif=peripheral; VP-16=ETO

Ninety five patients received chemotherapy associated to G-CSF and 5 patients received only G-CSF (3 patients with MM and 2 patients with NB) in order to mobilise PBSC. Mobilisation algorithm data is available for only 75 patients. (Table 3.).

The great majority of patients received a mobilisation regimen with high doses of Cy (MM and HD) or DHAP (HD and NHL). (Table 3.).

A number of CD34+ cells and CFU-GM (mean values 9.3 x 10^6 CD34+/kg and 59 x 10^4 CFU-GM/kg) higher than high doses of Cy regimen (mean values 4.8 x 10^6 CD34+/kg and 161.6 x 10^4 CFU-GM/kg) was obtained by DHAP mobilization. For patients mobilised

with high doses of Cy, cell viability after thawing decreased 50%, much more than comparing to patients DHAP mobilisation (38% decrease) or neupogene (25% decrease). (Table 3.).

The influence of mobilisation algorithm

Patients having received chemotherapy and G-CSF achieved better results (greater number of CD34+ cells/kg/sample and CFU-GM/kg/sample) comparing to those receiving only growth factor as follows: 5.7 x 10^6 CD34+/kg respectively 240 x 10^4 CFU-GM/kg comparing to 2.1 x 10^6 CD34+/kg respectively 133 x 10^4/kg mean values (Table 4.).

No significant difference was found in cell viability before and after adding DMSO solution between the two mobilisation procedures: chemotherapy + G-CSF (97% → 83 %) and G-CSF alone (94% → 85%).

Peripheral CD34+ cells number before leukaphaeresis

Peripheral CD34+ cells number before leukaphaeresis has an important role in starting harvest procedure. The great majority of the procedures were started at the concentration of CD34+ cells > 20 x 10^6/L. A positive correlation was noticed between peripheral CD34+ and the values of CD34+ cells number obtained/procedure (cc = 0.85) respectively number of CFU-GM/procedure (cc = 0.80). These data are similar to those available in literature, where cc was found 0.80.

Table 4. Studied parameters values per procedure by growth factor mobilisation alone or associated with chemotherapy method

Regimens	CD34+ perifx10^6/L	CD34+ recx10^6/kg	CV%	CV%+ DMSO	CFU-GMx10^4	CV% thw	CFU-GM x10^4 thw/kg	dgn
Neupogen (G-CSF)	33	2.1	94	85	133	70	9.9(1.8-18) only 2 samples	MM, NB
G-CSF+ Chemo	47	5.7	97	83	240	65	67	all dgn.

Abbreviations

CV=cell viability; CVthw=thawing CV; DMSO=dimethylsulfoxide; CFU-GM=colony forming unit granulocyte-macrophage; perif=peripheral dgn=diagnosis; MM=multiple myeloma; NB=neuroblastoma

For the 29 leukaphaeresis started at CD34+ cells values between 10 20 x 106/L (mean value 15.2), a harvest of 1.4 x 10^6 CD34+/kg and 73 x 10^4 CFU-GM/kg was obtained (Table 5.). Those values were smaller comparing to leukaphaeresis started at peripheral blood CD34+ cells value > 20 x 10^6/L (mean value 89.5). The correlation coefficient between CD34+ cells and CFU-GM/procedure was 0.64. No significant cell viability variations regarding number of peripheral CD34+ cells at starting leukaphaeresis procedure was found.

The LVL procedure and starting stem cells harvest at CD34+ cells values > 20 x 10^6/L proved to be efficient by obtaining a higher number of CD34+ x 10^6/kg and apheresis number/patient decrease.

Table 5. Graft evaluation by number of CD34+ preleukapheresis peripheral blood cells - mean values of studied parameters

CD34+ perif x10^6/L	CD34+yieldx10^6/kgc	CV%	CV%+DMSO	CFU-GMx10^4/kg
<10 (mean 7.2)	0.6	94.3	85.2	35.5
10– 20 (mean 15.2)	1.4	95.8	87.8	73
>20 (mean 89.5)	7.6	96.6	80.8	324

Abbreviations

CV=cell viability; DMSO=dimethylsulfoxide; CFU-GM=colony forming unit granulocyte-macrophage; perif=peripheral

Haemogram influence

Haemogram and blood smear are very important in graft quality contol. This is to determine the number of total mononuclear cells/bag. These data are necessary to calculate number of CFU-GM x 10^4/kg. Different results cand be explained by varying different processing conditions: many types of laboratories equipments, harvest procedure ending time, thawing cryotubes procedure time. From 165 leukaphaeresis, haemogram, and blood smear before and after cryopreservation were carried out for only 62 cases. In 11/62 cases a higher total mononuclear cells number/bag in thawed product (cryotube/graft), mean values 201 x 10^8/bag (75.5-3772.2) comparing to native product, mean values 168.4 x 10^8/bag (13.8 - 362.8). In 2/11 harvests, CFU-GM number was higher than after thawing (666 and 347 x 104/kg), comparing to CFU-GM from native product (89 and 126 x 10^4/kg). For the rest of harvests (n = 51), the number of total mononuclear cells/bag in native product was 242.7 x 10^8/bag (14 - 741.9), respectively 146 x 10^8/bag (11.9-442.1) after thawing.

Cryopreservation influence on graft quality

For only 80/165 leukaphaeresis products, cell viability was determined both for native product and for thawed product. The stem cells grafts were stored in electric freezer (-1340C) most of the time. After the electric freezer broke, stem cells grafts were moved in nitrogen liquid (-1300C). The following mean values were found: CV 97% (90 100), CV+DMSO 82% (32 100), CVths 62% (13 97). Freezing and cryopreservation brought to a cell viability decrease in 16/80 cases and CVths was smaller than 50% in the end.
In 52/165 procedures the number of CFU-GM was found for both native product and cryotube/graft thawing. The obtained mean values are: native CFU-GM 237.4 x 10^4/kg (8 - 1704), CFC-GMths 80 x 10^4/kg (1 666). In 10/52 cases, CFU-GM number after thawing was higher comparing to native CFU-GM and this cannot be explained.

Some authors conducted a comparative study about cells recovery between cryopreserved grafts immediately after leukaphaeresis procedure (28 grafts) and next day cryopreservation after a +40C storage during night long (106 grafts). In the first situation, number of cells pre and post freezing was similar (mean value of CV =81% and all thawed cases having more than 50%). In the second situation mean value of CV decreased to 60%. For 46/106 cases CV value was smaller than 50%. CFU-GM assay showed that 1/28 products recently frozen presented less than 30% CFU-GM recovery than frozen products maintained at +40C during night long.

Conclusions

Peripheral blood haematopoietic stem cell graft obtaining and its parameters quality knowledge is the result of a team work: TMO Department group, Transfusion unit (harvest, freezing and stem cells storage), and the following research laboratories: flow cytometry, haematopoiesis, cytomorphology, bacteriology. The research laboratories have had the important tasks of accurate and in time analyses to appreciate graft quality before and after cryopreservation

The aim of this study was to demonstrate the importance of laboratory tests in an efficient collect of CD34+ x 10^6/L cells. Harvest procedure is conditioned by an available CD34+ x 10^6/L cell number for the beginning step. It is also important to evaluate freezing and cryopreservation procedures on graft quality referring to the following laboratory parameters: number of mononuclear cells, CD34+ cells, CFU-GM, percent of viable cells evaluated by trypan blue stain method and bacteriological control.

A precise evaluation of the graft quality was disturbed because not all leukaphaeresis products were completely analysed as regards to the above-mentioned tests before and after cryopreservation.
One hundred patients were studied in this paper and 165 procedures were completed to obtain stem cells. Quality control summarized next data: CV% - 143 procedures, CV%+DMSO 133 procedures, CFU-GM -94 procedures, CD34+ - 96 procedures, CV%thw -94 procedures, CFU-GMthw -83 procedures and CD34+thw -48 procedures.

The following conclusions could be drawn:

1) A positive correlation was found between circulating CD34+ cell number before leukaphaeresis and CD34+ cells number (cc 0.85), respectively CFU-GM number (cc 0.80). A better CD34+ harvest was obtained when leukaphaeresis procedures started at a circulating CD34+ cells value > 20 x 10^6/L. When CD34+ cells value < 10 x 106/L, harvest is not recommended. When CD34+ cells value is between 10-20 x 10^6/L (frequently met for patients treated before with multiple chemotherapy doses with not a good mobilisation), leukaphaeresis procedure starting is tried by LVL method.

2) For the patients receiving chemotherapy + G-CSF, a better graft was obtained (5.7 x 10^6 CD34+/kg and 240 x 10^4 CFU-GM/kg) comparing to patients who

received only G-CSF for mobilisation (2.1 x 10^6 CD34+/kg and 133 x 10^4 CFU-GM/kg) Between these two mobilisation procedures no significant cell viability was found before and after DMSO solution mixing (97% → 83% and 94% → 85%)

3) DHAP procedure applied to HD and NHL patients is more efficient both to peripheral stem cell mobilisation (mean 113.7 x 10^6/L) and graft quality. So, convenient CD34+ cells and CFU-GM values were obtained.

4) Correlation between graft quality parameters and poor recovery in thawed state is shown both in scientific papers and in this study as follows: cell viability malignant diseases, Cy usage for mobilisation; CD34+ cells malignant disease, storage time; number of CFU-GM malignant disease, Cy usage for mobilisation.

An important decrease cell viability after thawing was observed (CVths 49% mean) comparing to other mobilisation regimens (mean value CVthw 60% with DHAP mobilisation respectively 70% neupogen mobilisation)

5) Haemogram is recommended to be evaluated with the same equipment, leucocytes to be counted in Türck haemocytometer and a 1/10 dilution to be made with automated micropipette to avoid errors.

6) During freezing and graft cryopreservation, a significant decrease of cell viability, the number of total mononuclear cells/bag, CD34+ cells and CFU-GM is observed, with average values of 26%, 36%, 45% and 72% (Table 1. and 2.).

Scientific literature showed that above mentioned behaviour happens because of graft freezing after +40C storage over night long. In our study cell viability before and after cryopreservation was carried out for only 80/165 HSC products.

We found out that temperature variations from the electric freezer and cell freezing next day may be two important factors for cell viability decreasing and mainly for the number of CFU-GM.

Taking all the above into consideration we can conclude that stem cell graft quality control after harvest and especially after cryopreservation and thawing is very important through laboratory analyses.

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ANAGRELIDUM
Guidelines for Diagnosis and Treatment of MPD-T

I. Definition of the disease

Essential thrombocythemia

MPD are characterized by disturbances of stem hematopoietic cells. These disturbances are caused by the clonally expansion of the stem hematopoiatic cells and determine a group of diseases that include four entities: essential thrombocythcmia, polycythemia vera, chronic myeloid leukemia and osteomyelofibrosis.

Essential thrombocythemia is characterized by an increase of platelets number above the normal limit that is considered by the majority of the laboratories to be between 150,000 and 450,000/pl (Petrides 2001). Generally, the increase of the platelets number, high age and additional risk factors like hypcrcholesteremia and/or diabetes determining vascular alterations are associated with high risk of thromboembolic complications.

Essential thrombocythemia is considered a middle age disease, beginning in 5th and 6th decades of age and easily increased preponderance for women (3, 4). The disease is also frequent diagnosed to the asymptomatic patients, young adults and even children (approximate 10 - 25% from the patients diagnosed with essential thrombocythcmia are adults aged below 40 years old). We have to remark that, from the total number of the asymptomatic patients, approximate 7% will become symptomatic. The disease incidence in general population is 2, 5 cases/] 00000persons/year.

II. StaEes of the disease

There are no specific stages described in medical literature for this disease. Increased number of platelets determines the installation of the thromboembolic events that determine the morbidity increase and, if the coronary, cerebral or pulmonary arteries are involved even the increase of mortality. For this reason, primary and secondary prevention of thrombosis by decreasing the platelets number has a critical importance for the patients diagnosed with essential thrombocythemia. It is estimated that 25% from the total number of patients suffering from essential thrombocythemia will have thrombocmbolic complications (Beykirch et al, 1997).

III. Including criterions (AEe Sex Clinical and Para clinical parameters etc)

The internationally adopted PVSG criteria make ET a diagnosis by exclusion, and attempt to exclude cases with secondary (reactive) thrombocytosis and other MPDs, based on recognition of some specific positive criteria of the diseases to be excluded (Ph chromosome for chronic myeloid leukemia, the increased red cell mass or hematocrit for PV, excessive collagen fibrosis for IMF) and some nonspecific criteria to exclude secondary thrombocytosis (e.g., in inflammation or in iron store deficiency), but do not offer a single criterion of positive recognition for ET.

In sharp contrast, the novel diagnostic criteria of MPDs, elaborated by Michiels et al and the European Working Group on MPD and the new ECP criteria (as the extension of the pathological WHO criteria) are based primarily on a positive recognition feature of each MPD subtype (i.e., on boric marrow histopathology). It has been shown by Thiele et al that histopathology may distinguish between ET, PV (including its prepolycythcmic stage), and IMF (including its prefibrotic and early fibrotic stages IMF-0 and IMF-1), and, in addition, it may even distinguish cases with secondary thrombocytosis or erythrocytasis.

Following the information described above, some conclusions are important and necessary:

1. Nosological diagnosis of MPD-T according to the ECP or WHO criteria is strongly recommended. We emphasize that biopsy must be performed in specialized centers before treatment is administered. Only in patients already pretreated with cytoreductive drugs and when no diagnostic biopsy was performed, and in elderly patients and those in poor clinical condition is the diagnosis according to the PVSG criteria acceptable.

2. The aim of management of MPD-T is to overcome the possible fatal complications and to prevent or alleviate the clinical symptoms. The most important goal is to prevent thrombosis and thromboembolism as the main cause of morbidity and mortality.

3. Treatment must be adapted to the individual patient's risk of thrombosis and ma sjor bleeding. Bleeding can be prevented easily by keeping the platelet counts below 1000 X 10 /L (or below 1500 X 109/L in the patients younger than 40 years) by using cytoreductive drugs and avoiding antiaggrcgants at these high counts.

The following criteria are recognized as major risks of thrombosis and embolism:

a) Age older than 60 years
b) Previous thrombotic event
c) Platelet count (350-2200 X 109/L with a peak at 900 X 109/L), as inferred from the meta analysis made by Michiels et al, and from the finding that cytorcductivc treatment prevents thrombotic complications.

d) Additional risk factors include inherited thrombophilia (protein C and S deficiencies, the Leiden mutation of TV, antithrombin deficiency, etc). Very high levels of FII and FVIII, as well as low levels of FXII may be taken into account if (optionally) tested. Further recognized risk factors include antiphospho-lipid syndrome; clinically serious forms of atherosclcrosis of the coronary, cerebral, and lower limb arteries; any hypercoagulable state in pregnancy; systemic infection; additional malignancy; and major surgery.

e) Treatment should not be harmful to the patient (the principle of non nocere). If we consider the possible Icukemogenicity of any cytostatic drug, including HU, the drug may be administered for prolonged periods of time only in patients whose life expectancy is not substantially longer than the median time of transition to s-AML (-15 years). Arbitrarily, HU may be given as frontline therapy to patients older than 60 years.

IV. Treatment (doses, doses adjustment, treatment period)

The therapeutic goal of thromboreductive therapy with HU, ANG, or IFN should be the normalization of platelet counts (below 400 X 109/L) in high-risk patients with an indication to thromboreducing agents, especially in those with additional thrombophilic risks. In low-risk patients without additional thrombophilic risk factors (whose indication to cytoreductive therapy was based solely on the excessive platelet count), the goal to reach the counts below 600 X 109/L seems satisfactory. Maintenance treatment is always necessary. Treatment is necessary for life duration.

On the basis of the above-summarized principles, we conclude on:

Primary Treatment Algorithm for MPD-T, Based on Individual Risk Estimates

Platelet Count (x 10^9/L)	**18-60 Years Asymptomatic/ Negative History of T-E Events, Thrombophilia Negative**	**18-60 years Symptomatic/ Positive History of T-E events, Thrombophilia Positive**	**>60 Years**
400-1000	(0) or ASA	**IFN or ANG+ASA**	(HU*)+ASA
600-1000 progressive	I FN or ANG+ASA	**IFN or ANG+ASA**	HU+ASA
1000-1500	I FN or ANG or ASA*	**IFN or ANG (+ASA*)**	HU(+ASA)
1500-2000	**(HU ^) IFN or ANG**	**HU IFN or ANG**	HU
>2000	**HU (±TAF) IFN or ANG**	**HU(±TAF) IFN or ANG**	HU
> 2000 +major bleeding	**HU + TAF IFN or ANG**	**HU+TAF IFN or ANG**	TAF + HU
	Standard risk	High risk	

*ASA allowed in very yung patients or in older patients with cardiological indication for ASA
Progressive thrombocythemia with increments of platalet couns >200 x 10^9 L in 2 monts. *HU must be given to patients with an additional thrombophilic state; in others it is optional, MPD-T, myeloproliferative disorder with trombocythemia; T-E, thromboembolism; ASA, acetylsalicylic acid; INF, interferon a; ANG, anagrelide; HD, hydroxyurea; TAF, thrombapheresis; O, optional.

Comments:

1. We have introduced the category MPD-T with progressive thrombocythemia, based on the experience with those patients with increases of platelets >200x109/L in 2 months will always achieve counts for which thromboreducing therapy is indicated. Early introduction of therapy minimizes the time when they are at higher risk of thrombosis, according to the study showing that the thrornbotic risk is dependent on the time of exposure to elevated platelets.

3. The recommended dosages are the following: ASA, 50 to 100 mg daily (or 100 mg every other day); ANG, 0.5 to 5.0 mg/d; IFN, 1 to 30 M1U/wk; HU: 0.5 to 2.0 mg/d.

3. In case of an insufficient effect of these doses or the occurrence of major side effects, IFN may be a substitute for ANG and vice versa. In case of an insufficient effect or slight toxicity or side effects, another drug (of the three thromboreductivc agents) can be added in combination, allowing the reduction of the dosage of the first-line drug.

4. The choice between ANG and IFN is left to the treating physician, adapting the treatment according to the case's particularities (e.g., his or her compliance to self-administer IFN, etc). In true ET, ANG may be the drug of choice in the younger patients, without representing an absolute recommendation.

5. At high platelet counts (> 1500 X 109/L, and especially at >2000 X 109/L) exists both the hemorrhagic and thrombotic risks in parallel. As soon as possible is mandatory to achieve platelet counts below 1000 X 109/L. At this level, the sole risk is thrombosis, which can be further reduced by addition of ASA. For the purpose of quick platelets reduction, we advocate HU even in younger patients (< 60 years). Once the platelet count achieves < 1000 X 109/L, we switch from HU to either ANG or IFN.

6. If the patient receiving ANG or IFN therapy has no significant side effects and exceeds the age of 60 years, continued administration of the respective medication is allowed.

7. ASA may be optionally discontinued in low-risk patients if thromboreductive maintenance therapy steadily keeps the platelet counts below 400 X 10'/L. ASA is not given to patients receiving anticoagulants (warfarin and similar drugs), which is planned to be life-long therapy in patients with a history of venous thromboembolism. ANG will not be administered to pregnant women or to women that plan the pregnancy.

8. Bleeding has to be managed using ethamsylate, plasma derivatives, and nonspecific agents. Antifibrinolytics or activated coagulation factor concentrates should be avoided or used exceptionally and cautiously.

This holds true especially in patients with a history of thrombocmbolism, given that these therapeutic interventions might provoke its recurrence. Naturally, administration of antiaggrcgants must be stopped.

Thromborcductin (anagrelidum) has to be individual dosed for each patient. Initial dose is 1 mg daily administered orally, two times per day. Initial dose has to be maintained at least one week. After one week, the dose can be increased gradually for each patient in order to obtain the minimum necessary and efficacy dose for reducing and/or maintaining the platelets number below 600 $x10^9$/L and, ideally, to values between 150 $x10^9$/L - 400 x 10^9/L . The increase of the dose has not to overpass 0,5 mg each week and the unique maximum recommended dose has not to overpass 2, 5 mg.

Therapeutical response has to be periodically controlled. If the initial dose is >lmg per day, the number of platelets has to be controlled once at each two days in the first week of treatment and at least once weekly after this period until the maintenance constant dose is obtained. Usually, it is observed a decrease of platelets number in 14 - 21 days since the beginning of the treatment and majority of patients do have and maintain a optimum therapeutic response with 1 - 3 mg daily dose. Changing of the previous treatment (HU or INF) has to be done gradually, by interpenetration. ANG is life - long administered drug. After the treatment is stopped, increased platelets number (to the values before the beginning of the treatment) will reappear during few days.

V. Treatment monitoring (clinical & paraclinical parameters and periodicity)

Treatment monitoring means strictly monitoring of platelets number. It is recommended regular determination of Hb, platelets count and white cell count. Evaluation of liver parameters (OGT, PGT) and renal tests (seric creatinin, ureea) has to be done regularly, especially in cases of liver or renal preexisting impairment. Any hemorrhagic or thrombotic event is an alarm signal for the patient to visit the physician.

It is not recommended to use at the same time ANG with fosfodiesteraze inhibitors (PDE III). It is recommended cautious for children administration. Due to lactose content of ANG, it is not administered to the patients with galactose intolerance, lactase's deficit or malabsorbtion syndrome for glucose - galactosis.

VI. Treatment exclusion criteria

AND is not recommended to patients with hypersensitivity to ANG or any of the product clecaiary. ANG has also not to be administered to patients with severe liver insufficiency and also to patients with severe renal impairment (creatininc clearance <50 ml/min).

In clinical studies, patients suffering severe heart alterations (degree III or IV) with a negative ratio risk/benefit were excluded. ANG has not to be administered during pregnancy or breast feeding.

In case of therapeutic resistance to ANG, physician has to consider other treatment. During treatment, platelets count has to be regularly performed.

VII. Treatment rerun (conditions) - it is not applicable - ANG has to be recommended for unlimited time period.

VIII. Prescribers - hematologists and oncologists

ORAL SESSIONS

NEW AGENTS FOR THE TREATMENT OF ACUTE MYELOID LEUKAEMIA

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The clinical trials from the last 20 years, using conventional cytotoxic agents, could not provide evidence of significant improvements in survival, particularly for adults and elderly patients. It is necesary to develope new therapeutic strategies to improve the remission rates of the patients, and this is becoming possible thanks to the increased understanding of the molecular basis and heterogeneity of the disease as well as major advances in biotechnology.

New therapies used in the clinical trials are: new cytotoxic agents, nucleoside analogues, antibody-directed chemotherapy and molecular therapies that target genetic abnormalities of the leukaemic cells. Although most of these new compounds are active when they are used as monotherapy, clinical development programmes are trying to fiind ways to incorporate them into conventional chemotherapy regimens, to increase their leukaemia cells destroi capacity.

This report provides an overview of some of the more promising new agents in development for use in Acute Myeloid Leukaemia (AML).

Class	Agents under evaluation	Clinical Trials
Cytotoxic agents	Troxacitabine	Phase I/II
	Clofarabine	Phase II/III
	Cloretazine	PhaseII/III
	Amonafide	Phase II/III
Monoclonal antibodies	Gemtuzumab ozogamicin	Phase II/III
FLT3 Inhibitors	PKC-412, CEP-701, MLN-518	Phase I-III
Farnesyltransferase Inhibitors	Tipifanib, Lonafanib	Phase I-III
mTOR Inhibitors	Sirolimus, Temsirolimus, Everolimus	Phase I/II
Antiangiogenesic agents	Bevacizumab, PTK-787, Lenalidomide	Phase I-II
Histone deacetylase Inhibitors	Depsipeptide, Valproic acid, Vorinostat	Phase I/II
Proteasome Inhibitors	Bortezomib	Phase I/II
Apoptosis modulators	Oblimersen	Phase II/III
Cell cycle Inhibitors	UCN-01	Phase I
Hypomethylating agents	Azacitidine, Decitabine	Phase II/III
MDR Modulators	Zosuquidar	Phase II/III

Cytotoxic agents

Several new drugs are under investigetion such as the nucleoside analogues: Troxacitarabine and Clofarabine.

Troxacitarabine is the first L-enantiomer nucleoside analogue with anticancer activity. It is active in refractory AML when combined with cytarabine, idarubicin and topotecan, and also in patients with adverse cytogenetic abnormalities.

Cloforabine, structurally similar to fludarabine and cladribine, is a second-generation purine nucleoside analogue. It was studied in acute leukaemias, includig refractory AML, in elderly patients, as single agent or in combination with conventional chemotherapy.

Alkylating agents

Cloretazine is an agent with promising antileukaemic activity in patients with advenced AML, both as single agent or in combination with chemotherapy.

Topoisomerase II inhibitors

Amonafide is a catalytic inhibitor of topoisomerase II that is different from the classical topoisomerase II inhibitors, with good rresults in patients with high risk AML and in secondary AML, both as monotherapy and in combination with cytarabine.

Monoclonal antibodies

Lineage-associated antigens axpressed by the leukaemic cells are the target for the treatment with monoclonal antibodies thatcan be used in the unconjugated form or conjugeted to cytotoxic drugs, toxins or radionuclides.

Most clinical studies refer to the monoclonal antibody

targeting the CD33 antigen, which is expressed on normal haematopoietic precursors and on more than 90% of AML blasts. Good results have been obtained with gemtuzumab ozogamicin (GO) used in AML in first relapse, elderly patients or new diagnosed AML patients. It is also active in patients with acute promyelocytic leukaemia and in reduced-intensity conditioning for allograft.

FLT3 and other thyrosine kinase inhibitors

FLT3 belongs to the receptor thyrosine kinase class III family and plays an important role in an early stage of haematopoiesis. FLT3 is expressed by normal myeloid and lymphoid early progenitors as well as by leukaemic cells in 70-90% of patients with AML. Activating mutations of the FLT3 gene occur in approximately 30% of patients with AML and confer a poor prognisis. There are several identified molecules to inhibit this thyrosin kinase; those were used in newly diagnosed AML cases or in relapse, mostly in combination with chemotherapy.

C-kit, a receptorthyrosine kinase, is expressed in most myeloid blasts and mutations of c-kit have been described in approximately 30% of AML cases and may worsen the prognosis.

Farnesyltransferase inhibitors (FTY)

Activating mutations of the RAS family proteins have a pathogenetic role in the development of myeloid leukaemias. The biological activity of the RAS proteins depends on post-translational farnesylation. Farnesyltransferase inhibitors (Tipifarnib) were used for their antileukaemic activity, expecially in advance phase AML, refractory or relapsed AML, high risk newly diagnosed AML and older adults.

mTOR inhibitors

The m TOR inhibitors are involved in the critical regulatory pathway of many cellular processes including matabolism, proliferation and apoptosi. The m TOR inhibitors have e role in leukaemic cells death, but not of normal CD34+ cells. Rapamicine (sirolimus) was efficient in patiens with high risk AML, relapsed and refractory AML.

DNA methyltransferase inhibitors

DNA methylation abnormalities are present in AML and increase with disease progressin. Azacitidine and decitabine are powerful inhibitors of DNA methyltransferase with established clinical activity in myelodisplastic syndroms and also in AML, mostly used in refractory and elderly AML patients.

Conclusions:

Improved understanding of the biology of AML has driven the development of new agents used in the clinical practice with promising activity and lower toxicity than traditional chemotherapy.

Because of the good bioavailability and the generally favourable side effect profiles this agents are particurarly attractive treatment options in the older population or in patients not suitable for conventional chemotherapy.

The future studies will serve to define the optimal roles of these molecules in the treatment of AML and the most effective regimens in which to use these agents.

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PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIACURRENT DIAGNOSTIC AND THERAPEUTIC ASPECTS

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Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid cells that occurs in all age groups, from infants to elderly. Philadelphia (Ph) chromosome-positive ALL is an area of unmet medical need. The Ph cromosome has been detected in approximately 15-30% of adults with ALL; only <5% of children have Ph+ disease. The incidence of Ph+ ALL increases with age, rising to approximately 53% in older patients, compared with about 20% in young adults. The

frequency of Ph+ ALL is similar in men and women. Ph+ ALL is characteristically found in disease affecting the B-cell lineage.

BCR-ABL fusion protein activates downstream signaling pathways and ultimately impairs the proliferation and diferentiation of lymphoid precursor. Among BCR-ABL fusion proteins denoted by their molecular weight, the p190 BCR-ABL is the predominant form in Ph+ ALL (60-80% of cases), p 210 BCR-ABL is found in about 20% of cases, and the remaining Ph+ ALL patients have both fusion proteins. The p 190 BCR-ABL fusion protein has higher tyrosine kinase activity than the other molecular variants, and this activity is correlated with the ability to stimulate lymphoid proliferation. The frequency of the BCR-ABL gene rearrangement (molecular analysis using RT-PCR) is found to be higher than the frequency of the t(9;22).
At diagnosis, older age, high WBC count, certain immunophenotypes (CD10 negative precursor B-cell type), and certain cytogenetic abnormalities are the most important prognostic factors. The presence of BCR-ABL rearrangement has been recognized as the most adverse prognostic factor for ALL. Although complete remission (CR) is achieved in 50-80% of patients after intensive chemotherapy, which is slightly inferior to those without this anomaly, long-term outcome is dismal with overall survival of approximately 10%. The most common cause of treatment failure is relapse, and most patients suffer a relapse within the first year after achieving CR.

Currently, as a target therapy, imatinib and other new tyrosine kinase inhibitors (dastinib, nilotinib) lead to a considerable improvement of disease outcome. As single drug therapy, imatinib can induce CR in de novo Ph+ ALL. Combination of intensive chemotherapy and imatinib can rapidly induce high-quality CR for a majority of patients with newly diagnosed BCR-ABL-positive ALL, and about 50% molecular remission. Allogeneic hematopoietic stem cell transplantation (SCT) is thought to be the only curative therapy for the disease in adults, holding the promise of long-term survival, but many patients are ineligible and treatment-related mortality remains significant. It is extremly important that the transplant can be delivered during the first CR because the disease status at the time of SCT is a strong indicator for long-term survival. Outcome after SCT depends on pre-transplant and post-transplant minimal residual disease (MRD). In many cases, patients who successfully receive a SCT still have molecular evidence of disease and remain at risk for relapse. Post-transplant imatinib therapy contributes to a lower relapse rate.

Development of resistance based on imatinib domain mutations can lead to patients relapse. Attempts to reduce the incidence of mutations and to overcome r resistance (with alternative kinase inhibitors), may further improve outcome of Ph+ ALL. It is possible that inhibitors of other targets in the signal transduction cascade (e.g. farnesyl transferase inhibitors, mTOR inhibitors) may eventually be used in combination with drugs such as imatinib and nilotinib for the treatment of Ph+ ALL.

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. DISEASE OR SYNDROME?

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B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous entity from a a clinical and hematological point of view, having a variable clinical course. Even before the advent of the Rai and Binet stadialisation systems which delineate incipient, intermediary and advanced stages, it was observed that there are forms with a benign evolution and forms with a aggressive clinical course.

Recent studies targeting the variable region of the immunoglobulin heavy chain genes (IgVH) have described 2 entities with different prognosis according to the mutated or unmutated status of these genes. The group of patients with mutant IgVH status have a relatively benign clinical course, while the group with non-mutant IgVH status have a much more aggressive evolution. However, a subgroup of patients with an atypical mutation at IgVH 3' 21, with distinctive phenotypical and genotypical characteristics and poor prognosis was identified. Due to the difficulties encountered in the sequencing of the IgVH gene a several surrogate markers have been evaluated. The expression of thge CD38 antigen is correlated with the non-mutational status and signifies a worse prognosis. Similarily, the hyperexpression of the ZAP70 protein, an intracellular tyrosine-kinase with a critical role in T-cell receptor signalling is correlated with the non-mutant IgVH status and with a worse prognosis. Several cytogenetical abnormalities such as 17p- si 11q- are associated with poor prognosis.

Another recently studied aspect is the clinico-hematological and therapeutical implications of the expansion of cytotoxic T lymphocytes in B-CLL. It was observed that patients in which there is a decrease in activated T-helper lymphocytes and an increase in cytotoxic T cells and NK cells have a lower tumor burden (smaller lymphadenopathy, splenomegaly/

hepatomegaly) but often a more aggressive clinical course. Various T-cell and NK cell abnormalities are associated with morphologic variants of the leukemic cells. The number of CD4+ and CD8+ T-cells expressing cytoplasmic TNF-, play a role in disease proggression towards advanced stages. The expression of IFN- in CD3+, CD8+ T-cells also correlates with disease stage. The principal mechanism of anemia and thrombocytopenia of advanced stages is the immune, T-cell mediated suppression of erythroid and megakaryocytic progenitors and precursors.

These findings may constitute the theoretical basis for a more tailored therapy in advanced stages of CLL with small tumor burden, with immunosuppression/ immunomodulation rather than cytotoxic chemo-therapy.

ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS. 5-YEAR EXPERIENCE OF THE HEMATOLOGY DEPARTMENT OF THE "ION CHIRICUTA" CANCER INSTITUTE CLUJ-NAPOCA

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Introduction. Acute lymphoblastic leukemia (ALL) in adults represents about 15-20% of all newly diagnosed adult acute leukemia cases, involving especially younger patients. Despite significant progress over the past decades, results are still inferior to those obtained in children.

Material and Methods. 49 patients, 28 men (57%) and 21 women (43%), with a mean age of 35.8 years (16-68), diagnosed and treated in our department between 2003 and 2007 were studied.

Results. 37 cases were of B lineage (76%) and 12 cases were of T lineage 24%. Among the B-cell cases, 19 (50% of B-lineage cases) presented a preB (common) immunophenotype. 13 cases (28%) displayed extralymphatic involvement. Only in 11 patients a cytogenetical analysis was made; cytogenetics were not instrumental in therapeutical decision making. The first line induction treatment consisted of the local "standard" regimen in 16 patients (32%), of the HyperCVAD regimen in 13 patients (26%); in 8 patients (16%). Complete remission (CR) was obtained in 35 patients (71%). Relapse occurred in 17 patients (48%), on average at 14 months (4-56 months). A second CR was obtained in 10 cases (58%). The median overall survival was 25 months and the median relapse free survival was 12 months. Both CR rate and overall survival were significantly better in patients <40 years (p=0.03) and in those with preB immunophenotype (p=0.03 and 0.01 respectively). There were no significant differences regarding the initial induction regimen, sex, initial white blood cell count. Given the low number of tested patients no conclusion can be drawn regarding the value of cytogenetics. Allografting was performed in only 2 patients, one of them dying due to early relapse.

Discussion and conclusions. The results of adult ALL treatment are still unsatisfactory. Even though the CR rate is close to world standards, the early relapse rate is still unacceptably high. As future directions, the routine incorporation of cytogenetics in initial evaluation as well as of targeted treatments (such as rituximab in B-ALL and tyrosine kinase inhibitors in Ph+ ALL) in the induction protocols and a more widespread use of allografting may further improve overall results.

The role of hepatitis infection in the ethiopathogeny of chronic lymphoproliferative syndromes

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Key words: chronic lymphoproliferations, hepatitis virus infections, HBV, HCV

Introduction:

Patients with chronic lymphoproliferative syndromes (CLS) frequently associate infections with hepatitis viruses HBV, HCV, HDV. Although these viruses have a primary liver tropism, it is demonstrated that they can also infect and replicate inside haematopoetic cells.

Objectives:

This study is a part of Project LIMFOVIR, which proposes multidisciplinary research to identify the molecular mechanisms involved in lymphoproliferation oncogenesis. We analyzed a group of patients with CLS associated with HBV, HDV, or HCV infection, diagnosed and overseen between March 2005 and March 2008 in Haematology Department of SUUB. The main objectives of this study were: establishing the diagnosis of CLS through immunophenotipic and histopathologic examination, statistical analysis of clinical, paraclinical and immunophenotipic data, identifying correlations between these parameters recognizing prognostic clusters and establishing a relationship between the results in the data base of LIMFOVIR.

Material and method:

We analyzed 18 patients (12 males and 6 females); 3 patients were between 20 and 50 years old, 10 between 51 and 70 years and 5 patients were over 70 years old. The histological types were as follows: diffuse and follicular non-Hodgkin lymphoma (NHL) - 14 patients, T cell NHL - 1 patient, chronic lymphocytic leukemia (CLL) - 2 patients, Hodgkin Lymphoma - 1 patient. The distribution according to viral infection was: 7 patients with HBV infection, 9 patients with HCV infection, 1 patient with HBV + HDV, and one patient with HBV+HDV+HCV. The observed clinical parameters were: B signs of hematological disease, presence of enlarged lymph nodes (superficial and internal), enlarged liver or spleen, signs of worsening liver disease. The analyzed hematological and biological parameters were: presence of anemia, thrombocytopenia, lymphocytosis, hemolysis, crioglobulines, the status of the liver function.

Results, discussions, conclusions:

The analysis of a small group of CLS patients with hepatitis viral infection showed an increased frequency over 50 years, especially in females. There is an increased association of the viral infection with CLS with B lymphocytes - 14 patients present large or small B cell NHL or B-CLL. There is an equal occurrence of aggressive and indolent types of NHL, although the majority of the studies showed an increased incidence of indolent lymphomas in patients with hepatitic viral infections (imunocitoma, marginal lymphoma, non-gastric MALT lymphomas). The most frequent infection was with HCV, and the most uncommon pattern included the infection with more than one virus.

Most of the patients presented B signs of disease, enlarged lymph nodes and hepatosplenomegaly, and only a small number had liver disfunction. The alterations of the hematological parameters secondary to the CLS were insignificant at the onset. We intend to follow the clinical and hematological parameters through the chemotherapy associated or not with antivirals, as well as the influence of viral infection on the response to chemotherapy and the reactivation of viral infection.

Studiul flowcitometric al trombocitului in leucemia acuta mieloida

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Observatie: Prezenta lucrare a fost conceputa pe baza datelor culese in cadrul proiectului MULTRO (Grant CEEX 62/2005)

Introducere si obiective:

Hemoragia extinsa reprezinta una dintre cele mai frecvente cauze de deces in leucemiile acute mieloide (LAM). Studii anterioare asupra comportamentului trombocitar asociat acestor boli an identificat tulburari ale proceselor de activare si agregare. Scopul acestei lucrari a fost identificarea utilitatii examenului flowcitometric ca metoda de investigare a functiei plachetare dar si stabilirea unei posibile corelatii intre defectele proteinelor trombocitare de suprafata si alti factori clinico-paraclinici de rise hemoragipar (febra, sepsis, sangerare recenta, uremie, leucocitoza, hematocrit, tratament).

Material si metoda:

S-a efectuat analiza flowcitometrica a receptorilor trombocitari de suprafata in probe de sange periferic recoltate pe anticoagulant citrat la 22 pacienti diagnosticati cu leucemie acuta mieloida de diferite subtipuri conform clasificarii FAB (French- American-British) si aflati in stadii diferite de tratament. S-au analizat receptorii plachetari de suprafata implicat in procesele de adeziune (Glicoproteina Ib-IX [CD42b, CD42a]), agregare (Glycoproteina Ilb-Illa [CD41, CD61]) si activare (Pselectina [CD62P], granulofizina [CD63]) in lotul de pacienti comparativ cu on lot de 10 voluntari sanatosi.

Rezultate:

In urma analizei efectuate s-a observat o scadere semnificativa a nivelului de fluorescenta a markerilor de activare (CD63 [14.11% vs. 40.78 % P<0.05]; CD62P [15.26% vs. 28.23% P<0.05]); a markerilor de adeziune (CD42b [69.08% vs. 84.41% P<0.05]) si a markerilor de agregare (CD61 [83.79% vs. 98.62% P<0.001]) la pacienti in comparatie cu lotul de control. Nivelul de expresie al CD41 [80.62% vs. 86.31%, P=0.290] si CD42a [77.98% vs. 94.15%, P=0.99] nu a prezentat diferente in lotul de pacienti comparative cu lotul de martori.

Nu s-au identificat corelatii semnificative statistic intre receptorii plachetari luati in lucru si factorii de rise hemoragipar considerati.

Concluzie: Pacientii cu leucemie acuta mieloida prezinta modificari ale receptorilor trombocitari de adeziune, activare si agregare sugestive pentru un defect functional de suprafata sau o denaturare a caii de semnalizare intracelulara.

Datele expuse ilustreaza rolul examenului flowcitometric in identificarea efectiva si specifica a multiplelor defecte functionale trombocitare prezente la pacientii cu leucemie acuta.

Diagnosticul leucemiei limfocitare cronice prin flowcttometrie-profilul imunofenotipic complex

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Introducere.

Sistemele de stadializare clinica Rai si Binet raman cele mai utilizate metode de evaluare prognostica in leucemia limfocitara cronica (LLC). Imunofenotiparea limfocitara este metoda de diagnostic consacrata in LLC, cat si in determinarea unor marked de prognostic. Acestia se clasifica astfel: imunofenotip atipic FMC7, CD20++; imunofenotip aberant cu marked mieloizi CD14, CD36, CD13, CD33; marked solubili sCD23, sCD25, sCD27; marked de activare (CD38); surrogat pentru statusul mutatiei IgVH (ZAP-70); supraexpresia cyclin Dl, bcl2; molecule de adeziune: CD54 (ICAM-1), sCD44.

Materiale si metode.

Am analizat 162 pacienti diagnosticati cu LLC pentru a depista corelatii cu stadiul clinic, imunofenotip, evolutie. Diagnosticul s-a stabilit prin flowcitometrie cu asocierea clasica a markerilor CID 19, CDS, CD23 si CD20, CD79b, FMC7. La aoesti pacienti am anlizat si alti marked de suprafata CD38, CD27, CD43, si markeri intracelulari Cyclin Dl, Bcl-2 si ZAP70. Analiza s-a efectuat pe flowcitometrul BD FACS Flow Calibur, cu software de achizitie Ce1lQuest. Analiza statistica a fost efectuata utilizand SPSS software version 10.

Rezultate.

Pacientii cu LLC au fost stratificati pe baza stadializarii clinice astfel: 22,34% stage 0, 30,85% stage I, 26,6% stage II, 5,32% stage III, and 14,89% stage IV. Diagnosticul LLC s-a stability prin coexpresia CD 19/CD20-CDS-CD23-CD79b low. Am studiat markerii de prognostic pentru statusul mutatiei IgVH. am gasit corelatii intre expresia CD38 si evolutie dr: 0.541, p<0.05.), si intre ZAP-70 si CD38 (dr: 0.666;p=.018). Expresia BCL-2 se coreleaza cu evolutia (dr: .533, p<0.01) si raspunsul la tratament (dr: .420, p<0.01). De asemenea, s-a gasit o corelatie a ZAP-70 cu CD38 (to: 0.666; p=.018). Am gasit o corelatie putemica a expresiei cyclin Dl cu evolutia (ro: 0.667; p=0.14).

Analiza multiparametrica a markerilor imunofenotipici a descris un imunofenotip complex CD23 low - CD20 high - FMC7 positive - cyclin Dl positive, corelat cu markerii de pronostic rezervat CD38, ZAP-70.

Concluzii.

Flowcitometria este cea mai importanta metoda de diagnostic si evaluarea prognosticului in LLC. Cei mai utili markeri pentru primua treapta de diagnostic sunt CD19, CDS, CD23, CD79b. Markerii imunofenotipici CD38 and ZAP-70 an o corelatie putemica ciu evolutia nefavorabila. Corelatia markerilor CD20, FMC7, cyclin Dl si CD23 poate defini un imunofenotip de granita asocial cu prognostic rezervat.

THE RESULTS OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN 7 CASES OF ACUTE MYELOBLASTIC LEUKEMIA

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We present the evolution of 7 patients with autologous stem cell transplantation for AML4. In one case the transplant was performed 5 years ago, in 3 cases 3 years ago and in one case 1 year ago. The mobilization of stem cells was difficult in 4 cases, the patients needed double mobilization, the first one with Cyclophosphamide and growth factor and the second one with growth factor. The pretransplant conditioning treatment was done in the case of patients with poor

mobilization with Busulphan in monotherapy and in patients at which we obtained a quantity of CD34+ > 2,6x106/kg with Busulphan + Cyclophosphamide. The infectious complications that appeared in the aplastic phase responded well to the administrated treatment with the exception of one case with severe Pseudomonas aeruginosa infection of the skin and respiratory tract. All our patients had maintanance therapy with Purinethol and Methotrexat and they are at present in hematological and cytogenetic remission.

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN
AUTOIMMUNE DISEASES

We present 2 cases of severe autoimmune diseases of connective tissue: lupus and immune arthritis, in which due to bad response or no response to immunosupresive treatment with high dose methylprednisolon and azathioprim, autologous haemopoietic stem cell transplantation was indicated. The mobilization was done with Cyclophosphamide and growth factor (G-CSF) in both cases and we obtained a sufficient quantity of CD34+ cells. The conditioning treatment was done in one case with Fludarabine + Cyclophosphamide due to the anaphylactic reaction to thymoglobuline and in the other case with Cyclophosphamide + ATG. In both cases in the aplastic phase severe bacterial infections appeared. We present the evolution of the cases 1 year respectively 3 month posttransplant.

**AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN
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**THE RESULTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION
AT A CASE OF MULTIPLE SCLEROSIS**

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We present a case of a 59 year old patient with multiple scerosis transplanted in our Clinic. The disease started 7 years ago with optic nevritis and cervical myelitis. In september 2006 the motor deficit at the level of hands and feet agravated and corticosteroid therapy was started. The patient did not respond to the treatment and the simptoms agravated. Due to this hematopoietic stem cell transplantation was indicated. We present the results of the mobilization, the infectious complications that appeared posttransplantation (respiratory and urinary tract infections due to Pseudomonas aeruginosa) and the occurrence of engraftment syndrome. The evolution after 1 year posttransplant of the patient is favorable with the improvement of motor deficit of the limbs.

Identification of chromosomal abnormalities in acute leukemia with molecular techniques

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The outcome for children with acute lymphoblastic leukemia (ALL) has improved dramatically with current therapy resulting in an event free survival exceeding 75% for most patients. In addition, 25% of patients fail therapy and novel treatments that are focused on undermining specifically the leukemic process are needed. Acute myeloid leukemia (AML) is a relatively rare malignancy in the pediatric population, comprising only 15% to 20% of the acute leukemias.

Nevertheless, it remains a challenging disease with an inferior treatment outcome compared with pediatric ALL. Despite the introduction of new drugs, the aggressive use of allogeneic and autologous bone marrow transplantation, and improvements in supportive care, overall cure rates of AML remain below 60%. Further improvements in cure rates are likely to come from a better understanding of both the molecular abnormalities responsible for the formation and growth of the leukemic cells, and the mechanisms underlying drug resistance.
Material & Methods: 36 pts with age 2 mos - 18 y, 17 F/19M, admitted in Fundeni Pediatric Institute, Hemato-oncology & BMT Department between November 2006 - June 2008 were included in this study. Complex diagnostic morphology, cytochemistry, immunophenotyping, cytogenetic, FISH, molecular biology tests were performed in Gianina Gaslini Institute, Genova, Italy, "Mina Minovici" National Institute for Forensic Medicine and in Fundeni Clinical Institute. The molecular screening for BCR/ABL P190 and p210, MLL-AF4 was performed in all patients. For 12 patients we performed the molecular screening with Hemavision test.
Results: 30 patients (13F/17M) with ALL and 6 patients (4F/2M) with AML were identified. FISH technique applied in 7 cases identified: NUP98 deletion- 1 case, TEL/AML1 -1 case, PML/RARA 1 case, t(X;6)(p11.2;q23) 1 case. The molecular screening for BCR/ABL P190 and p210, MLL-AF4 was negative for all patients in this study. The molecular screening using Hemavision test identified a case with t(1;19).
Conclusion: identification of chromosomal abnormalities is mandatory for therapeutic risk. The diagnostic molecular tests are relatively rapid and allow the monitoring of treatment response.

ABSTRACTS
Acute Leukemia

ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS. 5-YEAR EXPERIENCE OF THE HEMATOLOGY DEPARTMENT OF THE "ION CHIRICUTA" CANCER INSTITUTE CLUJ-NAPOCA

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Introduction.
Acute lymphoblastic leukemia (ALL) in adults represents about 15-20% of all newly diagnosed adult acute leukemia cases, involving especially younger patients. Despite significant progress over the past decades, results are still inferior to those obtained in children.
Material and Methods. 49 patients, 28 men (57%) and 21 women (43%), with a mean age of 35.8 years (16-68), diagnosed and treated in our department between 2003 and 2007 were studied.
Results. 37 cases were of B lineage (76%) and 12 cases were of T lineage 24%. Among the B-cell cases, 19 (50% of B-lineage cases) presented a preB (common) immunophenotype. 13 cases (28%) displayed extralymphatic involvement. Only in 11 patients a cytogenetical analysis was made; cytogenetics were not instrumental in therapeutical decision making. The first line induction treatment consisted of the local "standard" regimen in 16 patients (32%), of the HyperCVAD regimen in 13 patients (26%); in 8 patients (16%). Complete remission (CR) was obtained in 35 patients (71%). Relapse occurred in 17 patients (48%), on average at 14 months (4-56 months). A second CR was obtained in 10 cases (58%). The median overall survival was 25 months and the median relapse free survival was 12 months. Both CR rate and overall survival were significantly better in patients <40 years (p=0.03) and in those with preB immunophenotype (p=0.03 and 0.01 respectively). There were no significant differences regarding the initial induction regimen, sex, initial white blood cell count. Given the low number of tested patients no conclusion can be drawn regarding the value of cytogenetics. Allografting was performed in only 2 patients, one of them dying due to early relapse.
Discussion and conclusions. The results of adult ALL treatment are still unsatisfactory. Even though the CR rate is close to world standards, the early relapse rate is still unacceptably high. As future directions, the routine incorporation of cytogenetics in initial evaluation as well as of targeted treatments (such as rituximab in B-ALL and tyrosine kinase inhibitors in Ph+ ALL) in the induction protocols and a more widespread use of allografting may further improve overall results.

THE ROLE OF CYTOGENETICS AND MOLECULAR GENETICS IN THE MANAGEMENT OF ACUTE MYEOID LEUKEMIA. THE EXPERIENCE OF THE HEMATOLOGY CLINIC CLUJ-NAPOCA

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Introduction. The overall prognosis of acute myeloid leukemias (AML) remains poor despite progress achieved over the past decade. Widespread cytogenetic analysis has led recently to a prognostic classification of AML in 3 risk groups: good (t (8;21), inv 16, t (15;17), intermediate (normal caryotype, 8+) , poor (abnormalities of chromosomes 5 and 7, multiple abnormalities and others). Molecular genetics may further subdivide the normal caryotype group.
Material and methods. For the past 10 months, thanks to collaboration between our institution and the University of Ulm, Germany, funded by the Jose Carreras Foundation, we applied these techniques systematically on our AML patient population. There were 32 patients (20 men, 12 women) aged 30-66 years (median 55 years). Besides the routine cytological, cytochemical and immunophenotypical analysis, caryotyping was applied in all cases and in those cases with normal caryotype, the mutational status of the NPM1 and FLT3 genes was assessed.
Results. 5 cases (15%) were in the good risk group (2 cases with t(15;17), 2 cases with t(8 ;21), 1 case with inv 16). 13 cases (40%) were in the intermediate prognosis group (2 cases with trisomy 8, 11 cases with normal caryotype). 14 patients were included in the poor prognosis group (11 cases with multiple abnormalities, 2 cases with trisomy 22 and 1 case with cu deletion of chromosome 7). Out of the 32 patients, 28 were treated

with curative intent. Out of these, in 11 patients (39%) complete remission (CR) was achieved. 3 out of 5 patients (60%) in the good risk group achieved CR, 7 out of 13 (53%) in the intermediate group, while in only one patient in the poor risk group (10%) achieved CR. The molecular analysis in patients with normal caryotype revealed the presence of the NPM1 mutation in one patient, the association of NPM1 and FLT3 mutation in one patient and the presence of a mutated FLT3 alone in another patient. The patient who had as unique molecular abnormality the NPM1 mutation achieved a near-CR (6-7 blasts in bone marrow) after low dose ARA-C combined with ATRA.

Conclusion. Our study represents the first attempt in our center towards a modern, systematic categorization of AML cases. Our results confirm the data in the literature, especially concerning the bad prognosis of cases with multiple cytogenetic abnormalities. Even though the number of patients studied is very small, the low percentage of patients positive for the NPM1 mutation (generally associated with a good prognosis) may explain in part the unsatisfactory results of chemotherapy. Complex, cytogenetic and molecular diagnosis is essential in the 21st century, especially considering the fact that targeted small molecules (such as anti-FLT3) may become available in the near future.

HEMATOLOGICAL RESPONSE IN A PATIENT WITH ACUTE MYELOID LEUKEMIA NPM1+. CASE REPORT

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Introduction. The prognosis of patients with acute myeloid leukemia is strongly influenced by acquired genetic abnormalities in the leukemic cells, which include chromosomal aberrations, gene mutations. In the present, genetic abnormalities detected at the time of diagnosis are the key element in defining the prognosis. Recently, cytogenetic analysis has been globally used for diagnosis, which led to a new classification of patients in prognostic groups: good (for example t(8;21)), intermediate (trisomy 8), sever (anomalies of chromosome 5). In cases of normal karyotype techniques of molecular biology can show mutations of NPM1 gene (most frequently seen in AML).

Case report. We present the case of a female patient, 61 years old, diagnosed in our department in January 2008 with AML, unclassified FAB or WHO. At presentation: normal leucocytes, but 10% blasts. Bone marrow aspirate and biopsy showed fibrosis. The karyotype was normal, but there was mutation of the NPM1 gene. Considering the age of the patient, we started low dose chemotherapy (15 mg cytosine arabinoside 21 days) associated with ATRA (45 mg/m2, D6-8, 15 mg/m2, D9-21). The protocol was common with Universitatklinikum Ulm. 2 courses of chemotherapy were administered obtaining a very good response (6-7% blasts and the disappearing of fibrosis). There two other more courses of chemotherapy to be administered.

Conclusion. The data from the literature shows a favorable prognosis for patient with AML and mutation of NPM1 gene. The case we present confirms the good prognosis, even in association with low dose chemotherapy. The molecular biology techniques are important as new agents targeted against specific mutation are being studied.

PLASMA CELL LEUKEMIA: CLINICAL, LABORATORY AND THERAPEUTICAL ASPECTS

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Plasma cell leukemia is a rare form of plasma cell dyscrasia.The diagnosis is based on hematological features,including a plasmacytosis exceeding 2X 10(9)/l .Plasma cell leukemia has two variants:the primary form, in patients with no previous history of multiple myeloma,and the secondary form witch consists of a leukemic transformation in a previously recognized multiple myeloma.From 1993 to 2008 we diagnosed 17 cases of plasma cell leukemia(10 primary and 7 secondary) and a total of 385 cases of multiple myeloma.The median age of the patients with plasma cell leukemia was 61 years, and the sex ratio(M/F) was 1,5/2.All this patients had anemia and trombocytopenia and 75% presented hypercalcemia.The extramedulary involvement vas present in 69%(11 patients),6 with primary and 5 with secondary form.All the patients were treated with combination chemotherapy:9 with VCAP,6 with VAD and 2 with Bortezomib.The median survival was of 6,6 mounths.We report also two cases,one with primary plasma cell leukemia and another with the secondary form,occurred after 11 mounths of evolution of an IgG kappa multiple myeloma stage IIIB.

ACUTE MYELOBLASTIC LEUKEMIA IN ELDERLY PATIENTS, RETROSPECTIVE STUDY ABOUT CLINICAL PRESENTATION, PROGNOSTIC FACTORS AND THERAPEUTICAL RESULTS

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Background: Acute myeloblastic leukemia (AML) apears more in elderly patients. Conventional therapy for AML in elderly patients can be some times harmfull by its toxicity. Also this patients have biological risc factors and multiple clinical comorbidyties.

Aims: We retrospectively studied the efficancy and toxicity of conventional therapy in AML, clinical presentation and prognostic factors in elderly patients.

Methods: We analized a group of 72 patients diagnosed and treated in the Hematology Clinic of Timisoara during 10 years (june 1997 june 2008). We colected: age- more than 65 years, performance status, the presence of infections, hemoleucogram values at diagnoses, bone marrow blasts value, hematological disease background, comorbidites and therapeutical protocols. We used the univariate analisys (Log Rank) and multivariate analisys (Cox - modd) for prognostic factors.

Results: Median age was 72 years (range 61 89), 68% patients had more than 70 years and 15% mone than 80 years; 71% were de novo AML, and 29 bad secondary AML after mielodisplastic Syndrom (MDS). Performance status (PS) was: 12% patients PS1; 38%-PS2; 32%-PS3 and 18%-PS4, 59% of the patients had concomitent diseases that needed special therapy (cardiac, hepatic, chronic pulmonary diseases). Also 10% had associated neoplastic disorders (prostate, skin, colon and lung cancers); 8% of this with active neoplasia. 41 patients presented hystory of hematological disorder. Documented infections at diagnosis presented 34% the patients. One line of therapy was used in 42% of the patients based on conventional therapy using antraciclin-aracytin regimens and 35% of the patients received 2 lines of induction therapy. Were excluded from induction therapy patients over 80 years, low PS, severe comorbidities that needed therapy, asociated active infections, active neoplastic disorders and denial of the patients. Suportive therapy was used in all patients, and Hidroxiurea was used in patients with leucocytosis . Response to therapy was: 38% major responses (28% complete remisions and 20% partial remisions) after HCI criteria. 20% patients were excluded from induction therapy and 42% patients had no response to therapy. Global median survival was 6,7 months (3,8 14,01 nonths), median survival in responding group was 11 months and 1,8 months for the patients with only suportive therapy. Prognostic factors at univariate analisis were: infections (p=0,21), grade 3-4 anemia (p=0,00029); more than 50% blasts (p=0,00069).

Conclusions: Conventional therapy can improve AML patients status. Infections, anemia, high blasts percent have a bad influence in the prognostic of the patients. Asociation of comorbidyties excludes patients from obtaining hematological response.

ABSTRACTS
Chronic Leukemia

EXTRAMEDULLARY BLASTIC PHASE IN A CASE OF CHRONIC MYELOID LEUKEMIA CONVERTED IN CHRONIC PHASE AFTER THE INTRODUCTION OF IMATINIB MESILAT

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Natural evolution of chronic myeloid leukaemia is characterized by 3 phases: chronic (CP) accelerated (AF) and blastic (BP), the survival with classic chemotherapy being between 35-65 months in CP, 12-24 months in AF and 3-12 months in BP. The disease transformation implies in the majority of cases the myeloid lineage-50% -, the lymphoid lineage in 25% cases, and the extramedullary determinations occurring rarely.

We present the case of a 50 years old man, diagnosed in 1998 with CML-AF (without cytogenetic exam or molecular analyses because of the lack of the technical possibilities), with reserved prognostic factors (relative risk Sokal and Hasford high). With classical chemotherapy regimen, the disease was converted in CP, but, after that, the patient passed another 4 AF. In the period between Oct.1998-Oct.2004, the patient was treated with hydeea, the interferon introduced in Jan.1999 after the second AF was stopped because of the patient's intolerance. In Oct.2004, 72 months from the diagnosis, the cytogenetic exam showed 100% Ph+ cells in 36 metaphases analysed and the ratio BCR-ABL/ABL was 1,27456. Because of the lumbosacral pain, it was made the RX and Ct exam, that showed L1 vertebra modifications, highly eloquent for secondary determination. The correlation of clinical, paraclinical and imagistic investigations sustained the diagnosis of extramedular blastic phase of CML. The patient started the treatment with imatinib 400 mg daily; with this therapy the complete haematological response(CHR) was achieved in 3 months and sustained for 24 months, but just with a minimal cytogenetic response at 12 months and suboptimal molecular response. 48 months after the initiation of the therapy with imatinib, the patient is in CP-CML, but, because of the suboptimal cytogenetic and molecular response, the problem of the therapeutic attitude appears: the increase the dose of imatinib, the association or change with another tyrosine kinase inhibitor, or allogeneic stem cell transplantation? To conclude with, the imatinib 400 mg daily, although introduced lately in the disease evolution, improved the survival at 48 months from the initiation of therapy and overall survival is of 10 years.

THE IMPORTANCE OF MOLECULAR RESPONSE MONITORING AT PATIENTS WITH CHRONIC PHASE - CHONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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The identification of Philadelphia chromosome-t (9; 22) - and its product, the fusion gene BCR-ABL, represented the beginning in the development of targeted therapy in chronic myeloid leukemia (CML), the research in this field leading to the discovery of the first tyrosine kinase inhibitor imatinib mesilate. According to the results of the Iris trial, after 5 years of continuous observations, imatinib induced 98% complete hematological response (RHC) and 87% complete cytogenetic response (RCC). The main determining factors of the subsequent disease evolution are: RCC at 12 months and major molecular response (RMM) at 18 months. We present the results of a retrospective study, made during a period of 24 months (July 2006-July 2008); the study included 17 patients (10 male and 7 female) with CML treated with imatinib, the majority as a second line therapy (88.3%). It has included just those cases who made minimum 2 molecular exams during the imatinib therapy. We observed a correlation between relative risk, imatinib dose and type of molecular response. The monitoring of the level of BCR-ABL transcript in patients with CP-CML treated with imatinib for 3 months in the first year and every 6 months later allows the identification of the suboptimal response and the relapse.

THE RESULTS OF THE TREATMENT WITH FLUDARA IN CHRONIC LYMPHOCYTIC LEUKEMIA -THE EXPERIENCE OF THE DEPARTMENT OF HEMATOLOGY BRASOV

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During 2007-2008, in the Department of Hematology Brasov, 40 patients (31 males, 9 females), with age between 40 and 80 years, were treated with fludara. Fludara was administered as first line therapy at 15 patients (37%). Fludara was administered at 7 patients with stage I, 25 patients with stage II and 8 patients with stage IV; the leucocytes count (WBC) between 20.000-50.000/mm3 at 63% of cases, between 50.000-100.000/mm3 at 35% of cases and over 100.000/mm3 at 2% cases. Fludara was administered alone at 20% of cases, associate with ciclofosfamida (FC) at 67% of cases and associate with mitoxantrona and ciclofosfamida (FCM) at 13% of cases. We made 2 cures at 13 patients, 3 cures at 8 patients, 4 cures at 5 patients and 1 cure at 14 patients. The results were good, the WBC decreased under 5000/mm3 with LF normal at 19 patients and under 10.000/mm3 with lymphocytosis at 18 patients, under 2000/mm3 at 3 patients. The 5 cases were relapsed at 3-5 months (the therapy was stopped when the WBC was 5000-10.000/mm3 with lymphocytosis). We registered 4 deaths, 2 cases had severe cytopenia with subsequent complications, one patient was refractory and one patient died by another cause (non-hematological).

The patients will be monitory in further and the finally conclusions we'll have in September 2008.

CHRONIC MYELOMONOCYTIC LEUKEMIA WITH EOSINOPHILIA-CASE PRESENTATION

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BACKGROUND

Chronic myelomonocytic leukemia(CMML)is a heterogeneous group of disorders with features both of myelodysplasia and of myeloproliferation.The World Health Organisation (WHO)group has been divided CMML into three types,based on the percentage of blasts in the peripheral blood and bone marrow and the associated eosinophilia:CMML-1,CMML-2 and CMML-1 or CMML-2 with eosinophilia.

MATERIALAND METHODS

We present the case of a 67 years-old male admitted in Fundeni Clinic of Hematology for the analysis of the progressive leucocytosis with monocytosis.The clinical exam and the paraclinic investigations of this patient were performed in Fundeni Clinic of Hematology:the hemogram with cytology,morphological and hystopathological exam of bone marrow,cytogenetic study,the cells cultures,flow-cytometry and molecular biology tests.

RESULTS

The patient presented in Fundeni Clinic of Hematology in march 2008 with hepatosplenomegaly and anemia-related symptoms.The hemogram showed leucocytosis (42000/mmc)with deviation left of leukocyte formula to 1%myeloblast,eosinophilia(2000/mmc) and monocytosis(8000/mmc).The bone marrow aspirate and biopsy showed hypercellularity with trilineal myelodysplasia.The cytogenetic study revealed the absence of Philadelphia chromosome.In cells cultures observed autonomous GM-CFU growth in bone marrow and immunophenotyping revealed the next cellular populations:11% monocytes,79%myeloid cells.The molecular biology tests performed in Germany were negative for BCR/ABL MAJOR,FIP1L1/PDGFRA,PDGFRB and JAK2V61F. The patient presents criteria of CMML-1(WHO criteria)with eosinophilia.

The patient followed treatment with Hydreea with favourable response.

CONCLUSIONS

The presence of this rare disease,CMML with associated eosinophilia underlines the importance of performing complete paraclinic investigations for elucidation of this entity.

ANEMIA IN LYMPHOPROLIFERATIVE DISEASES - DATES FROM EVIDENCE PROGRAM OF PATIENTS WITH ERYTROPOIETIC STIMULATING AGENTS TREATEMENT

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Anemia in lymphoproliferatives diseases is a constant

challenge for hematologists for various mechanisms and therapeutic possibilities.

Recent published studies discuss the limitation in treatment indications for erythropoietic stimulants agents, the adverse effects of this treatment and the rigorous selection of patients for a real patient advantage.

For a good evidence of patients with anemia and lymphoproliferatives disorders with chemotherapic treatment who receive erythropoietic stimulant agents we propose an informatic program who contains : general data of patient , with patient's consent and respecting of data confidentiality, diagnosis and treatment data (chemotherapic schedule, number of applications), anemia treatment data (type of erythropoietin, doses, schedule of administration, the eventually iron supplementation and the route of administration), treatment results estimation with hematological parameters, transfusions need, and parameters who evaluate quality of live fatigue, depression, physical activity , social activity, adverse events of erythropoietin stimulation agents administration.

The reason of this study is initiation of anemia in patients with chemotherapy and malignant hemopathies (chronic lymphoproliferatives diseases and monoclonal gammapathies) registry, the real identification of patients with erythropoietin stimulation agents therapy and the use of these data for establish a treatment indication in accord with local reality.

CITOGENETIC ANOMALIES IN THE CHRONIC LYMPHOCYTIC LEUKEMIA

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Aim of study: We study the citogenetic anomalies in chronic lymphocytic leukemia and the association with another unfavorable prognostic factors.

Materials and methods: We have observed a group of 181 patients suffering from chronic lymphocytic leukemia in Medical Clinic I in Târgu-Mureş and we have analised the cytogenetic anomalies from periferic blood in 22 patients.

Results: Out of these 22 patients, we have discovered normal cariotip in 12 patients, 8 showed one or more cytogenetic anomalies and 2 were technically uninterpretabile. The cytogenetic anomalies found were deletion of chromosom 11 in 1 patient, trysomia 21 in 3 patients, monosomie 4 in 2 patients, trysomia 12 in 2 patients and hiperdyploidie in 1 patient. The median age was 67,4 years, with limits between 46 and 86 years. In our group we have 19 mens and 1 women. The patients suffering from chromosomial anomaly show LDH growth, low value of the hemoglobin at the diagnosis, increased percent of lymphocytes in the marrow and low value of the imunoglobulins. About the stady, we observed in our group, that are not a correlation with avansed stady, Binet B and C, the patients don't have more freqvently citogenetic anomalies.

Conclusions: The chromosomial anomalies are a very important prognostic factor in chronic lymphocytic leukemia and we have observed the correlation with other negativ prognostic factors.

FLOW-CYTOMETRY, HISTOLOGICAL BONE MARROW INVASION PATTERN AND STANDARD CYTOGENETIC EXAM IN CHRONIC LYMPHOCYTIC LEUKEMIA. SINGLE INSTITUTION EXPERIENCE BETWEEN 2006-2007

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Background: Flow-cytometry (FCM) became indispensable for the proper positive diagnosis of chronic lymphocytic leukemia (CLL). The histological pattern of osseo-medullar tumor invasion and the cytogenetic study may reveal useful prognostic information.

Patients and method: For 44 consecutive, newly diagnosed (between 2006-2007), typical morphology B-cell CLL cases, FCM and bone marrow biopsy (BMB) were performed; blood samples from 23 cases were analyzed using standard cytogenetic exam. FCM panel included CD19, CD19/5, CD20, CD20/5, CD23, CD10, FMC7, CD79b, sIgM, cut-off point for positivity 30%. BMB analysis included standard morphology and immunohistochemistry. Median age of the study group was 68.5, male/female ratio 3.4:1, Binet stage A=17 (38.64%), B=11 (25%) and C=16 (36.36%) of cases respectively.

Results: FMC positive diagnosis score for CLL (markers CD19/5, CD23, FMC7, CD79b, sIgM) was 5 for 27 (61.36%), 4 for 15 (34.09%) and 3 for 2 (4.55%) of analyzed cases. CD5/CD20 coexpression was found between 16.5%-96.8% (median value 74.8%), CD3

expression between 1.2%-31.2% (median value 7.75%). 27 of cases (61.36%) presented diffuse pattern of BMB tumor invasion. Among 23 cases evaluated, only 5 (11.36%) presented cytogenetic abnormalities, with or without known prognostic significance.
Conclusions: FCM and BMB are mandatory for the positive diagnosis accuracy in CLL cases, even if additional cost is inevitable. In our study group, standard cytogenetic exam using blood samples was able to detect abnormalities in only few cases.

COINCIDENCE OF CHRONIC MYELOPROLIFERATIVE AND LYMPHOPROLIFERATIVE DISEASES.A RARE PHENOMEN ?

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Chronic idiopatic myelofibrosis (CIMF) or myelofibrosis with myeloid metaplasia is a clinico-pathological entity characterized by a stem cell derived clonal myeloproliferation, extramedullary hematopoiesis, splenomegaly, bone marrow fibrosis and a leuco-erythroblastic blood picture.

It is un uncommon disorder, with a reported annual incidence ranging from 0,5 to 1,3 per 100,000. Its prognosis remains poor when compared to other BCR-ABL negative chronic myeloproliferative disorders, with death resulting from hemorrhage, infection, cardiac failure and leukemic transformations., with a median survival of only 4 years. Cure is only possible following bone marrow transplantation.

Chronic lymphocytic leukemia (CLL) is a progressive accumulation of mature appearing, functionally incompetent lymphocyte B in peripheral blood bone marrow, lymphnodes, spleen, liver or other organs. CLL is sometimes associated with solid tumors but rarely coexists with other hematologic neoplasias in the same patient.

The coincidence of chronic myeloproliferative and lymphoproliferative diseases in the same patient is a rare phenomen. According to the relevant literature 7 cases with a combination of CIMF-CLL have been reported. Possible pathomechanisms for the development of such coincidences are: 1) a bilineage manifestation of a pluripotent stem cell proliferation 2) independent proliferatios of two distinct cell lines under a common leukaemogenic stimulus or 3) an accidental association.

We reported two cases of myelofibrosis with myeloid metaplasia associated with chronic lymphocytic leukemia. Both cases are documented by clinic, bone marrow biopsy and immunologic studies.

CLL followed CIMF in both cases and a commune complication was autoimmune haemolytic anaemia.

CLADRIBINE IN HAIRY CELL LEUKEMIA-IO CLUJ NAPOCA-HEMATOLOGY EXPERIENCE

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Hairy cell leukemia is a rare chronic lymphoproliferative disorder characterized by circulating B lymphocytes that display prominent cytoplasmic projections. The neoplastic cells infiltrate the marrow and spleen in a characteristic way. For patients requiring treatment, cladribine is the drug of choice because of the very high complete remission rate and prolonged duration of remission in many patients.
We use cladribine for our HCL patients since 2003.14 out of the 35 HCL patients diagnosed and treated in IOCN Hematology in a 7 year period(2001-2007) received cladribine(Litak) 0,1 mg/kg/day, subcut, 5 days.

11 were men, median age 46,5 years(31-72) ;diagnosis was made by clinical exam, cytology of blood and marrow, histology and imunohistochemistry of bone marrow.

13 had a complete response, 1 a partial one. In 10 cases the treatment was followed by a period of aplasia, complicated with infections that required antibiotherapy(7/14), GCSF(7/14) and symptomatic anemia that required blood transfusions(3/14).There were no deaths related to the cladribine therapy. All 14 patients are alive and free of disease, max follow-up 4,5 years, min.0,5 years.

FIRST LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): FLUDARABINE AND CYCLOPHOSPHAMIDE (FC) VERSUS CYCLOPHOSPHAMIDE, VINCRISTINE AND PREDNISONE (CVP)

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Background: The introduction of fludarabine into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission rate (CR), overall response rate (OR) and progresion free survival (PFS) compared with alkilator based regimens. Its synergistic action with cyclophosphamide has demonstrated significative advances as front line therapy in untreated

CLL patients. Aims: To evaluate the response rate, time to disease progression and survival of the patients of FC (arm A)versus CVP (arm B) as first line CLL treatment. **Methods:** Starting from 2004, 94 untreated patients with CLL, 61 male and 33 female, were randomised into the two treatment arms, each 47 patients. The diagnosis of CLL was established according to the criteria of International Workshop on CLL (IWCLL)1989. The median age was 68,5 years, range (44 75), we studied patients with ECOG performance status 0-II with high risk category (RAI stage III IV or RAI stage I II if they have at least one of the followings: one or more of the disease related symptoms, progressive marrow failure, massive splenomegally or lymphadenopathy or progressive lymphocytosis). Arm A received: Cyclophosphamide 250mg/m² iv D1to D3 and Fludarabine 25mg/m² D1 to D3. Arm B received: Cyclophosphamide 400 mg/m² iv D1 to D3, Vincristine 1,4mg/m² D1 and Prednisone 100 mg/m² D1 to D5. Cycles to be reapeted every 21 days. Hematological toxicity was recorded according to the NCI-WC for diagnosis and treatment and evaluation of response was done according to the NCI-WC response criteria. Were excluded from the study patients with stable or progressive disease after 3rd cycle. While PR and CR cases continued to 6 cycles of the same treatment. To confirm the response to treatment were performed Bone marrow biopsy and immunophenotyping. **Results:** Thirty six patients had stage IV, 32 patients had stage III and 26 had stage II and I. The median TLC was 93x109/l, the median lymphocyte count was 75x109/l, the median hemoglobin level was 8,9gr/dl, the median platlets count was 130x109/l. Bone marrow biopsy showed diffuse pattern in 81,91% (77 pts) and the median lymphocyte in the bone marrow was 90,5%. Complet clinical remission was reported in 33/47 patients in Arm A (70,21%) compared to 12/47 patients in Arm B (25,53%) p=0,015. Confirmed CR by bone marrow biopsy was reported in 13 patients in Arm A (27,66%) and only in 3 cases in Arm B (6,38%). Partial response with nodules was reported in15 patients (31,91%) in Arm A and 6 cases (12,76%) in Arm B. Median time to progression was 27 month in arm A and 8 month in arm B (p=0.03). In therms of haematological toxicity in Arm A 8 patients developed grade IV neutropenia and received G-CSF treatment while 2 patients developed severe anemia (grade III and IV) that reqiured red blood cell transfusion. Four patients developed a transient febrile neutropenia of unknown origin, which require hospitalization. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in 11 patients during the treatment in boath Arm A and B.
Conclusion: The combination of FC is able to induce higher response rate at the level of bone marrow biopsy. The hematological and extrahematological side effect were mild and manageable. There was a statistically significant difference in time to disease progression in favor of FC regimen .

IMATINIB MESYLATE THERAPY IN Ph+ CHRONIC MYELOID LEUKEMIA CHRONIC PHASE

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Background. Imatinib mesylase (Glivec) is powerfull and selectiv inhibitor of p210 Bcr-Abl tirozin kinase, with important efficiency in chronic myeloid leukemia (CML) therapy.
Aims. We studied the efficiency, tolerance and compliance of imatinib in CML patients.
Methods. We studied 58 CML patients in chronic phase treated in the hematology clinic of Timisoara from ianuary 2004 till january 2007. 12 patients were previously treated with Interferon alfa and 46 patients were new CML cases with previously 1-3 months of Hydroxiureea. All patients receved initialy 400mg Imatinib daily per os. The patients were 37 males and 21 females, with a median age of 50 years (range 18-70 years). Sokal Scale: 38% low risk; 42% intermediar risk and 20% high risk.
Results: Hematological complete response (CR) was seen in 95% of the patients with cytogenetic CR-72% patients after 12 months of therapy. After a median follow up 38 months of cytogenetic CR the response rate increased al 81% . Molecular response was performed in a few cases. Side effects were: peripheral and orbitary edema in 38% of patients; muscular cramps-27% of patients, diarhea-21% of patients; fatigue-15 % of patients. Only 3% of patients had grade 3-4 cytopenias and high hepatic enzimes. 7% of the patients died due to disease progresion in blastic crisis, patients who did not respond to high Imatinib doses (600-800), chemotherapy regimens (DAT) or evan Dasatinib therapy (1 case).94% of the patients are still alive; 89% of those are still on Imatinib therapy; but in 7% the therapy is discontinued due to grade 3-4 cytopenia and progresive disease.
Conclusions: This data prove a very good efficiency, tolerability and compliance of Imatinib in CML patients and are similar to other national and international results.

HAIRY CELL LEUKEMIA CLINICO-EVOLUTIVE ASPECTS AT 73 PATIENTS ADMITTED IN FUNDENI CLINIC OF HEMATOLOGY BETWEEN 2000-2008.

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Background : hairy cell leukemia (HCL) is an indolent B cell lymphoproliferative disorder that affect middle aged males; the main symptoms at diagnosis are : fatigue, splenomegaly, pancytopenia, hemorhagic syndrome, infections. The morphological marker of the disease in hairy cell, an activated memory B cell with characteristic immunophenotype (panB+, CD5-, CD23-, CD25+, CD11c+, CD103+, CD123+). The new methods of diagnosis (flowcytometry, immunohistochemistry) allow the diferrential diagnosis between classical an variant form, and/or another lymphomas (eg. Splenic marginal zone lymphoma, with villous cells). The response to the treatment with interferon and/or purine analogues is very good, with long term remissions.

Material and method: clinical and epidemiological retrospective study of 73 patients diagnosed in Fundeni clinic of Hematology between 2000-2008.

Results : 62 patients were diagnosed with classical form, 11 with variant form of HCL; most of the patients were males, with a medium age of 57,1 years; the main features at diagnosis were: splenomegaly (>12 cm diameter at abdominal ultrasound) -55cases; infections (24 cases), cytopenias (mono-, bi- or pancytopenia) all of the 73 patients, hemorrhagic syndrome - 15 cases; 11 cases had autoimmune manifestations.

The treatment methods were: alfa interferon - 44 cases, Cladribine - 12 cases, combined secvential therapy with alfa interferon and Cladribine - 8 cases, splenectomy 8 cases, alkilants agents 5 cases. The response to the treatment was evaluated at 57 patients: 19 partial responses, 34 complete responses, 4 without response. 7 patients died, 3 because of severe toxico-septic shock. The complications in evolution were: infections (21 cases), hemorrhagic syndrome (2 cases), cardiovascular disease (2 patients); 2 patients achieved a second malignancy.

Conclusion: HCL is an indolent lymphoproliferative disease, with well established diagnostic criterias (clinical, morphological, immunological), good response to the treatment, long remissions, but high incidence of infections, that appear at diagnosis or whenever through the evolution.

ABSTRACTS Varia

EXTRANODAL INVOLVMENT IN FOLLICULAR LYMPHOMAS

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INTRODUCTION: The follicular lymphoma is part of the indolent lymphomas and represents about 20-25% of the total cases of de nonhodgkin malign lymphoma both in the USA and Europe. At diagnostic, most cases are in the fourth stage with bone marrow involvement. Extranodal localizations such as the gastrointestinal tract or skin were rarely described. Beside the known prognostic factors currently used like the FLIPI score, recently additional molecular markers have been studied. The treatment of follicular lymphoma is made according to clinical, histological and molecular prognostic factors.
MATERIAL AND METHOD: Considering the data existing in the literature, we analyzed 9 follicular lymphoma cases with extranodal involvement: skin (3 cases), salivary (2 cases), gut (1 case), pleural (1 case), mouth ceiling (1 case), orbital (1 case). Cases were assessed considering the following: clinical features: age, numbers of lymph node areas involved, clinical stage, liver and spleen size; biologic features: peripheral blood findings, LDH level, seric albumin, seric calcium, beta2microglobulin values; histopathologic findings, immunohistochemistry (CD20, bcl2, bcl6, CD10, Ki67, lambda/kappa light chains); response to combined chemotherapy regimens with or without Rituximab.
CONCLUSIONS: Considering that primary extranodal localizations in follicular lymphoma are rare, we intend to identify if there are any differences regarding clinical, biological, and histological findings between the follicular lymphoma with nodal involvement and those with extranodal involvement and also to evaluate if the prognostic markers identified in follicular lymphoma with extranodal determination have any impact on treatment outcome.

FLUDARA ORAL THE EXPERIENCE OF THE DEPARTMENT OF HEMATOLOGY BRASOV

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Fludara, oral formulation, as first-line therapy, in monotherapy, for Chronic Lymphocytic Leukemia (CLL), has inserted in the Department of Hematology Brasov since January 2008. We treated 10 patients (7 females, 3 males), 8 patients with CLL and 2 patients with Small Lymphocytic Lymphoma. Fludara was administrated as first line therapy at 70% of cases. The patients received, at home, fludara 40mg/m2 p.o. Days1-5 every 28 days, well tolerated. Fludara was administred at 50% of cases with CLL stage I (RAI), 25% of cases with CLL stage 0 and 25% of cases with CLL stage II. 1/3 of cases had WBC under 20.000/mm3 and 2/3 of cases had WBC under 50.000/mm3. 6 patients made 2 cures, 3 patients made 3 cures and 1 patient made 5 cures. We registered WBC decreased under 5.000/mm3 at 2 patients (NHL), under 10.000/mm3 at 5 patients (3 patients with LF normal), under 20.000/mm3 at 3 patients (2 patients with 2 cures and one patient with 5 cures).

The patients will be treated further and the finally conclusions we'll have in September 2008.

THE PHASES OF PSYCHOLOGIC ADAPTATION/ADJUSTING TO THE DIAGNOSIS OF MALIGNITY

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Introduction: The psychooncology defines coping as a sum of behaviour and cognitive activities, which appeared as a response to an overwhelming event of life, with the purpose of overcoming it. These "response behaviours" the pacient develops after learning the diagnosis, appear as a subconscious way of fighting psychologically against it, in order to eliminate the cause (neoplasia). The most appreciated and used model of coping with cancer implies five phases all the pacients make and was described by Elisabeth Kubler Ross (1926-2004), doctor in psychiatry, in her work

"On Death and Dying". The phases , described initially as being made by neoplasic pacients in terminal stage, were afterwards applied on pacients (and their relatives) who have been recently diagnosed with the desease. The five phases are: denial (of the diagnosis, the unfavorable prognosis, death scale and emotional reactions caused by the disease), anger (against the medical staff or family), by which the pacient draws the attention towards himself, agreement with the Divinity, depression (reactive, secondary to the disease or prior to death) and acceptance of the diagnosis and prognosis.

Maerial and method: During the study of these phases the method used was the interview. I developed a set of interview-questions with general data on the pacient, questions referring to the evolution of these phases, described above, factors which influenced the quicker assimilation or avoiding of these phases, factors which helped the pacients overcome more easy the ineffective coping (long term denial, anger, secondary depression). The purpose of the interviews was also to explore the emotional states and analyze the emotional rections of pacients during these phases. I have interviewed 50 pacients with acute leukemia, the results being processed with the statistical program SPSS.

Results: 58% of the pacients went through a period of denial, the 42% left didn't go through this process due to different factors (critical symptomatology, confirmed diagnosis by different doctors, they had been cured of other serious diseases or the rapidly applied treatment confirmed the disease). 25% of the pacients admit going through the phase of anger, 50% admit the phase of agreement with the divinity, wishing to prolongue their life for reasons mostly connected with their families. 94% of the pacients accepted the diagnosis of acute leukemia, others instantly and some others needed a few hours, days or weeks of interior dialogue.

Conclusions: The knowledge of the coping phases by the medical staff is important for an adequate behaviour with pacients. In all cases, the medical staff must be supportive towards them, show understanding and respect for the pacients' grief and suffering. In the phase of denial , the pacients need explanations regarding the disease, arguments for its existence by the means of the symptomatology, time for a dialogue with themselves. The pacient's anger should not be interpreted as a personal attack, but as a way of drawing attention to himself. During the phase of prior depression the pacient's feelings must be respected, because he needs more a non-verbal physical presence of those who support him morally. The reactive depression must be approached through psychotherapy, encouraging of the pacients and antidepressive medication.

THE ADVANTAGES OF HOMOGENOUS GROUPS OF PSYCHOLOGICAL SUPPORT IN CONTRAST WITH THE HETEROGENOUS GROUPS

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Introduction: The psychologic support group is considered as being the most efficient form of psychological support in psychooncology. An advantage of the group therapy consist in the fact that the recently diagnosed pacients learn from the experience of those who had been hospitalized more often („veterans"). The result is a diminuished fear at the new pacients towards the cancer diagnosis, implicitely a raised confidence in the efficiency of therapeutical means and medical competence. It has been proven that the experience of knowing and interacting with another person suffering of a similar disease, who shares the same feelings is more valuable than an exposure made by the doctor or psychologist about a person with the same experience.

Material and method: Since three years there have been meetings of homogenous and heterogenous support groups in the Hematology Department, Cluj. The support groups are open (they involve as well the hospitalized pacients, as outside pacients and relatives).

The diversity referrs to pacients with different age, gender, personality, cultural level, background, education, as well as to the pathology, with acces to pacients' groups regardless of their diagnosis, stage, evolution of the disease or therapy type. After two and a half years of experience in organising the groups, we decided to form homogen groups with on a main pathology. We accomplished so far groups dedicated to acute leukemia and malign lymphoma.

The support groups have two mediators, the department Psychologist and a hematologist with training of psycho-social counceling.The psychological approaches used within the support group are the **informational- educational** one (by which the therapeutical compliance and pacients' motivation raises and the false preconceived opinions are defeated and the pacients receive the pertinent sources of information), the **cognitive-behaviourist** approach (with the purpose of identification and improvment of coping, control of certain symptoms and assuming of an active role by the pacient during treatment) and the **emotional support** offered.

We want to compare the efficiency and advantage of the two group types by analysing the feedback from the pacients and their relatives at the end of the group

sessions, as well as the group mediators' observations.
Results and conclusions: In a heterogenous group, the more participants (various in personality, disease i.e.) the more different are the problems discussed and the experiences the participants share. The disadvantage of this group is represented by the fact that many times general problems are discussed, the specific disease problems being better handled in homogen groups, dedicated strictly to one pathology. The approach of a detail or a specific problem of a disease in a non-homogen group helps only one group participant, the others may feel left aside, isolated, not understood or may consider the fact that their problems are not important. Discussing in detail the specific apsects of a certain disease in the heterogenous group led to confusion, so that the pacients tried to apply the information discussed on their own disease.

LABORATORY FEATURES OF MIXED MYELODYSPLASTIC SYNDROME ASSOCIATED WITH B-THALASSEMIA INTERMEDIA (CASE REPORT)

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Introduction. Myelodysplastic syndrome is a group of disorders which affects mainly the elderly and thalassemia is manifest mainly in the young people. Therefore, the association of these diseases in the same patient is rare and, when it happens, rises difficult problems of laboratory diagnosis, follow-up and treatment.
Case report. A 33-years-old woman known as suffering of heterozygous β-thalassemia intermedia form since her childhood has been admitted for the first time on May 2005 presenting pallor, asthenia, headaches, dizzeness and splenomegaly at 9 and hepatomegaly at 2 cm below costal margin, respectively. The peripheral blood showed Hb 7.1 g/dl, Ht 21.9%, MCV 47.1 fl, MCH 15.3 pg, MCHC 32 g/dl, RDW-CV 26.5-33.3%, WBC 5,5x103/μl (bands 2%, neutrophils 56%, eosinophils 6%, basophils 1%, lymphocytes 8%, erythoblasts 3/100), Plt 900 x103/μl with anisocytosis, reticulocytes 0.17%, marked poikilocytosis, abnormal RBC distribution (dimorphic population), stippled cells, cells with Cabot rings and Howell-Jolly bodies, , moderate hypochromia with target cells and 3/100 erythroblasts, hypogranular neutrophils. The serum total bilirubin was in normal limits during the follow-up, serum ferritin was 609 μg/l, serum erythropoietin was 9.2 mUI/ml, soluble receptor of transferin 5.05 mg/l (N: 0.83-1.76), serum lactic dehidrogenase 254UI/l, HbA2 was 5%, Hb F was 0% and JAK-2 mutation negative. The cytological and histological examination of the bone marrow showed moderate hypercellularity, marked erythroid hyperplasia with predominance of basophil and polychromatophil erythroid precursors, macromegaloblastic features, myeloblasts 3%, hypogranular neutrophils, polymorphous megakaryocytes and no fibrosis. The hemosiderin was much increased in macrophages with 15% ringed sideroblasts, spontaneous and stimulated cell cultures were closer of "dysplastic" type and the kariotype was normal. The CT scan revealed no ectopic hematopoietic masses. The diagnosis of refractory anemia with ringed sideroblasts, with bilineal dysplasia and thrombocytosis (RARS-T) associated with β-thalassemia intermedia has been established and the treatment with monthly 3 units of packed blood cells has been instituted until October 2005. At that time, when the ferritine level reached 1640 μg/l, erythropoietin (EPO) therapy (NeoRecormon) has been initiated. The level of Hb increased up to 10.1 g/dl, of hematocrit up to 29%, the reticulocyte count up to 3.2%, the ferritine level decreased up to 834 μg/l, without iron chelation therapy, and Hb F remained unchanged. The persistent and important thrombocytosis was initially treated with hydroxyurea, but has been well controlled by interferon (IFN) 3 MU x 3/wk.
Conclusions. The interest of the case consists in a rare association of two different kinds of anemia, one hyporegenerative and the other regenerative. The cytological findings in both peripheral blood and bone marrow, as the other alterations of laboratory parameters, were responsable for the "intricated" aspect of the hematological data and for difficulties in diagnosis. The EPO therapy induced independence of transfusions without apparent deterioration of thalassemia. IFN controlled optimally the thrombocytosis and hydroxyurea had no effect on both diseases.

ABSTRACT (Rolul proteinelor de faza acuta in limfoame si mielomul multiplu)

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Aim of study
Aim of our study is to establish the role of inflammatory process in malignant hemopathies from beginning until final stage.
Material and method
We have included in our study 50 patients, hospitalized

in 1 Medical Clinic from Târgu Mureş, during 2000 - 2008 period, diagnostized with Hodgkin lymphoma (n=10), non Hodgkin lymphoma (n=20) and multiple myeloma (n=20). We determined the inflammatory markers using ELISA and electrophoresis methods.
We studied the values of inflammatory markers during disease evolution, before and after the application of chimiotherapy and we correlated the results with pacient's immunological status and the complications.

Results

We obtained high values of the acut phase proteins even from the beginning of the disease: α1 antitripsin (60,4%), haptoglobin (64,5%), ceruloplasmin (58,4%), α1 glicoprotein (62,1%) and C reactive protein (70,3%). Increase was significant in multiple myeloma (69,4%) and non Hodgkin lymphoma (61,8%), compared to Hodgkin lymphoma(32,4%). Transferrine value was low in Hodgkin lymphomas (58,2%), non-Hodgkin lymphomas (64,3%) and multiple myeloma(65,8%) . 37,6% of patients had decreased values of immunoglobulins. After the application of the cytostatic treatment we observed the normalization of acute phase protein values: multiple myeloma (92%), non Hodgkin lymphoma (76,4%) and Hodgkin lymphoma (98,7%).

Conclusion:

Acute phase proteins increase significantly in lymphomas and multiple myeloma. Cytostatic therapy influence disease evolution in a positive manner by reducing the tumoral mass and normalizing the inflammatory markers.

Key words: inflammatory markers, malignant hemopathies

HAIRY CELL LEUKEMIA (HCL) LIKE SYNDROME INDUCED BY SPLEEN TUBERCULOSIS

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Keywords Tuberculosis Hairy cell leukemia Spleen

Abstract text:

We are presenting the case of a male patient, age 69, taken into our clinic after repeated admissions into infectious diseases and dermatology clinics, for a disease characterized by prolonged febrile syndrome, splenomegaly 22/21cm, rheumatic syndrome, paresthesia, skin eruptions forming micropustules with sterile pus, skin vasculitis augmented by histopathological exam.

Biologically, we observed: the presence of cryoglobulins with a cryocrit of 7%, pancytopenia: 200-300 neutrophil granulocytes, 900-1200 lymphoid cells represented by amoeboid lymphocytes, lymphocytes with kidney shaped nuclei, clear cytoplasm and irregular and fine cytoplasmic projections, the absence of monocytes, platelets count of 40000 90000/mmc. The bone morrow biopsy indicates focal lesions made of mononucleate cells with halos and nests of large sized histiocyte cells and the partial preservation of normal hematopoiesis.

The IHC exam though is inconclusive. Evaluations of images from abdominal CT confirms splenomegaly and reveals nodular spleen lesions compatible with determinations of lymphoma / caseification / spleen abscesses.

The hemocultures for aerobic and anaerobic germs and for fungi are all negative. Trans-esophagus ultrasonography evaluations of the cardiac valves excludes the presence of any vegetations and the serology and PCR evaluations for mycobacterium tuberculosis are negative, a context in which a splenectomy is decided as absolutely needed. Immediately post-splenectomy, the hematological parameters are being significantly corrected and the febrile state is being remitted.

The histopathological evaluation and the Ziehl-Nielsen colouring indicate patognomonic lesions of spleen tuberculosis and the absence of any other malignant lymphoproliferative lesions.

Tuberculostatic therapy: rifampicin, INH, etambutol and pyrazinamide, associated with prednisone 0.5 mg/K, pneumococcal vaccine all being well tolerated, and the patient has a favorable hematological and clinical evolution.

THE CURATIVE EFFECT OF ERADICATION OF H PYLORI IN A GASTRIC MALT (MUCOSA-ASSOCIATED LYMPHOID TISSUE) LYMPHOMA

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Keywords: MALT lymphoma Helicobacter pylori

Abstract text:

Most of the MALTomas occur in the stomach, and roughly 70% of gastric MALTomas are associated with H pylori infection. We report a case of gastric MALT Lymphoma in a woman associating H pylori infection.
Case presentation: A 58 years old woman with a more

then 6 mouths distress associating weakness, dyspeptic syndrome and weight loss thereupon laboratory tests showed iron deficiency anemia and evidence of iron malabsorption; gastric endoscopy fallowed by echoendoscopy showed disorganization of gastric mucosa and submucosa with thickening of those, especially in greater curvature (7 to 11 mm); histopathological and immunohistochemical assessment showed "mucosa associated lymphoid tissue" lymphoma CD 5 -, CD10 -, BCL 2-, Pan B markers+; CT scan sowed absence of intra-abdominal lymphadenopathy and confirmed excessive thickening of gastric wall. In the context of positive H pylori test, we initiated triple therapy with clarithromycine, amoxicillin and esomeprazole, with good tolerance and favorable evolution. Hematological and imagistic reassessment at 35 days showed partial improvement of anemia and iron malabsorption and important regression of modifications showed at echoendoscopy with regression and restriction of the lesions to a zone of 6/4 cm diameter where persist a light thickening (6.5 mm) of the structures.

Conclusion: we confirm the curative effect of triple therapy anti H pylori in gastric MALT lymphoma, continuing to follow up (clinical, hematological and imagistic) the patient; we indicated H pylori serologic evaluation for her husband.

ERYTHROPHAGOCYTOSIS EXTENSIVE IN BONE MARROW, HEMOPHAGOCYTOSIS FROM A PATIENT WITH SEVERE ANEMIA WHO DEVELOPED ACUTE MYELOID LEUKEMIA M4/M5b.(CASE REPORT. PATOGENESIS)

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By using May Grünwald-Giemsa stain for conventional light microscopy, the marrow smear showed the presence of macrophage with 4-7 red cells erithrophagocytosis, from a patient with severe anemie (Fig,1,Fig,2,Fig.3,Fig.4,Fig.5,Fig.6. Clinical Central Laboratory Department of Hematology, Târgu-Mures).

The hemoglobin level was 3.7 g per deciliter.The patient developed an acute myeloid leukemia M4/M5b (Fig.7,Fig.8,Fig.9,Fig.10,Fig.11. Oncological Institute „Ion Chiricuță , Laboratory of Hematology Department,Cluj-Napoca).

The erythrophagocytic cells show abundant vacuolate cytoplasm, with 4-7 red blood cells or remnants of red blood cells and contain round ,oval or kidney-sharped nuclei with lacy chromatin and inconspicuous nucleoli. In malignant histiocytosis, unlike reactive processes, phagocytic histiocytes are atypical and pleomorphic, and may show hyperchromatism, prominent nucleoli and multinucleation.

Phagocytosis of the hematopoietic cells by macrophages has been observated in a variety of neoplasic and non neoplasic conditions. The most prominent phagocytozed cells are erythrocytes. However, erythrophagocytosis in often associated with phagocytosis of other cellular elements, such al platelets and neutrophils. In a number of viral infections, such as herpes simplex virus, cytomegalovirus, EBV, adenovirus, parainfluenza and measles, a significant number of reactive macrophages may display erythrophagocytosis.

Reactive histiocytosis with hemophagocytosis has been reported in association with a variety of malignant neoplasms, particulary lymphoid malignancies, but also acute myeloblastic leukemia and acute monocytic leukemia.

Acute mixed lineage leukemia M4/M5 with an inv(8)(p11q13) resulting in fusion of the genes for MOZ (monocytic leukemia zinc finger protein) and TIF2 (transcriptional intermediary factor 2) have been associated with prominent erythrophagocytosis.

The MOZ-TIF2 fusion in one of a new family of chromosomal rearrangement,that associate HAT domain (hystone acetyltransferase), transcriptional coactivation,and acute leukemia. Two distinct clinical syndromes have been associated with 8p11: a chronic myeloproliferative disorder complicated by T-cell lymphoblastic leukemia/lymphoma and peripheral blood eosinophilia and M4/M5 acute monocytic leukemia with prominent erythrophagocytosis.

In HLH (Hemophagocytic Limpho Histiocytosis) is impaired or absent function of natural killer (NK) cells and cytotoxic T cells (CTL).

The cytotoxic activity of NK cells and CTLs (cytotoxiy T-cells) is mediated by the release of cytolytic granules (containing large amounts of perforin, granzymes and other serine like proteases) via the immunological synapse to the target cell.

POSTSPLENECTOMY LATE RESPONSE IN IMUNE THROMOCYTOPENIC PURPURA. A SINGLE INSTITUTION EXPERIENCE FOR 15 YEARS.

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Background: Idiopathic thrombocytopenic purpura (ITP), also reffered as immune or autoimmune thrombocytopenic purpura, is an acquired disease characterized by low platelet count, normal bone marrow, usually with an increased number of megakaryocytes and the absence of any other disease.The majority of patients respond, on short term, at an initial corticosteroid therapy, which is used as first line therapy. Some times after steroid therapy patients relaps and in this case splenectomy can be the second line of therapy. Aims: We tryed to evaluate the therapeutical results in a group of patients with ITP and the duration of their remision after splenectomy. **Methods:** From may 1990 to may 2005 were hospitalised and treated in the Hematology Clinic 165 patients with ITP. The median age of patients at diagnosis was 38,18 ± 16,44 years (range 15-72 years).

The distribution on sex was 112 females and 53 males. The mean time from diagnosis was 21,61 month. Follow up was minimum 32 month and maximum 156 month.The mean platelet count before tretment was 21,252 ± 2,336/µl with limits between 5000 and 115.000. Our patients 39% presented gastrointestinal bleedings, 47% had sclerotegumentary bleedings, 9% had bleedings in the central nervous system and 5 % other bleedings. The most of the patients (78%) were treated with corticosteroids, 12% received steroids and immuno globulins and the remaning of the patients were treated with steroids, vinca alkaloids and rituximab. The patients with severe thrombocytopenia received platelet transfusions. We consider a sustained response a platelet count above 50.000/µl or above 30.000/µl without hemorrhages or only with minor purpura. A complet response was considered a platelet count above 150.000/ µl after a discontinuation of therapy. Splenectomy was consider after 3 to 6 month in patient resistent to corticosteroids or erlier at patients demand. 45% (75 pts)had a splenectomy because they relapsed after steroids or they needed very high doses of steroids for a safe number of platelets. From those patients with splenectomy, 53 were females and 22 were males. Mean age at the time of the splenectomy was 36,41±16,88, the medium time from diagnosis to splenectomy was 3,5 years (0,6-96 months). The response to splenectomy was defined as followes: complete response (CR) a number of trombocytes higher than 150.000/mms for more that 4 weeks, partial response (PR) trombocytes between 50.000-150.000/mms lastig more that 4 weeks and relapse a number of trombocytes under 50.000/mms. Results: The overall response was 75%, with 53% of CR and 22% PR. From 75 patients with splenectomy 20 patients relapsed and 4 of this 20 were in CR after steroid therapy following splenectomy. The long therm follow-up in CR and PR proves a good, stabil and durabil response in time for more than 7 years. Post splenectomy complications in the study group were not significant.

Conclusion: Our study proves that patients with chronic imune thrombocytopenic purpura who failled corticotherapy get a safe and durable response in time after splenectomy. It is now important to use modern therapies (i.v. immunoglobulines, Rituximab) in relapsed patients after steroid therapy, before surgery.

ERITROPOIETIN THERAPY OF ANEMIA IN PATIENTS WITH NON-MYELOID HEMATOLOGICAL MALIGNANCIES RECIVING CHEMOTHERAPY.

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Background: Anemia is a common feature in patients with cancer resulting from the disease it self and the effects of myelosupressive treatment. In the European Cancer Anemia Survey [ECAS] with included cancer patients at various stages of treatment from 24 European countries, 73% of 2956 patients with lymphoid malignancies were found to have anemia (hemoglobin [Hb]<12g/dl) at some point during the 6 month evaluation period (Ludwig H. et all, Blood 2002). Anemia has a detrimental effect on patient quality of life[QoL] including debilitating fatique, dyspnaea, cardiovascular complication, weakness and depression. Anemia is also a negativ pronostic factor for survival in patients with lyphoid malignacies. Epoetin beta (NeoRecormon) has been shown to be effective in increasing Hb levels, reducing transfusion reguirements and improving QoL when administered three times weekly (t.i.w) subcutaneously (S.C). However the option to administer epoetin beta once weekly (QW) would provide a more convenient treatment schedule for patients. Aims: To evaluate the efficacy and safety of

epoetin beta 300000IU QW in patient with lymphoproliferative malignancies.

Methods:

This was an single-arm study from a single center experience about patients treated from january 2002-june 2007. Adult patients with non-myeloid hematological malignacies and anemia (Hb levels 8-11g/dl), a WHO performance status 0-2 % and a life expectancy > 6 month who scheduled receive chemotherapy were enrolled. Patients received epoetin beta 300000 IU sc QW over 16 weeks. Follow-up visits scheduled after each chemoterapy cycle.The end point included change in Hb level during epoetin beta therapy.The clinical response was defined as an increase in Hb concentration of ≥ 2g/dl during the treatment phase without transfusion requirement after the initial 4 weeks of tretment. Hb response was defined according to patients baseline Hb level.

Results:

A total of 173 patients were included in the intention-to-treat population. Patients with anemia (Hb≤11g/dl) associated with multiple myloma 62 pts(35,84%), non Hodgkin lymphoma 68 pts (39,3%) and chronic lymphocytic leukaemia 43 pts (24,86%) were eligible for the study. Mean age was 63 years, M/F ratio was 98/75, mean serum Hb (SD) g/dl = 9,2 (1,1), mean hematocrit (SD)% = 28,2(4,7), mean transferrin saturation (%) = 38. The median duration of the treatment was 14 weeks, Hb response was observed in 65 % of patients during the study. The median time to response was 53 (range 25- 120)days. Hb response was seen with all types of chemotherapy used in the lymphoproliferative malignancies hat we studied. The mean Hb level at study end point was 12.0(SD 2,2g/dl). Epoetin beta treatment was well tolerated. Five percent of patients presented tromboembolic events, other few adverse events were related to the study medication but without major significance.

Conclusion:

In patients with non-myeloid hematological malignancies, Epotein beta 30000UI s.c.QW effectively and rapidly increased Hb to target level, reduced the need for blood transfusions with their associated risks and was well tolerated.

BIOLOGICAL INVESTIGATION IN THROMBOPHILIC PATIENTS: PROTEIN C AND PROTEIN S DEFICIENCY

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Background:

A hereditary thrombophilic state must be differentiated from an acquired one and it is suspected in a patient with characteristic anamnestic data: thromboembolic episodes at an early age (15-45 years) with familial distribution, occurrence in unusual sites such as mesenteric, cerebral and axillary veins, reccurence in the same site or, characteristically, in separate sites, with or without triggers, despite an adequate therapy, warfarin-induced skin necrosis.Because of the discusions about the necesity of screening tests in hereditary thrombophilia, about the management of the patients and their family, it is necesary to enlarge the database with new studied cases.

Aims: In this study we report the results of the measurement of functional activity of activated protein C and protein S in a strictly selected cohort of patients suspected of hereditary thrombophilia.

Methods: Were studied a group consisted of 79 patients examined in the Hematology Department of the City Hospital, Timisoara within five years. The selection criteria were: age under 50, recurrent thrombosis, sometimes in different sites from one episode to another, without an apparent cause, all of them having no clinical, imagistic or laboratory signs of paraneoplastic, inflammatory or infectious activity. Global plasma activity of protein C and S were determined using an ACL 2000 nephelometric centrifugal analyzer and IL Test Kitts provided by Instrumentation Laboratory (IL SpA, Viale Monza 338-20128 Milan, Italy).

Results: The studied cohort (79 pts) included 51 males (64,56%) and 28 females (35,44%) with recurrent thrombotic events in personal and family history. The affected sites are:10 pts presented clinical signs of pulmonary embolism (PE), 45 patients thrombosis of legs veins (TLV) from which 31 superficial thrombophlebitis and 14 deep vein thrombosis, 11 patients were diagnosed with axillary thrombosis (TAV), 8 patients thrombosis of the mesenteric veins (TMV), 3 patients inferior cava vein thrombosis (TCV) and 2 with renal arteria thrombosis (TRA). Note that 2 of the TLV were complicated with PE. Eleven patients (13,92%) presented functional protein C deficiency, other 13 patients (16,45%) presented functional protein S deficiency and 2 patients (2,53%) was diagnosed with associated deficiency of protein C and protein S.No deficiency was seen in 67,1% of the patients. None of the patients with functional protein C or S deficiency had associated functional deficiency of antithrombin III or resistance to activated protein C. All the positive results were confirmed after six month by repeating all tests, in the same conditions.

Conclusions: This study demonstrates that the incidence of functional protein C and S deficiency in patients with recurrent thromboembolic disease

increases when the selection is carefully made, making the test useful and cost-efficient. Finding a hereditary protein C and/or protein S deficiency has to be followed by searching it in blood-related individuals in order to take an adequate prophylactic and therapeutic attitude.

A SINGLE CENTER EXPERIENCE DURING 5 YEARS IN 120 NEW MDS PATIENTS.

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Background: Myelodisplastic syndroms (MDS) are clonal hematopoietic stem-cell disoders characterised by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterised by peripheral blood cytopenias and high risk of progression to acute myeloid leukemia (AML). Aims: We gathered data on patient characteristics and treatment of 120 MDS patients seen in our hematology center during five years period (2001-2006).
Methods: We studied all new cases diagnosed and treated since january 2000 in our clinic. All patients were studied on admission with full blood count , bone marrow aspirate smears (BMA) and some were studied with cytogenetics and bone marrow biopsy specimens (BMB).We evaluated morfological features for all MDS patients including the availability of May Grunwald-Giemsa well stained BMA smears and BMB specimens. BMA were examined for dyserythropoesis (DE), dysgranulopoesis (DG) and dysmegakariopoesis (DM) as defined by WHO criteria. For all MDS patients we analised the percentage of blasts and ringed sideroblasts.
Results: From the 120 patients 91,66% (110pts) had primary MDS and 8,33% (10pts) were diagnosed as treatment related MDS. The distribution among MDS types was 7 - RA, 3 - RARS, 36 - RCMD, 14 - RCMD-RS, 13 - RAEB I, 15 - RAEB II,12 - CMML, 3 patients with 5q sindrome and 17 patients with RAEB-t. The majority of the patients were with a normal karyotype. According to the International Pronostic Scoring System (IPSS) 35% of the patients belonged to the low risk, 30% to the Intermediate-1, 20% to the Intermediate-2 and 15% to the high-risk group.There were 68 males and 52 females. From the 120 patients that we studied 98 patients (81,66%) were treated in our hematology departament; 25% of those treated in our clinic (30pts) were treated in day care hospital and 75% were admitted to the hospital. Reason for hospitalisation were high-risk group patients, disease progression, disease complications like infections, hemorrhages and bad general conditions. From all treated patients, in 41 cases patients were admitted for intensive chemotherapy and any kind of treatment that requires inpatient care. None of the patients received a Stem Cell Transplantation. Eighty five percent of patients received at least one unit of packed red cells and 65% received at least one unit of platlets. The median number of hospitalizations per patient was 8 (1-27). Thirty five patients (29,16%) died during first year of evolution, 41 patients (34,16 %) showed progression to AML.
Conclusion: With regard to MDS subtype distribution, patients seen in our hematological center did not differ much from the MDS population as a whole. Our patient needed hospitalisation inpatient care either for management of MDS related complications or intensive treatment of the underlying bone marrow disease. It is necesary that youngh patients with high risk to be submited for Stem Cell Transplantation

EPIDEMIOLOGY OF ANEMIA IN PATIENTS WITH MULTIPLE MYELOMA. RESULTS FROM A SINGLE CENTER EXPERIENCE

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Background: Anemia is a common and frequent complication of multiple myeloma (MM). There are a lot of studies about anemia in malignant diseases, but there are a few informations about the evolution of anemia in MM related to the clinico-biological characteristics, pronostic factors for anemia and the therapeutical methods in this patients.
Aims: We tried to identify the incidence, prevalence and evolution of anemia during an up to 6 month follow up period, to analise the possible corelations between anamia and clinico-biological characteristics , to identify some possible risk factors for evolution of anemia and to present the treatment of anemia in MM patients from our department.
Methods: We performed a prospective study of 143 with MM treated in our clinic from january 2003 till june 2007. Mean age was 63 years (range 45-86), male/female ratio was 78/65 patients. Twenty five percent of the patients were younger than 60 years, 42% were with age 60-69 years, 33% were > 70 years. The Imunoglobuline (Ig) isotype was : IgG in 60%, IgA in 20,5%, Light Chains in 11,5% and nonsecretory MM in 8%. Bone lessions were presents in 75% of patients and Durie-Salmon Staging (DSS) was: stageI in 3%, Stage II in 21% and Stage III in 76% of the cases. Follow-up visits were scheduled after each chemotherapy cicle for

6 month period for every survivig patients. Results: In the first 6 month of follow-up 12% of the patients died. The treatment used was: 62% received chemotherapy (CT), 27% received combined CT with radiotherapy or only radiotherapy and 11% were not receiving any cancer treatment. At enrollment 78%of patients were anemic (Hb<12gr/dl), 25% had Hb between 12 10 gr/dl, 33% had Hb between 10 8gr/dl and 20% had less than 8gr/dl Hb. Patients from the age group under 60 years were anemic in a proportion of 83%, patients of 60-69 years were anemic in 92% of the cases and patients older than 70 years were ever anemic. Refering to the performance status 61% had a WHO score of 2-4. The incidence of anemia in MM increased with increasing age >70 years MM patients were 100% anemic. Adverse WHO score correlated with low Hb (r=0.346) . In the studied group, 15 of the anemic patients (Hb 10-12 gr/dl) received no treatment, the other received: 11% iron preparates, 20% blood transfusions and 32% Epoetin. The severity of anemia was correlated with females, stage III DSS and complexe chemotherapy. **Conclusions:** The frequency of anemia in MM remains high and important: :prevalence of anemia in MM is high (78%), increases with age and correlates with poor PS. Adequate treatment of anemia was not available for all anemic MM patints. It is necesary to use Epoietin in anemia therapy of MM patients. With the identification of risk factors, anemia management in MM patients could be improved. Funding: Study supported by grant TD 22 no.27690/14.03.2005 of CNCSIS (Romanian National Council of Scientific Research)

Heparin Monitoring

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Heparins (UFH) are a mixture of glycosaminoglycans with heterogeneous molecular weight, size, anticoagulant activity and pharmacocinetic properties. Its molecular weight ranges from 3000 to 30.000 and only one third aof an administered dose bind AT, responsible of its anticoagulant effect. Heparin/AT complex inactivates FII, FXa, FIXa, FXIa FXIIa. FII and Fxa are more sensitive to inhibition by heparin/AT and thrombin is about 10-fold more sensitive to inhibition than factor Xa.

The anticoagulant profile and clearence of UFH is influenced by the chain length of the molecules. The higher molecular weight species are cleared from the circulation more rapidly than the lower molecular weight species. The relationship between the dose of heparin administered and its efficacy and safety has been demonstrated and is a standard practice to monitor UFH. The anticoagulant effect of heparin is monitored by the aPTT when usual therapeutic doses are used and by ACT when higher doses are used in association with percutaneous interventions and cardiopulmonary bypass surgery.. Because of lack of standardization therapeutic aPTT should be calibrated specifically for each reagent/coagulometer by determining the aPTT values that correlate with therapeutic heparin levels (equivalent 0.3-0.7UI/mL by factor Xa inhibition for the tratment of venous thromboembolism).

Low molecular weight heparin (LMWH) are derived from UFH by chemical or enzymatic depolymerization. LMWH have anti-factor Xa/anti-IIa ratios between 2:1 and 4:1, depending on their molecular size distribution. Several clinical trials have demonstrated the equivalence or superiority of LMWH over UFH for the prevention and treatment of venous thromboembolsim and for non-ST elevation acutre coronary syndromes.

LMWHs are administered in fixed or weight adjusted doses. LMWH Laboratory monitoring is debated. In fact some authors suggest that monitoring should be done in obese patients, in patients with renal insufficiency and in pregnancy. Some studies reported that high anti-Xa levels are associated with an increased bleeding risk , others failed to show this relationship. For treatment of venous thromboembolism, a conservative peak anti-Xa level with twice daily enoxaparin or nadroparin is 0.6 to 1.0 U/mL.

From a methodologically point of view commercial anti Xa assay are not standardized and anti Xa peak level could be different for the molecule in use. Farthermore, anti Xa activity represents partially LMWH pharmacological antithrombotic actions.

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ABSTRACTS
Transfusions

Transfusin transmitted infections (HBV, HCV, HIV, HTLV) - Current situation, limits and possible ways to reduce the transfusion risks

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The value of the residual risk of TTI is determined by the lenghth of the negative window period, which depends itself on the sensitivity (precocity) of screening tests, as well as on the growth rate and the maximum level reached by the viremia, which in its turn depends on the magnitude of the infecting inoculum, and on the other side, on the incidence of a given infection in the donor population.

HBV and HCV infections are the most frequent in first-time donors (FTBD), the incidence being 10 to 100 times higher than in Western EU, whereas the incidence of HIV-1 infections is similar and rises at the same pace than in Western EU; the incidence of HTLV-I remains at consrant levels specific for an area of moderate endemy (1‰).

At 17, 12 and 9 years respectively (1990 for HBV and HIV-1, 1995 for HCV, 1999 for HTLV-I) after the introduction of EIA screening, the incidence and the value of residual risk, though lowered, are still higher, by an order of 102 , as compared to Central-Western UE, with important local variations. Allthough the sensitivity of screening tests is a lot better as compared to the moment of EIA introduction, the values are similar to those reported for the central western area of UE before the introduction of screening by NAT.

Upon analysis of residual ITT cases, there is a tendency, in central-western UE, to supplement the HBV screening with anti-HBc (EIA) and anti-HBs (EIA), in an effort to ensure, somehow, the detection of „variant" HBV, and to direct the plasma towards fractionation in order to produce specific anti-HBs immunoglobulin. Finally, the commercial NAT tests are available for the most important ITTs only, which leaves uncovered the area of transfusion dedicated to supporting the organ and tissue transplantation.

The use NAT/GAT in detecting transfusion transmitted infections: benefits and limits

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Serological diagnosis of transfusion transmitted viral infections is either indirect, punand în evidenta rather the exposure to an infectious agent (HIV, VHC, HTLV) or direct, detecting the agent itself as is the case with HBV. The residual risk of transmitting a viral infection by transfusion is mainly linked to the existence of „ negative serological window", viral variability and the incidence of a specific infection in the target population for donor selection. Molecular diagnosis is direct, adressing the genome of the virus, detected by nucleic acid amplification techniques (NAT/ GAT) and was adopted to overcome the limits of serological diagnosis, reducing / excluding the the negative serological window, mainly.

Molecular diagnosis relies on various technilogies, PCR based or non-PCR based (target vs. signal amplification, such as TMA,NASBA, LCR, RCA). The use of these technologies for rutine screening of blood donations was hindered by the difficulties derived from the technique and equipment complexity, the need for highly skilled staff and the high cost of the overall process.

The current procedure adopted for blood screening is the "pool" testing, establishment of pool sizes being dependant on the sensitivity of the method of choice, the prevalence of a specific virus in the donor population and the avalability of complementary procedures for virus concentration such as ultracentrifugation and immunocapture.

Development of "real time " PCR, of "multiple target" methods (duplexes, triplexes) simultaneously detecting two or three viruses, and of automated platforms for sample processing resulted in dedicated systems for single blood unit screening, enhancing the detection sensitivity and allowing a more rapid validation by avoiding the pooling process.

The main benefits of NAT/ GAT technologies for blood unit screening derive from their ability to detect

earlier the agent during the first stage of infection and the detection of some cronic carriers with serological markers below the detection limit of serological tests. The sensitivity gain is dependent on the pool size and higher on single unit testing.

The high specificity of these methods may represent a drawback when viral variability is considered. On the other hand, chronic infection with transient viral replication or without active replication is recognized and diagnosis of such cases relies on serologic methods only.

Romanian External Quality Assessment Scheme in Immunohematology

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Introduction. In the frame of the European project "Strengthening the capability of the Romanian blood transfusion system to comply with EU requirements for quality and safety of blood and blood components" and in cooperation with INSTAND WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine, External Quality Assessment Scheme in Immunohematolgy has been initialized in 44 Romanian Blood Establishments and in Romanian National Institute of Transfusion Hematology . The study presents the results of the first 2 exercises.
Materials and methods. Each time, 2 serum samples and 2 erythrocyte 4% samples were distributed to 44 blood establishments from Romania and to Romanian National Institute of Transfusion Hematology . Instructions for use and instructions for reporting results accompanied the samples. The participants were asked to perform the following tests: ABO grouping, Rh factor D, A subgroup, Rh phenotype inclusive e , Irregularly antibodies-screening, Direct Coombs test, Kell antigen, Antibodies identification.
Results. All participants (100%) accurately determined ABO grouping and Rh factor D in both exercises. A subgroup was determined correctly by 51,11 % respectively 64,44% of the participants. Rh phenotype inclusive e was identified by 73,33% and respectively 80,00%. Screening for irregularly antibodies has been done by 86,67% of the participants in the first exercise and 95,56% participants in the second exercise. Direct Coombs test has successful passed by 88,89% and respectively 97,78 participants. Kell antigen was identified by the same percentage of participants (95,56%) in both exercises. Only a low percentage of participants passed successfully the antibody identification: 22.22% in the first exercise and 37,78% in the second exercise.
Conclusions. 17,77% respectively 28,00% of 45 participants passed the scheme successfully. While there is a high safety in ABO grouping and Rh factor D determination, A subgroup typing and antibodies screening and identification were found to represent major challenges .This seems to be due to technical shortcomings related to the availability of the test kits needed for these examinations. Only, Romanian National Institute of Transfusion Hematology, Regional Blood Transfusion Centers and some County Blood Transfusion Centers have kits for antibody identification. Additionally, failure in the scheme is often related to editorial mistakes in the documentation of the scheme results. The results of the two exercises reveal the educative role of the External Quality Assessment Scheme.

The value of dna typing for organ and tissue transplantation

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Introduction. The serological typing method is usually used to define the HLA antigens on the surface of the cells. The molecular typing techniques represent a radical change to define more exactly the HLA polymorphism. The rapid development of these new techniques permitted the direct study of the genome.
Material and method. 50 persons (recipients and donors for kidney and bone marrow transplantation) previously serologically were retyped by PCR-SSP (sequence specific primers) for class I: HLA A, B, C, class II DRB1, DRB3, DRB4, DRB5, DQB
Results. The serological and molecular typing of patients and donors releved both concordances and discordances between these techniques and so we could correct the last ones.In most cases the serological and molecular typing SSP low resolution was similar. It was difficult to type A30 in the presence of A31, A66 in the presence of A26, B52 in the presence of B51, Dr16 in the presence of DR15, DR 103 in the presence of Dr15 and DR16. The most difficulties were found for locus C. Many blanks for this locus were typed at the molecular level.
Conclusion. The DNA typing was very useful for homozigotism state, establishing the compatibility at the allelic level and to elucidate the ambiguities in serological typing.

The corelation between the number of platelets and the presence of the antibodies to the patients with trombocitopenia

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Introduction: Anti-platelets antibodies are involved in many diseases with low number of platelets.
Material and method: 178 patients with thrombocytopenia of different etiologies (idiopathic thrombocytopenia purple, chronic hepatitis, viral infections, lymphoproliferative diseases) were investigated for the presence of ant platelets antibodies.The number of trombocytes was variable between 10-150.000/mm3. The anti-platelets antibodies were detected by ELISA techniques, kit PAK PLUS which relived antibodies against glycoprotein's GP IIb/IIIa, GP Ia/IIa, GPIV and anti-HLA.
Results: 38 patients had anti-platelets antibodies and 8 patients were immunized in HLA system(39 PTI, 2 lymphoprolipheratives diseases, 3 chronic hepatitis, 2 pregnants ladies). 36 patients with thrombocytopenia had antibodies platelets, which could explain the low number of platelets. 10 patients had antibodies platelets with thrombocites over 100.000/mm3. For 8 patients the presence of anti HLA antibodies could be correlated with thrombocytopenia, because of high quantity of HLA molecules on the surface of platelets.
Conclusion: The determination of anti-platelets antibodies is useful for the clinician for establish of etiopatogeny of diseases and for treatment. For establishing a real correlation between the number of platelets and the presence of anti-platelets antibodies there are necessary more studies with more cases.

The contribution of a NAT based sorting method for providing a higher level of transfussion safety against the infections with HIV 1, HCV, HBV

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Introduction. Any blood derivative product is in compliance with the E.U. regulations only if it is subjected to this test methodology.

While ELISA blood screening technology relies on the detection of serological markers, these markers may not appear in blood until up to three months after an infection with HIV 1, HCV, HBV , leaving a "window period" in which a risk of transfusion transmitted infection is increased.
The PROCLEIX ULTRIO Assay detects viral material in the earlier stages of infection, reducing the „window period", and providing a higher level of confidence and safety for the blood supply.
Study's objective.
1. To compare the prevalence of viral serology markers (HBs Ag, anti-HCV, anti-HIV) with that of molecular markers (HBV-DNA, HCV-RNA, HIV-1 RNA) in first time and repeat blood donors.
2. To determine the prevalence of occult HBV infection among the Romanian blood donors.
3. To set up a plasma sample repository of "NAT only" and "serology test only" to enable reliable supplementary tests procedures and further scientific studies.
4. To check the effect of lyophilisation or heat inactivation of viral standards on the analytical sensitivity in cps and IU/ml.
CONTENT AND METHODS.

The PROCLEIX ULTRIO ASSAY is an ,in vitro" nucleic acid amplification test for the qualitative detection of HIV-1 (RNA), HCV (RNA), HBV (DNA) simultaneously in human plasma. The ULTRIO ASSAY involves three main steps that take place in a single tube: -Sample preparation; - HIV-1 (RNA) and HCV (RNA) and HBV (DNA) target amplification by transcription mediated amplification (TMA); - detection of the amplification products (the specific amplicons HIV-1/HCV/HBV) using a probe with a specific slow light emission.

Samples found to be reactive in the ULTRIO ASSAY may be repetead in duplicate and run individual HIV-1, HCV and/or HBV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three. The Discriminatory Assay may be run on the same system: PROCLEIX TIGRIS.

These assays are suitable for screening both pooled and individual human donor samples. The internal control added to each tube verifies if specimen processing, amplification and detection is working. The system is designed as a batch analyzer with the capacity to perform 500 tests in less than 9 hours and 1000 tests in les then 14 hours.
Case studies. Utilizitaion of Procleix Ultrio Assay on a significant number of blood donors (about 2,000).
Conclusions. Comparing to the Ab or/and Ag tests, the Nucleic Acid Testing (NAT) reduces the infectious "window period" as follows: with 63 % for HIV-1, with 92 % for HCV and with 36% for HBV.
In the world of blood screening, confidence in results is of critical importance.

Phenotyping in ABO and Rh systems using microplates Comparative study

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Introduction. Phenotyping in ABO and Rh systems are considered the routine selection of blood for transfusion.The procedure using compact microplates haemagglutination technique allows autoprocessing of large series of samples per day.

The present study tries to emphasize the advantages of using microplates in semiautomated analyzer Zenyx (Biotest) and fully automated system Qwalys (Diagast). The test was carried out in comparison with Ortho BioVue cassette technique based on the same principle of haemagglutination.

Echipaments and results. Echipaments: semiautomated analyzer Zenyx (Biotest), fully automated system Qwalys (Diagast), Ortho BoiVue **System.** A number of 12.096 samples were taken and processed from donors (2 x 96 samples/day . The advantages of using microplates were: - a full traceability using barcode control from the primary tube to the result, - reduced operating time by delivering 50 groups + phenotypes/ hour, - optimized specificity, - high resolution results due to the new nanotechnology based on magnetized red blood cells(E.M. Technology Qwalys), - reduced error rates because the magnetized red blood cells are not exposed to centrifugation and to the stresses associated with this process, - clear reactions through using high quality monoclonal reagents (Zenyx) and a new nanotechnology (Qwalys), - 93% (Zenix) and 95,5% (Qwalys) accuracy in reading Rh D weak, - computer controlled evaluation by means of a photometer (Zenix) and the CCD camera (Qwalys) and image analysis for optimum automatic interpretation of the results, - friendly software for optimum automatic interpretation of the results, - reagents are pre filled in the microplates to eliminate any pipetting errors, - using a small amount of the samples ensures optimum use of the reagents and lower costs, - safer storage of the results on a computer , - more time for the medical personnel to get involved in other operations.

Conclusions. Various configuration in the assignment of the microtest plates satisfy a very wide range of requirements adapted to all needs. All of the following tests are possible : Grouping, Phenotyping, Cross-matching and Antibody Screening. In conclusion, every laboratory with a large number of samples per day should use the microplates haemagglutination system. For a smaller number of samples per day it is better to use the column agglutination on the cassette technique.

A possible Ah phenotype in a young woman

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The aim of this study is to present the immunoserologic investigation performed in a particular case with discrepancy in ABO blood type.

Material and methods. Case reported a young healthy Arab woman investigated in our laboratory because two other laboratories in Bucharest did not succeed to establish the correct ABO blood type. Immunoserologic test: ABO phenotyping by routine tests, using both haemoagglutination on the plate and in column, direct anti-globulin test, titration of the serum against O, A, B red cells, titration of a commercial anti-I reagent against the own red cells and against normal type O adult and cordon red cells, the elution tests on the red cells of the respective case sensitized with anti-H, anti-AB, anti-A and anti-B sera.

Results. The routine tests showed 1) a total absence of red cells agglutination with anti-AB, anti-A, anti-B tests sera, but a reactivity of the serum against O, A, B red cells test; 2) the presence of an anti-B antibody in a high titre (1/64), and also the presence of anti-A (1/8) and anti-H (1/8) antibodies; 3) the presence of a normal I antigen and 4) the presence of a very small quantity of A and H antigens.

Conclusions. All these immunoserologic particularities lead us to classify this case as Ah phenotype.

The diagnosis and prevention of anaphylactic transfusion reaction due to the anti IgA antibodies

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Background. Anaphylactoid transfusion reactions due to specific antibodies for immunoglobulin A (IgA) are an established hazard in transfusion medicine. These IgA specific antibodies are found in a substantial number of patients with selective IgA deficiency and common variable immunodeficiency. Anti IgA antibodies may cause severe reactions during or following transfusion of blood components containing IgA. Therefore, patients with IgA deficiency demand distinctive transfusion needs: they should receive washed red blood cells, blood products from another IgA deficient donor, or from autologous blood donation.

Aim: To detect specific anti IgA antibodies in

pretransfusion screening and to identify IgA deficient blood donors.
Material and methods. The combined use of "ID PaGIA anti-IgA antibody test" and "ID PaGIA IgA deficiency test", on 100 subjects/patients and blood donors to identify IgA deficiency donors and subjects with anti IgA antibodies.
Conclusions. The use of "ID PaGIA anti-IgA antibody test" to detect specific anti IgA in pretransfusion screening and "ID PaGIA IgA deficiency test" to identify IgA-deficient blood donors provides an effective and safe strategy for the diagnosis and presentation of IgA anaphylactic transfusion reaction.

Evaluation study of HemoCue WBC system for leukocytes counting against the hematological analyzer Nihon Cohden

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Background. HemoCue WBC is a new leukocytes counting system that completes the existing ones in screening the hemoglobin and the free hemoglobin in blood donors and derivative products, useful in both pre-donation tests (donor selection) and post-donation (PSL quality control).
Aim. The present study for HemoCue WBC evaluation aims to control, in the same time, the leukocytes number in the capillary and venous donated blood and to compare the results with those issued from the Nihon Cohden hematological analyzer.
Materials and methods. The study was done upon 140 donors and 14 PSL (8 units platelets, 2 units fresh plasma and 4 units red cells), observing entirely the manufacturers recommendation for both counting system and working techniques.
Results. The results obtained from both systems have been analyzed, the final results showing, in linear domain, a good concordance between HemoCue WBC (0,3×109/L - 30×109/L) and Nihon Cohden systems. The average difference of leukocytes number between the two tests have been 0,37×109/L/test, on the behalf of Nihon system. The highest difference in leukocytes counting at the same donor (over 2×109/L) has occurred in 9 cases, probably due to both collection techniques (capillary or venous puncture) and possible calibration differences.
Conclusions. The present study showed a good concordance between the two leukocytes counting systems. All the recommendations regarding samples collection from the donor, as well as micro tubs fulfillment have been strictly observed. As a conclusion, we may say that HemoCue WBC system allows a very friendly screening either for technique and price, which is very important while in conformity with local and European regulations in force the leukocytes level of 4×109/L - 9×109/L is a must for blood donors' eligibility. Besides, the HemoCue WBC system is also useful in post-donation stage when appreciating the residual depleted/non-depleted leukocytes in the red cells concentrate. For the depleted concentrates with residual leukocytes less than 0,3×109/L, in the HemoCue WBC system the appreciation is performed by classic counting techniques in Nageotte chamber or by flow-citometry.

The Mobile Blood Collection Units in the European Union Vision BTCB application

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Introduction: Collecting blood using mobile blood collection units is recommended in order to get a closer approach to the working place of the potential blood donors.

The goal is to increase the number of mobile collection units as for to increase the percentage of blood donors per Romanian population. The procedure consists in describing the mobile blood collection, starting with the reception of the potential donor and ending with the actual blood collection process. All this will be done respecting the criteria of acceptance of a person (18- 65 years) for blood donation, this way assuring the biological security of the donor, as well as of the receiver of the blood or the derivates blood components transfusion.

The cycle might be described as follows: identifying the blood donor; completing the donation card with all the identification details; the result of the physical examination; the pre-donation examinations; the agree for collection; the blood quantity; specials mentions; carrying out the actual collection of blood; handing the papers that show the donation act; Thanking and the invitation for future donations.
Conclusions: In the C.T.S Bucharest two new mobile blood collection teams have been instructed by the European Union experts and the results were: The increase of mobile blood collection sessions in Bucharest. For the future there are expectations that two more teams will be instructed to act in the six districts of Bucharest and that the number of donors will increase up to 20 000 donations in the next four years.

A statistical study leukodepletion degree in blood products using integrate filter LST 1

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All the time, transfusion medicine specialists were looking for new methods to diversify blood products from whole blood collected from blood donor and used in transfusional therapy. In the same time, they are making permanent research for growing safety of blood transfusion and eliminate the possible deleterious effects. In present, leukocytes are considerate to be responsible for the majority of transfusion related side effects (transmitted viruses, bacteria, immunomodulation, etc.) , and for this reason, there are used several methods for different levels of leukocytes reduction from blood products. Because reduction of leukocytes number seems to be insufficient in all cases to avoid the adverse effects, there are used filters with high efficiency that are able to remove 99, 9% of whole blood leukocytes.

Our study present evaluation of an integrate leukodepletion filter (LST1) on 29 samples of whole blood.

For each sample of whole blood, we made hematological quality control before and after filtration (with CellTech analyzer), and residual leucocytes were numbered in Nageotte chamber, in this way filtration degree being estimated. In the same time, there was performed a statistical analyze of leucocytes population for those 29 samples before and after filtration in order to emphasize a semnificant statistical difference.

Results showed, in Nageotte chamber, a reduction of leukocytes number with 3-4 log, and a significant statistical difference, by using statistical t-student test, between whole blood samples before and after leukofiltration, which certified modification in filtrated samples. For leukofiltrated samples, leukocytes number average was between 0.2-0.49x106/unit, with a media of 0.316x106 and a coefficient of variance of 0.217. This very low value certifies the filter performances, because from any initial leukocytes number, it succeeds an obviously very significant reduction, all issues for leukofiltred samples being in European norms.

Conclusions: The present study shown the efficiency of this kind of leucofilter in the reduction of residual leucocytes number, and, in consequence, of all deleterious effects transfusion associated.

Rejection criteria and their frequency to potential donors presenting at the pre-donation group practice within the Regional Centre of Blood Transfusion Craiova

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Introduction. According to EC Recommendation no. 98/463/E.C. of June 1998, to EC Directive no. 2002/98/E.C. of the European Parliament and European Council of January 27th, 2003 and to EC Directive no. 2004/33 of March 22nd, 2004, the quality and safety standards have been established for the human blood and blood components collecting, testing, processing, storage and distribution, as well as the eligibility criteria for blood and blood components donors.

These criteria have been transcribed also into Romanian legislation, respectively Health Minister Order no. 1193/July 7th, 2007 which stipulate the regulations concerning the admissibility of human blood and blood components donors.

Throughout this material we will try to stress the most frequent causes of donation exclusion of the potential donors who presented during 01.01.2008-31.05.2008 at the pre-donation group practice within the centre.

Materials and methods. During the mentioned period, a number of 5239 persons presented out of which 3709 persons were admitted for donation, representing 71%, the rest of 1530 persons, approximately 29%, being rejected. The exclusion causes were various: anaemic syndromes, increased/low values, liver parameters/total bilirubin type pre-donation tests, various chronic diseases or acute infections, risk of achieving blood transmitted infections, pregnancy, serious surgical interventions, medicamentary treatment, other causes.

Results. Out of the 1530 persons excluded from donation, respectively 29%, have obtained the following results per groups of causes: * anaemic syndromes: - women = 243 cases (16%); men = 110 cases (7%); * increased/low values of blood pressure - 338 cases (22%); * liver parameters/total bilirubin type pre-donation tests - 246 cases (16%); * chronic affections - 101 cases (6,6%); * acute infections - 101 cases (6,6%); - risk of blood transmitted infections - 46 cases (3%); * pregnancy - 7 cases (0,45%); * serious surgical interventions - 23 cases (1,5%); * medicamentary treatment - 46 cases (3%); * other cases - 212 cases (14%)

Conclusions. Analysing the results obtained in absolute value and percents we can draw the conclusion that the addressability is high, but biologically the eligibility conditions have not been achieved in a high

percentage (29%) by the potential donors, respectively almost half of those admitted. The highest exclusion percentage of 22% was caused by blood pressure variations, an exam which mostly traces hypertension, unknown to donors.

We conclude by stating that promoting donation is essential and it can be considered an extremely important conclusion of people's health education, at national level.

Qualitative haematological examination performed . On the re-suspended red celled concentrate

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Introduction. Qualitative haematological examination of the unstable blood components is part of the quality assurance system within the blood transfusion system. The quality criteria of the blood components have been established according to the Guidelines concerning the blood components preparation and quality assurance published by the Europe Council and to Order no. 1237/July 10th, 2007 concerning the approval of the national list of classification of the human blood components for therapeutic use. The re-suspended red celled concentrate is a blood component obtained in a closed system, from an adult unit of total blood which is submitted to a centrifuging process and out of which plasma is decanted (without eliminating the leucocytes and thrombocytes layer) and then additive conservation solution is added. The red celled concentrate is used for the replacement of the red cell to anaemic patients or for the administration, in association with crystalloid or colloid substitution fluids to patients with acute blood loss. The role of the qualitative haematological examination of the re-suspended red celled concentrate is to obtain information on the red cell and, implicitly, on the haemoglobin supply in the blood transfusion.
Materials and methods. The tests have been achieved in the haematology laboratory within the Regional Centre of Blood Transfusion Craiova using the Nihon Kohden Celltac automatic analyser, consisting in determining the haemoglobin and haematocrit from samples of tubulature corresponding to the re-suspended red celled concentrate bag. According to the regulations in force, two units, randomly chosen, per week have been examined. During 01.01-31.05.2008 within the Regional Centre of Blood Transfusion Craiova a number of 2936 re-suspended red celled concentrates have been prepared. During this time a number of 42 samples have been the subject of the haematological examination, determining the haemoglobin value expressed in g/dl, using the analyser. The value of the haemoglobin measured with the analyser was multiplied with the volume of the blood component in order to obtain the total quantity of haemoglobin corresponding to the re-suspended red celled concentrate. The haematocrit value is that measured by the analyser.
Results. Out of the 42 examined samples a number of 10, representing 23%, had the haemoglobin quantity/product less that 45 g. It is worth mentioning that the non-conformity was almost surely determined by the manner of preparation of the tubulature used to measure the haemoglobin. The product homogenization and the repeated stripping of the tubulature lead to the increase of conformity between the values obtained while determining and the total haemoglobin quantity from the re-suspended red celled concentrate bag. The values obtained in determinations have been verified with the donor's haemoglobin determined before donation from venous blood, thus resulting their conformity.
Conclusions. In conclusion, the samples preparation in the process of determining haemoglobin and haematocrit is essential, proving once again the importance of the pre-analytic stage in order to obtain results as close as possible to results. At the same time it has been proved once again that the determination of the haemoglobin before donation is important both for donor (exclusion of those with haemoglobin lower than that foreseen in the regulations in force), and for the quality of the blood components obtained, as well.

Peg-intreferon and ribavirin treatment in patients with major thalassaemia politransfused HCV positive

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Introduction. Infection with hepatitis C virus is the commonest cause for liver diseases between polytransfused patients with major thalassaemia.The aim of our study is evaluation of biochemical response and virologic efficacy of peg interferon and ribavirin treatment.
Material & Method. Among patients with major thalassaemia registered in National Institute of Transfusion Haematology, 70% are positive for HCV.7 patients (3 female and 4 male) with major thalassaemia and chronic HCV infection received peg- interferon ± ribavirin treatment. Among them, 4 patients performed liver biopsy showing different stages of fibrosis and medium or severe liver haemocromatosis. Patients were monitories during treatment for AST, ALT, CBC monthly and ARN- HCV and serum ferritin every 3 month.

Results & Discussion. ARN- HCV initial values were between 385 000 2 300 000 units/ml.
Unfortunately, we didn't have the possibility to determine HCV genotype. Four of patients (57%) had elevated values for ALT, AST (2-3 times comparing to normal), the other 3 (43%) moderate elevated values. Because of haemolitical risk, association with ribavirin was possible only for 4 patients (57%). After 3 month of treatment ARN- HCV values decreased for all patients but only for 2 of them become not detectible. ALT, AST values decreased for this patients are between a minim 432,00 ng/ml and a maxim 4347,00 ng/ml correlating with results of liver biopsy. Transfusion request raised with 20% during interferon treatment comparing with anterior period, especially for those patients treated with interferon and ribavirin. Treatment was generally well tolerated, but all patients associated false-flu symptoms.
Conclusions. Treatment response was generally comparable with non-thalassaemic HCV positive patients. Because of haemolitical induction risk, ribavirin was prudentially introduced and not for all patients. Association of post transfusion liver haemocromatossis and C virus infection is the cause of extremely severe liver diseases, like cirrhosis and haepatocarcinoma, treatment of C virus infection and a good management of iron overloud could be extremely beneficial.

Difficulty in differential diagnosis of cerebral abscesses for a major thalassaemia patient

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Patient, 19 years old, male with major thalassaemia-severe clinical form, dependent of frequent transfusions, severe secondary haemocromatossis, secondary endocrinopathies, came for periodic transfusion complaining for severe headache, throw-up, fever, photophobia, alterate general status. Biochemical tests showed severe biological inflammatory syndrome, severe anaemia, leucocitossis, and elevated serum ferritin. Initially, our patient was suspected for acute gall bladder inflammation, surgery consult and echografic exam infirmed this diagnosis. For intracranial hypertension syndrome, our patient was investigated at Bagdazar-ArseniHospital, where CT and RMN shown a lot of cerebral abscesses. X-ray shown in the left lung a process of condensation. Initially, for this polytransfused patient , was considerate HIV infection for a reason for those abscesses, HIV serological tests were negative. After pneumological consult, our patient received antibiotherapy and anti-inflammatory therapy, but after a few days the patient came back with high fever and throw-up. The investigations continuated in Matei-Bals Hospital, general status worsted, even became comatose. They suspected a TBC cause for those abscesses- aggressive tuberculostatic therapy was immediately started, this improved progressively general status of the patient. Nowadays, our patient is tuberculostatic treatment and he feels good.

Our experience in the therapeutic plasmaferesis

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Introduction: The retrospective analysis of plasmaferesis that was asked by our clinical specialties: Hematology, I.R., and rheumatology for the following affections: Multiple Mieloma (M.M.) (4 cases), Waldenstrom Macroglobulinemy (W.M.) (6 cases), Rheumatoid arthritis (R.A.) with high rheumatoid factor (10 cases).
Material and method: In the period 1998- 2007, in a group formed by 20 patients who were hospitalized in the clinics of hematology, I.R. and rheumatology, patients that benefit of therapeutical plasmaferesis with emission of 600- 1000 ml plasma/ session, in intervals of 6 to 8 weeks in the hematological patients, and monthly to the rheumatological patients. The treatment was repeated after the clinical and biological evaluation. We took notice of: the clinical status of the patients, the hemoleucogram, proteinemy in patients with M.M., and the specific inflammatory probes (the rheumatoid factor; F.R) in patients with R.A.

The group was formed by 12 males and 8 females. We used the equipment PCS and PCS + with the afferent kits. In the aggravation periods, when the diseases were associated with severe anemia, the blood transfusion first to the plasmaferesis was imperative, in order to prevent the lipotimic stage that can occur during the procedure.
Results: * We observed a significant lowering of the pathological proteins in M.M. and M.W. after the 3-rd plasmaferesis with remission in 3-4 months. * The lowering in the F.R. and the inflammatory probes after the 4-th session, with an annual remission. * The insignificant changes in the hemoleucogram postplasmaferesis. Good tolerance of the patients. * The evolution of the patients was good after the plasmaferesis and the associated treatment according to the stage of the disease.
Conclusions: 1. The plasmeferesis procedure had a good tolerance among the patients, with the condition that the patients must be volemic and hydrostatic balanced. 2. A good venous abord is imperative. 3. The

good collaboration between the specialties gave good results in treating those patients, with the amelioration of the biological view, and the clinical view in all the three affections. 4. In the patients with R.A. we obtained the reduction of the AINS doses and even the articular destruction. 5. We obtained good results in patients whom we took 1000ml plasma/ session to patients that we took 600-800ml/ session.

Platelets transfusion new therapeutic strategies

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Abstract: Blood centers must develop new strategy for platelet production in order to ensure the increasing demand for platelet transfusion. Nowadays platelet products available for patients are safe due to the improvement of production technologies and reduction of infectious disease transmission risks. Platelet transfusion are beneficial and have permitted the use of more aggresive chemotherapy and bone marrow transplantation. Recent studies suggest that the threshold for prophylactic platelet transfusion may be safely lowered to 10x109/L from the previous standard of 20x109/L. Such restrictive platelet transfusion strategy decreases the patient´s risk of complication and considerable reduces the general costs.

Promotion of voluntary donation in the btc hunedoara district and the obtained results

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In compliance with 2002/98/EC Directive, 2005/61/EC Directive, 2005/62/EC Directive and the Council of Europe from January 2003, which establish quality and security norms for blood collection, control and labile product preparation, the aim of all members of UE is to encourage the voluntary, non remunerated blood donation.

Starting from the premise that blood can save or kill, it is the most important thing to provide the patient with harmless blood or to minimize the potential risks. It has been established that blood safety increases with the use of labile blood products (LBP) derived from voluntary non-remunerated donors and with the number of repeat donors.

Material and methods: We started from the analysis of numbers of donors in 2005, 2006, 2007 and we compared the first quarters of 2005, 2006, 2007 and d 2008. We have observed a decrease in the number of first time donors as well as in repeat donors. At the same time, the demand for LBP registered a progressive increase. We try to show here how we managed this situation.

We acted on: 1) Communication level: defining the message according to the seriousness of the situation. 2) Relationship with media. 3) We established a "kit" of communication with the donor as "an honorary citizen". 4) On the collection level: „we went towards the donor" establishing some new collection points in the towns of the county, since our BTC lacks the technical capabilities for the mobile collection.

The results became apparent by the first quarter of 2008 in the increase of numbers of first time donors as well as repeat donors, and in enlisting some of the first time donors as repeat donors.

Conclusion. Blood donation cannot be replaced by any other procedure, blood "being made by our hearts only". In the district of Hunedoara blood donation is voluntary, anonymous, and nonremunerated, but it involves us all.

Guide for covering the blood donation for the mass-media

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Introduction: Following several blood donation promotion campaigns, we noticed that the Mass-Media is neither informed nor educated in regards to the blood donation. Thus, the Mass-Media treats the subject without proffesionalism, which determines an unwanted reaction from the public towards the blood donation.

Goal. It is thus necessary to conceive a guide on how to relay the blood donation in order to avoid these situations in the future and to change the people's mentality, via the Mass-Media, determining a different approach for the blood donation.

Content: 1. Blood; what is the blood; what are the blood groups; compatibility; the RH sistem. 2. The blood donation; blood donation ethics; blood donation History; the blood path; the questionnaire. 3. What are the main risks for a blood donor. 4. What are the main risks for a blood receiver. 5. What happens to the blood after it is donated. 6. Announcing the donor about eventual annomalies in his or her blood. 7. Apheresis how a blood donation in apheresis occurs. 8. Who is transfuzes with what; red Blood cells; white blood cells; platelets; plasma. 9. Can we do without blood?; About the need for blood; About the self-transfusion; Where we can give blood. 10. Mobile collection; conditions for a mobile collection

Conclusions. Insuring blood safety. Demolishing excuses for not donating