Documenta haematologica

(New edition)

The Journal of the Romanian Society of Haematology and Romanian National Society of Blood Transfusion

VOL. XXXII, No. 1-2, 2014

Ed. MEDMUN

Address: ***Clinic of Haematology, Fundeni Clinical Institute, 258, Fundeni Road, Sect.2, 022328, Bucharest, Romania***

Subscription: individual 20 RON
institution 50 RON

RSH Account No. RO94RNCB0072049674870001, BCR Sector 1, Bucharest

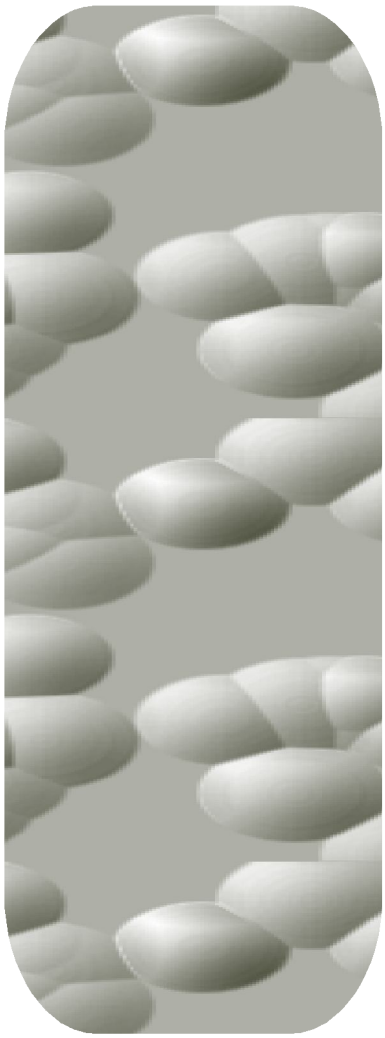
ISSN - 1582 - 196X **Ed. MEDMUN**

Documenta haematologica

REVISTA SOCIETĂȚII ROMÂNE DE HEMATOLOGIE
ȘI A SOCIETĂȚII NAȚIONALE DE TRANSFUZIE SANGUINĂ
DIN ROMÂNIA

ROMANIAN SOCIETY OF HAEMATOLOGY

CONTENTS



NATIONAL SOCIETY OF BLOOD TRANSFUSION FROM ROMANIA

Systemic Mastocytosis

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Abstract

Systemic mastocytosis represents a rare disorder with a wide spectrum of symptoms. It is difficult to diagnose, so the incidence could be higher than estimated. This article presents in a summarized manner the clinical, diagnosis and therapeutic options during adult systemic mastocytosis.

Key words: systemic mastocytosis, diagnostic, classification, terapeutic options.

Systemic mastocytosis represents a group of disorders characterized through excessive accumulation of mastocytes in one or several organs and tissues, accompanied or not by manifestations due to degranulation and release of vasoactive mediators (histamine, heparin, leukotriene, prostaglandin, tryptase, etc.). The clinical manifestations are heterogeneous, according to the tumor burden and visceral of mastocyte infiltration, as well according to the association with the mediator-related syndrome known as well as mastocyte activated synrome.
In accordance with the WHO classification we can distinguish two major categories:
1. Cutaneous mastocytosis, during which the clinical manifestations and the infiltration with mastocytes are exclusively limited to the skin, without any systemic involvement (without organomegaly, having serum tryptase with normal level);
2. Systemic mastocytosis, during which the pathologic mastocytes infiltrate at least one extra-cutaneous organ, affecting or not at the same time the skin; usually this includes also a high level of serum tryptase. The organs that are most often affected are the liver, spleen, lymph nodes, gastrointestinal tract, and rarely the lungS and the endocrine glands.[2]

These two entities must be differentiated from the syndromes of mastocytes activation, which do not meet the WHO criteria for systemic mastocytosis, but which clinically have similar signs and symptoms, caused by the release of vasoactive mediators, without the presence of morphologic and/or molecular abnormalities specific to systemic mastocytosis.[1]

Epidemiology

Systemic mastocytosis (SM) is a rare disease, at present it's incidence being uncertain; there is a possibility to under diagnose this disease because of its polymorphous manifestations and/or frequent confusion with other disorders that have an allergic pattern. SM affects both genders, regardless of the age. The cutaneous form is more frequent in children. In 50-80% of the cases, the diagnosis is established during the first year of life, and there are also forms that are exhibited since birth or peripartum. [1,2] Usually, during adolescence these forms may be attenuated or they even disappear.

The adults suffer more frequently from the systemic form. It has been acknowledged that it is more present in women and it starts after the age of 25-30 years.[1] There is a possibility for the disease to progress, from indolent forms to more aggressive ones, that is the reason why patients must be carefully monitored from the clinic perspective as well as by means of regular measurement of the serum tryptase level, marker which is well correlated with the mastocytes tumor load.[2,3,4]

Classification of systemic mastocytosis (WHO, 2001)[1,2]

1. Indolent systemic mastocytosis, with two sub entities:
   - isolated bone marrow mastocytosis, where there is only bone marrow infiltration, without any skin lesions and
   - smouldering form, with systemic affection, but without any signs of organ failure.
2. Systemic mastocytosis associated to a hematologic clonal disease (acute or chronic mieloproliferation, hypereosinophilic syndrome, chronic myelomonocytic leukemia, myelo-dysplasia, chronic lymphoproliferative disease.)

3. Aggressive systemic mastocytosis, where there are signs of infiltration of the organs associated with signs of organ failure; usually this form lacks skin manifestations. There is an included entity namely aggressive mastocytosis with lymphadenopathy and eosinophilia, with extensive bone marrow involvement, but without skin involvement and PDGFRA rearrangement.
4. Mast cell leukemia, with more than 10% immature mastocytes in the peripheral blood or the presence of more than 20% mastocytes with morphological abnormalities (nucleoli, atypical mitosis, bilobed nucleus, non-granular cytoplasm) in the bone marrow; a non-leukemic form is described, where less than 10% pathologic mastocytes can be found in the peripheral blood.
5. The mast cellsarcoma, with low incidence, characterized through the presence of mastocytes with morphological abnormalities in extra-cutaneous organs like the larynx, colon, meninges or inside the skin, rapidly followed by spread of the disease in organs, bone marrow and always ends in leukemic phase.
6. Extracutaneous mastocytoma, which takes place in a more frequent manner in the lung, consists in the aggregation of mature mastocytes, without any abnormalities. It has a benign evolution, it does not result in leukemia or progression in an aggressive form of systemic mastocytosis.[5] It has a good response to surgery.

Pathogenesis

Molecular abnormality most frequent in mastocytosis is the somatic mutation of proto-oncogene c-kit, which encodes a tyrosinekinase receptor for stem cell growth factor (SCF- stem cell factor). The mutation takes place in codon 816, where the valine is replaced with aspartate (Asp816Val). The consequence is an independent activation of c-kit receptor, characterized by auto-phosphorylation, followed by the activation of the STAT5, P13K, AKT. paths. The consequences are represented by the activation of uncontrolled proliferation and resistance to the apoptosis of the mastocyte cells of the skin and of the circulating ones in the blood or present in various tissues and organs. The abnormality can be present initially only in the dermal mastocytes, together with the progression and expansion of the diseases, being possible to detect it also in the mastocytes of the organs, blood, as well as in the other myeloid or lymphoid cells (aggressive forms of systemic mastocytosis).3,6 There are some other molecular abnormalities which can be found in patients suffering from systemic mastocytosis associated to a clonal non-mastocytary hematologic disease, like: FIP1L1-PDGFRά in the hypereosinophilic syndrome associated with mastocytosis, or RUNX/RUNX1, TET2, ASXL1, JakV617F in the associations with myeloproliferative disorders.[1,7]. These molecular abnormalities are translated in abnormal expressions on the surface of the mastocyte of the receptor for interleukin [5] (IL5R), interleukin [2] (IL2R, CD25) and for c-kit (Cd117).

The pathologic mastocyte has an abnormal co-expression for CD117, CD25,CD2 and cytoplasmic tryptase.

Clinical aspects

The clinical aspects differ according to the presence and severity of the skin affection, organ infiltration and the presence of mastocytary activated syndrome .

The skin involvement occurs in approximately 80% of the cases of systemic mastocytosis, being extremely polymorph: café au lait spots, pruritus, urticarial or blistered eruptions, with patchy or diffuse involvement of the skin. The Darier sign (reddening of the skin when being scratched with the nail or with a sharp object) is pathognomonic and relies on releasing vasoactive mediators (histamine) from the mastocytes of the derma. It has to be performed with prudence, as it can cause a severe crisis of de-granulation.

The signs of extra dermal affection are diverse: organomegaly (hepatomegaly, splenomegaly), adenopathy, skeleton lesions (osteoporosis, fracture of the pathologic bone, osteolysis), cytopenias due to the infiltration of the bone marrow as well as hypersplenism, malabsorption syndrome, gastro-intestinal injuries (peptic ulcer, irritable colon manifestation, mainly diarrhea, anorexia), as well as constitutional signs (weight loss, moving toward cachexia, mood disorder, tendency to depression, lack of concentration, insomnia).

Episodic release of mediators from mastocytes (the de-granulation syndrome) consist in flush, rash, headache, vomiting, myalgias, abdominal pain, diarrhea, fever and can even reach severe

manifestations, life threatening, like: arterial hypotension, angioedema, anaphylactic episode.

The triggers of mastocytes de-granulation or activation are very diverse: excessive heat or cold, temperature variations, emotional stress, infections, insect or snake bites, tissue tensioning, exploratory or interventional maneuvers (colonoscopy, superior digestive endoscopy), skin friction, food products, chocolate, spices, seafood and medication from diverse classes (Aspirin and anti-inflammatories, usual anti-pyretic, local or general anesthetics, etc.) All these manifestations can also occur in the absence of systemic mastocytosis, in simple allergies or in the case of syndromes which are mastocyte activated and which do not meet the WHO diagnostic criteria for systemic mastocytosis, that is why we require a rigorous differential diagnosis, determined by an experienced allergist.

Diagnosis of systemic mastocytosis

It is without no doubt difficult and it requires interdisciplinary collaboration: dermatologist, allergist at least during the initial stages, of suspicion or when there are certain dermal and general manifestations, together with a hematologist in order to exclude or diagnosis of systemic and more aggressive forms. The clinical suspicion must be confirmed through morphologic, immunohistochemical, biochemical and molecular testing in order to establish the WHO criteria, as well as in order to frame in the clinical form of the disease and exclude a hematologic non-mastocyte simultaneous disorder.

The diagnostic of systemic mastocytosis can be made when it is observed the association of a major criteria and of a minor criteria or the mandatory association of 3 minor criteria.[1,2]

Major criteria:

1. Multifocal infiltration with mastocytes (≥15 aggregate cells) in the hematogenic bone marrow and/or in an extra-cutaneous organ or tissue.(the aggregates are positive for the immune-histochemical coloring of the tryptase or with toluidine blue). Confirming the singular cutaneous infiltrate with mastocytes is not a major mastocytosis criteria!

Minor criteria:

1. 25% mastocytes with atypical morphologic aspect (spindle shaped) in the hematogenic bone marrow or in extra-cutaneous tissue;
2. Co-expression of CD25,CD117, CD2, for immune-histochemical coloring or through flow-cytometry ;
3. c-kit D816V mutation detected in the blood or bone marrow aspirate;
4. The level of serum tryptase > 20 ng/ml, persistent; the abnormality is not detected if it is associated with a clonal myeloid disorder.

The framing in different forms of systemic mastocytosis and the need for treatment rely on two categories of signs, in accordance with the WHO indications[1,2,3,8]

B signs ("burden", correlated with the mastocitary mass)

- >30% mastocytes in the bone marrow biopsy (focal, dense aggregates) and /or serum tryptase >200ng/ml
- Hyper-cellularity of bone marrow, with signs of myelodysplasia or discrete myeloproliferative signs, but not fulfilling the WHO diagnostic criteria for the mentioned disorders
- Organomegaly during the clinical and/or imaging examination (hepato- splenomegaly, or adenopathy with a diameter of more than 2 cm at ultrasound or CT scan), with organ failure /disfunction.

The presence of these only signs does not represent an indication to start the cyto-reductive treatment.

C Signs ("cytoreduction", shall be correlated with the impaired visceral function due to the infiltration with pathologic mastocytes). Their presence indicates the start of the cyto-reductive therapy.

- Cytopenias (neutrophils <1000/mmc or hemoglobin < 10g/dl, or thrombocytes <100000/mmc)
- Hepatomegaly with functional impairment such as hypoalbuminemia, ascites, portal hypertension
- Splenomegaly with hypersplenism
- Severe Skeletal involvement (osteoporosis, pathologic fracture, osteolysis)
- Malabsorption, cachexia, weight loss.
- The proof of organ infiltration with pathologic mastocytes is obtained by organ biopsies. We

can observe spindle shaped mastocyte clusters, elongated, with hypo-granular cytoplasm. The accurate confirmation and evaluation of the mastocyte infiltration level shall be performed with the help of special coloring (toluidine blue) and immuno histochemical stains for tryptase and CD117, and for neoplasic mastocyte the immunohistochemical stains for CD25 and CD21,2.

The prognosis of systemic mastocytosis

It depends on: the clinical form, onset age, presence and severity of the activation syndrome , the presence of C signs. Usually, the indolent forms have a good prognosis. There is the possibility of progression toward the aggressive form, yet there are few cases that were reported to this regard. The subcategory of smouldering indolent mastocytosis, characterized by a high mastocyte load, dysplasia and/or myeloproliferation signs (yet without fulfiliing the WHO criteria for histologic diagnostic for these entities), organomegaly has a slightly different evolution; it can be maintained in this state for a long period of time, but in a certain moment a transformation can take place, turning it into an aggressive form or in mast cell leukemia [1,8,9]. There is the possibility for this form to be named in the future smouldering systemic mastocytosis in progression, precisely in order to underline its unpredictable outcome. The evolution toward aggressive forms or toward the aggressive form of mastocytic leukemia can be explained through the accumulation of certain additional molecular abnormalities, often accompanied by the disappearance of the initial D816V mutation, probably due to a phenomena of clonal selection and transformation into a more agressive sub-clone.[8,9] Monitoring patients is mandatory, by using serum tryptase, whose level is well correlated with the mastocyte tumor load. The most reserved prognosis is that of, elderly patients, suffering from aggressive forms, those with associated clonal hematologic diseases, with hypoalbuminemia, or mast cell leukemia.

Therapeutic options

The main objectives of the treatment of systemic mastocytosis are improving the quality of life and reducing symptoms. The patients must be trained to adapt their life style and food diet so that they would avoid the triggers of the mastocyte activation syndrome. The prophylaxis of mastocyte de-granulation is performed through the continuous administration of inhibitors H1 (cetirizine, loratadin, diphenhydramine, hydroxyzine) and H2 (ranitidine, cimetidine, famotidine). The prophylaxis of osteoporosis consists of administration of bisphosphonates. In the event in which antagonists H1 and H2 do not control the clinical manifestations, the addition of mastocytary membrane stabilizers is indicated (cromoglycate), proton pump inhibitors, in order to control the digestive manifestations, or short treatments, or pulse therapy with oral or intravenously corticoids.

The treatment of aggressive and systemic forms with C signs is a challenge. At present there is no therapeutic or curative standard. There is still a desire to try and find focused therapies with fast cyto-reductive potential and with minimum secondary effects, especially in regard to the precipitation of a mastocyte activation syndrome, especially during the first administrations.

The available therapeutic options are alfa interferon, associated with corticosteroids therapy during the first weeks, in order to prevent the mastocyte activation syndrome. It represents the first line of cyto-reductive therapy, reducing the infiltration of organs; the pegylate forms are preferred for their easily administration as well as for the minimum risk of provoking the mastocyte activation syndrome.[8,10]

The second line cyto-reductive therapy is represented by Cladribine, administered to interferon intolerant or resistant patients. The risks are especially related to myelo-suppression and infections. The indicated tyrosine kinase inhibitors are Dasatinib and Midostaurin. Imatinib does not act on the D816Vmutation, but it can be used in aggressive mastocytes which do not have this mutation, as well as in mastocytosis associated with hypereosinophilia PDGFR/FIP1L1, the minimum doses of 100 mg/day being efficient, as it happens with the primary hypereosinophilic syndrome.[8] Hope is generated by the non-tyrosine kinase inhibitors of c-kit (rapamycin and analogs, Geldanamicine), or by the anti-CD25 monoclonal antibodies, but only in clinical studies. The young patients, suffering from aggressive forms, can undergo aggressive chemotherapy, in order to

reduce the total mastocyte tumor burden. The desire is that in the future we would be able to find methods that would control mastocyte proliferation, including the possibility to include the transplant in the therapeutical strategy.[8,10]

CONCLUSIONS

Systemic mastocytosis is a heterogeneous disorder, a continuum of disease clinical forms, with various manifestations and an unpredictable evolution. The difficulty to diagnose it is doubled by the difficulty of administering treatment; it is mandatory to collaborate within multidisciplinary teams comprising medical specializations, as well as surgical ones (allergist, dermatologist, pathologist, internal medicine specialist, hematologist, orthopedist or surgeon), as the patient suffering from mastocytosis is a complex and difficult to manage .

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Bleeding prophylaxis with minimum doses of Factor VIII versus "on demand" therapy in hemophilia A

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Abstract

Taking into account the permanent danger of bleeding, not related by age for hemophiliacs and the serious disability by arthritis, which definitely affect the quality of life, the authors make an evaluation of their experience in prophylactic treatment with minimal doses of factor VIII recombinant (250u/weak), for a 2 to 6 years period on a cohort of 23 patients, previously treated in "on demand" regimen.

An outstanding decrease of bleedings has been noted, from 50,8/year to 4/year ($p < 0,01$) and consequently, a consistent decrease of hospitalizations, from 26/year to 0,58/year ($p < 0,01$), both aspects being considered as a very encouraging fact. At the same time the joints status remain in a stand by and the inhibitors are noted in a lower rate, for only 4,4%. Because the magnitude and frequency of bleedings are related to hemophiliac phenotype but not of his age, the half-life of factor VIII in vivo overpass 72 hours to a secure concentration (>2%), and at least for adults the doses of F VIII are of lesser value for bleedings control, the authors conclude that minimal doses of Factor VIII, as a continuation of child prophylaxis, could change the adult hemophiliac's life by bleeding control, especially in the countries with economic difficulties.

Key words: hemophilia A prophylaxis "on demand" treatment

Introduction:

The use of recombinant factor VIII in the prophylaxis of bleeding at the beginning of the 90's, as well as for the treatment of hemorrhagic accidents, extremely frequent in hemophiliacs, represented the key into solving a continuous drama that is life threatening and leads to invalidity, which actually transforms the said patient into a socially assisted person having a more than precarious life quality [(1)]. Besides these aspects which are very important for the patient, as well as for the society, the reduction of the huge risks for hemophiliac shall be added, risks which were established through the utilization of the plasma and its derivatives (frozen plasma, cryoprecipitate, freeze-dried plasma etc.) or repeated transfusions with whole blood, in the contamination with HIV/AIDS, Hepatitis B or C, which led to the substantial reduction of the incidence of these diseases among hemophiliacs, even to their disappearance when prophylaxis is performed accordingly [(1)]. If the utility of prophylaxis in pediatric age (0 – 18 years) is unanimously accepted at present and even applied in most countries in the world, substantiated by the frequency of inherent accidents due to the lack de judgment, growth and development of the body as additional risks [(2)], the situation of the adult represents another area of debate and controversies [(3)]. These controversies start with the utility of prophylaxis, supported by the presence of the same risks as those faced by the child and adolescent [(4)], up to the moment when treatment with factor VIII is administered only in the case of bleeding episodes (on demand), motivated by the fact that aging a certain level of stability, to which the economic and financial rationale shall be added which is argued by some people through the high costs of prophylaxis [(5)]. We shall also add to this aspect the fact that the use of recombinant F VIII led to a new clinical and curative challenge through the appearance of inhibitor antibodies as an immunogenetic reaction, which has imposed specific modalities of approaching the patient, yet not less costly [(6)].

Paper objective: The paper aims to bring into fruition our experience in regard to the approach utilized in the case of hemophiliacs, by utilizing a minimum prophylactic scheme, imposed by the financial constraints that are part of our realities. The paper comprises a timeframe of 6 years (2008-2014), it has a county coverage and it monitors the evolution in time of the main parameters (blood and hospitalization) that have an impact on the evolution of the disease, quality of life and risk of appearance of inhibitor anti F VIII antibodies.

Material and methods: Our study group included 23 patients suffering from a severe form of the disease, with the age between 7 and 76 years, diagnosed during childhood in different hospital units and having their diagnosis confirmed in the hematology centers of Iaşi, Bucharest and

Timişoara. All these patients were administered a minimum prophylactic scheme with recombinant F VIII (Recombinants - Baxter and Kogenate – Bayer) for a unique dose of 250 u/week, regardless of the age and weight, during variable periods between 2 and 6 years. These minimum dose was imposed by the financial constraints mentioned before, especially due to our desire of casting away any form of discrimination in the way the hemophiliac is approached, including based on the age criteria.

The following parameters were taken into account: age, environment, number of joints affected at the beginning and at the end of the study, associated chronic diseases, number of bleeding episodes (hemorrhagic episodes/year) before and during the administration of the prophylactic scheme, frequency of annual hospitalizations before and during prophylaxis and incidence of the inhibitor anti F VIII antibodies. The study was based on the patients evidence and monitoring charts, patients questionnaires regarding their evolution before and during prophylaxis, hospitalization in the hematology centers or in the Buziaş center (prof. dr. Marghit Şerban) and the evaluation of inhibitors with the support of the Novo Nordisk company in the Synevo laboratories of Germany (dr. Cristina Farţade). Absolute and percentage values were utilized as calculation and manifestation modalities for each parameter, as well as the synoptic presentation through tables and graphs, and for the statistic implication the Fischer test was utilized.

Results: The 23 patients which are the subject matter of our paper, with the age between 7 and 76 years, are presented in table I: as age structure, environment, clinical and evolutional parameters in regard to joint disorders, bleeding and hospitalization frequency, doses administered annually, presence of inhibitor antibodies and prophylaxis duration.

Table II presents the basic parameters which express the actual state of the patients included in the study. Therefore, based on the age criteria we can state that we are dealing with a young group, the average age being of 31.9 years, among which 4 children (17.4%), 7 patients with the age between 18-30 years (30.4%), 5 between 31-40 years (21.7%), 6 between 41-50 years (26.1%) and only one higher than this age (4.4%), namely case 6 having the age of 76 years, a teacher in the rural environment (see table I). 74% of the cases (17 patients) come from the rural area which raises serious problems regarding access to emergency services when being face with a hemorrhagic emergency. The number of interested joints is higher than 3 (between 4 and 7), in 9 patients (39.2%) they concern major joints, mainly the unilateral or bilateral knee, elbows, scapulohumeral joints and ankles, 5 patients with 3 interested joints (21.7%), 2 joints in 3 patients (13%), one joint in 4 patients (17.4%) and 2 patients without any affected joint although they presented articular bleeding without reaching the disability state (8.7%). It can be observed in the same table (II) that 43.5% of the patients suffer from associated diseases: 7 patients with hepatitis C, 2 patients with hepatitis B and one with bronchial asthma. Our group does not include any patient suffering from HIV/AIDS. The share of hepatitis C and B refers to the period when transfusions and plasma derivatives were utilized.

The level of factor VIII (table III) was variable, between 0.35 and 1.3%, most of them having a value between 0.50 – 0.99%, 16 cases (69.6%), under 0.5% in 5 cases (22.1%), 2 cases (8.3%) having a value of 1% (case 23) and respectively 1.3% (case number 20). It is interesting to note in this case that the patient having the level of F VIII of 1.3% (42 years) does not present important bleeding episodes until the past 2 years, and they are completely remitted under our prophylactic regime. The quantity of administered factor VIII varies from one patient to the other, as mentioned above, therefore the annual average dose was of 241 u/kg body weight; 8 patients (34.8%) received 218.4u/kgc/year, 5 patients (21.7%) with 182 u/kgc/year, 5 in-patients (21.7%) with 254.8 u/kgc/year, 3 patients (13%) with 291.2 ukgc/year and 2 patients (8.3%) with 600 u/kgc/year. The same table (III) shows that the inhibitor antibodies have been displayed in only 2 patients, one of them (case 10) having an insignificant titer of 3.76 u Bethesda which was not modified in time and the other one (case 16) for a titer of 230 u B, which under "by passing" therapy with Novo Seven for one month led to obtaining the titer of 23.3 u B, after which prophylaxis with factor VIII was not restarted (dr. Gabriela Dorohoi).

In regard to the evaluation of evolutional

parameters (Table IV) for which a comparison was made between bleeding episodes, hospitalizations and the type of bleeding episode before and during prophylaxis, things are surprising because the differences are more than evocative. Therefore, if the bleeding average before prophylaxis was of 50.8 hemorrhagic episodes/year, the majority (55.8%) with more than 40 episodes/year (reaching even the level of 104/year), during prophylaxis have reduced their average to 4, which represents a reduction of 12.7 times ($p < 0.01$). It should be noted than the bleeding episodes are easier and are mainly summed up to the associated ones (dermal, muscular, sub-dermal and rarely visceral) and rarely they interest the joints, therefore the number of affected joints at the end of the study remains constant. The immediate effected can be retrieved in a very convincing manner in the frequency of hospitalizations, which dramatically decreases from an average of 26/year to 0.58/year, which represents a 45 times reduction ($p < 0.01$).

Discussions: Even if the literature still includes debates regarding the usefulness of prophylaxis, regardless of the age [7.8], some aspects have become extremely clear at present, when recombinant factor VIII is available. First of all it provides the possibility of fast substitution when faced with a hemorrhagic accident without having to use the blood transfusion or blood derivate. Thus, the risks of viral contamination were eliminated (especially the hepatic B and C viruses or retroviral viruses (HIV/AIDS), the last one representing a price extremely hard to be paid by the hemophiliacs through thousands and thousands of victims [1] during the 80's. Secondly and of great importance for the hemophiliac's quality of life, is the possibility of the prophylactic treatment which in many studies proved to be incomparably more economic than "on demand" therapy, observation which is supported by numerous researches to this regard. Therefore Aledort and associates present in 1994 in a study including 477 patients under the age of 25 years, that had received variable prophylactic doses, that the hemorrhagic episodes decrease significantly, the incidence of arthropathy is reduced considerably, the number of hospitalizations and school absences are greatly reduced, without any correlation with the administered dose, and the higher doses do not have any benefit. [9]. In 1995 Luscher recommends prophylaxis for the entire life-span in order to prevent complications [10], and in 1998 Miners and associates show that prophylaxis reduces the frequency of bleeding episodes three times (from 37/year to 13) with all the benefits that result, thus, by evaluating the cost-efficiency of prophylaxis it is determined that it is clearly superior to on demand therapy [11].

Following these studies, in 2001 Medical and Scientific Advisory Council (MASAC) recommends prophylaxis as election treatment for hemophilia at any age, after the World Health Organization and the World Federation of Hemophilia had already established the need of prophylactic treatment, and countries like Sweden (which is a leader in the field)), Canada, Netherlands, USA, Germany, France, Italy and Great Britain take over the idea and introduce prophylactic treatment in their countries. There are neighboring states like Hungary, Poland, Slovenia, the Czech Republic, Bulgaria, Austria, Lithuania, that already have experience in applying the prophylactic regime in the way they manage hemophilia.

However the professional literature still includes large debates regarding the moment and modalities of introducing prophylaxis in adults [8], as it has become obvious that substituting factor VIII has led to increasing the average life expectancy from less than 20 years to 70 years, let alone the quality of life, even when small doses are administered to persons that do not present any risks [12,13,14]. Astebnmark makes a very pertinent observation [4] which in 2003 concludes that **"whenever possible prophylaxis must be continued for the entire life-span because the risks of traumatic bleeding episodes do not disappear in the adult, the severity remains unchanged and the benefit of prophylaxis remains the same for the entire life-span"**. In fact 91% of the 22 European centers recommend and practice prophylaxis [15]. In 2005 Coppola and associates in a study on 84 patients (30 adolescents and 54 adults) for which the on demand treatment is replaced with prophylactic treatment, notice a reduction of the bleedings episodes with 70% with a moderate increase of the costs, yet with a significant improvement of the quality of life [5]. Focusing on the same topic, Togliaferi and associates observe that in 20 adolescents and adults

there is a decrease of the bleeding episodess from 26.1/year to 3.4/ year, followed by all the economic and individual consequences that result [16]. The same results are obtained by Walsh and associates on a group of 479 patients over 18 years of age, concluding that regardless if prophylaxis is started at this age, they are introduced in prophylaxis after an interruption or they continue the childhood prophylaxis, and the benefits are similar to those obtained in the child, namely the bleeding episodes are significantly reduced, the effects on joints are diminished and they crucially contribute to improving the quality of life [7]. In 2013 the preliminary results of a multinational study (SPINART) involving as well 3 Romanian specialists (L Rusen, M Ghinea and V Uscătescu) demonstrate like in children, that the prophylactic treatment is superior in adults as well, in regard to the frequency of bleeding episodes, evolution of joint effects, as well as in regard to the quality of life [17].

Yet maybe the most interesting study in our opinion is that of Collins and associates which on a lot 20 hemophiliacs with ages between 30-45 years which are switched from „on demand" to prophylactic treatment show that haemarthroses are reduced from 15 to 0, the Gilbert score of arthroses decreases from 25 to 18, and by researching the concentration of circulating factor VIII after the prophylactic administration it is determined that 48 hours after being injected 75% of the patients have the titer of factor VIII higher than 5%, and after 72 hours its titer is higher than 2% [18]. This last finding demonstrates that what is known in practice, namely that in vivo the semi-life of factor VIII is incomparably longer, and the frequency of administration and quantity can be personalized depending on clinical compliance and typology, actually according to the hemophiliac's phenotype [19.20]. In general the analysis of the results of our study assert the same realities in what concerns the main targets focused on through prophylaxis. Our study has demonstrated that there is a significant decrease of the number of bleeding episodes (over 12 times), from 50.8/year to 4/ year ($p < 0.01$).

Besides the high statistical significance of the results we would like to underline that during prophylaxis the bleeding episodes are incomparable easier, practically eliminating the vital risk of an important bleeding, especially when it is open. We would like to note that in this case the frequency of bleeding episodes is not lower in adults, as it is wrongly determined, because the four children in our study (cases 4, 17, 21 and 22) have an annual average of bleeding episodes of 36 as compared to the average of the group 50.8.

The first consequence of decreasing the bleeding episodes is the dramatic decrease of hospitalizations which are reduced form 26/year to 0.58/ year, which is more than 40 times ($p < 0.01$), aspect which from the economic perspective is more than significant as importance if we take into account the higher costs of hospitalization. If we add to all these the high quantities of factor VIII which are necessary in order to stop a hemorrhagic episode (3500-7000 u) in adults, we can establish rather easily the magnitude of expenditures that are implied by avoiding prophylaxis in this category of patients. We should also take into account that access to health services is not always easy, as is the case of our group, where 17 patients (74%) are from the rural environment, especially settlements that are difficult to access. We shall add here two other aspects that are of great importance and which concern the hemophiliac's quality of life. First of all the study shows that during prophylaxis the bleeding becomes episodically, rare and light from the perspective of frequency, concerning the muscle and dermal system, the mucosa and the bowels, totaling only 20 episodes, and the articular ones are present in only 6 patients, they are usually moderate, without causing invalidity, therefore at the end of the study the patients had the same number of affected joints which they had experienced when they were included in the study. Secondly prophylaxis provides the hemophiliac the possibility to have an almost normal life, without the spectrum of the vital risk that bleeding has, that of discomfort produced by pain, immobilization and hospitalization.

Although the utilized doses are minimum and they vary between 182-600u/kgc/year with weekly administration, our results show that the administered doses and their frequency are not the ones that matter but actually their continuity [9]. Our statement is supported by the findings of Collins and associates which show that the titer of factor VIII maintains the patient within the safety limits (>2%) even after 72 hours [18]. We would also like to

mention as an argument supporting this statement the classic observations which placed the patients with factor VIII titer between 1-2% in the moderately severe category, whose percentage represents approximately 20%, of the occasional bleeding episodes, the coagulating capacity of blood being sufficient for the regular need [19]. By means of minimum doses administered constantly and frequently we have managed to place the hemophiliac in this category (moderately severe) which radically changed its perspective. In our case what is evocative is the patient with H C (case no. 20), whose initial concentration of factor VIII was of 1.3%, who from unknown reasons started bleeding two years before (haemarthroses, gingival bleeding, lingual and conjunctival bleeding) requiring 2 hospitalizations monthly. By following our prophylactic regime the bleeding episodes become extremely rare and only occasional, allowing it to have a normal productive activity.

It can be observed in table III that most of our patients have concentrations of factor VIII with values between 0.50-0.99% and only 2 of them have a concentration of 1%, respectively 1.3% (the lowest concentration being that of 0.35%). Therefore, by administering a minimum dose of 250 u/week (namely 30-35u/day or 0.5-0.8 u/kg c/day) we can increase the titer of factor VIII within the safety limits (1.5-2%), therefore the cost per patient do not exceed the amount of 30.000 lei per patient allocated through the program for the "on demand" therapy [21]. In fact our economic calculation has illustrated that our expenditure for prophylaxis was of only 27058 lei/patient/year with all the inferred clinical and incremental benefits. As a matter of fact the objective of the prophylactic treatment is exactly that of placing the patient in the safety area (2-5%), and in the vision of the states that can afford costly programs the value is between 3-9 u/kg c/day, although there are studies that show that high doses do not bring additional benefits to the patients as compared to small doses [22]. Moreover, there are numerous studies that show that one of the major deficiencies of regular prophylaxis with recombinant factor VIII, especially when high doses are used, the administration is continuous or frequent (2-3/week), is the appearance of anti-factor VIII [23.24] inhibitor antibodies that reach 20-30% of the patients or even more [24]. The only case in our group (case no. 16, see table I) with 230 u B, which required by pass therapy with factor VII during which the level of inhibitors is reduced to 20.3 u B, showing a low incidence of 4.4%, which can support the idea that the low doses utilized in prophylaxis may reduce the risk of appearance of inhibitors[25].

Conclusions:

1. On a group of 23 patients suffering from a severe form of hemophilia A, the authors research the impact of the prophylactic treatment with minimum doses of factor VIII administered weekly, for a period between 2 to 6 years, under the aspect of incidence of hemorrhagic episodes, hospitalizations and appearance of inhibitor antibodies, as compared with a previous period when they were being administered „on demand" therapy.

2. The frequency of hemorrhagic events is reduced from an annual average of 50.8/patient to 4 ($p < 0.01$), and a reduced rate of articular effects, having a lower severity level, which has an extremely positive effect on the quality of life.

3. The immediate consequence of reducing the frequency of bleeding was the significant reduction of hospitalizations, which decrease from an annual average of 26/patient to 0.58 ($p < 0.01$), with all the medical and economic advantages which result in such a situation, at personal and social level.

4. The number of affected joints, as well as the level of the disability, did not change during the study which can mean that the status of the hemophiliac arthrosis was constant.

5. The risk of inhibitor antibodies proved to be extremely low (4.4%) as compared to the percentages of 20-30% cited in the professional literature when prophylaxis with high doses is administered.

6. Bleeding is still a constant threat, regardless of the hemophiliac's age, with all its negative consequences, therefore administering a prophylactic treatment only in the child, leaving the adult to hazard, is a form of discrimination that not accepted in the civilized world, moreover because minimum prophylaxis can bring benefits for the hemophiliac from the perspective of life quality, as well as to society from the socio-economic perspective, especially because in our study the actual expenditure was under the forecasted level

for "on demand" therapy.

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Iconography:

Table I - Structure of the studied group and evolution of the researched parameters
Table II - and **graph 1** Distribution of the cases according to age, environment, number of affected joints and associated diseases
Table III - and **graph 2** Initial Level of Factor VIII, administered quantity and F VIII inhibitor antibodies
Table IV - and **graph 3** Number of bleeding episodes and hospitalizations before and during prophylaxis.

TABLE I. Structure of the study group and evolution of the researched parameters

No.	Name	Age	Environment		No. of affected joints		Associated diseases			No. of bleeding episodes per year		Type of bleeding episodes during prophylaxis				Yearly hospitalizations		Inhibitor antibodies Bethesda units	Factor VIII		Prophylaxis duration
			Urban	Rural	Before prophylaxis	During prophylaxis	Bronchial asthma	hepatitis B	hepatitis C	Before prophylaxis	During prophylaxis	Articular	Mucosa	Muscular and sub dermal	bowels	Before prophylaxis	During prophylaxis		Initial conc. Administered	units / kg body weight	/year
1	C P	20	x		4	4	x			36	1	x	x	x		24	3	0	<1 %	21 8.4	6 years
2	D I	42	x		3	3				36	4			x		36	0	0	0.7 0%	29 1.2	6 years
3	D G	21		x	2	2				36	12		x			24	0	0	0.5 0%	18 2	6 years
4	C C	17		x	2	2				12	1			x		12	0	0	<1 %	36 5	6 years
5	C S	23	x		3	3			x	52	12		x			24	0	0	<1 %	21 8.4	5 years
6	D O	76		x	6	6		x		72	1	x		x	x	12	3	0	<1 %	18 2	4 years
7	B M	44		x	7	7			x	52	12			x	x	6	0	0	0.5 0%	18 2	6 years
8	M V	33	x		1	1				52	3		x			3	0	0	<1 %	21 8.4	4 years
9	M V	44		x	3	3			x	104	4	x	x	x		48	4	0	0.3 5%	21 8.4	3 years
10	A A	36		x	6	6			x	48	1			x		12	0	3.7 6	<1 %	25 4.8	6 years
11	I D	27		x	4	4				104	4		x			4	0.17	0	<1/ %	21 8.4	6 years
12	V L	21		x	1	1				104	4				x	12	0.33	0	<1 %	25 4.8	6 years
13	I G	30		x	3	3				24	1					12	0.6	0	<1 %	18 2.0	5 years
14	A C	34	x		7	7			x	24	1			x		12	0	0	0.5 0%	18 2.0	5 years
15	A V	42	x		3	3		x	x	60	3	x	x	x		12	0	0	<1	25	4
16	G C	45		x	5	5				56	24			x	x	3	0.17	230	<1 %	21 8.4	4 years
17	A A	15		x	1	1				52	4		x			24	0.17	0	<1 %	36 5.0	5 years
18	M P	31		x	5	5				24	1	x	x			12	0	0	0.5 0%	25 4.8	3 years
19	P D	30		x	1	1				12	1		x			12	0	0	<1 %	21 8.4	3 years
20	H C	40		x	2	2				84	2		x			36	0	0	1.3 0%	21 8.4	2 years
21	B B	13		x	0	0				36	2			x		24	1	0	<1 %	60 0.0	2 years
22	C D	7		x	0	0				36	2	x		x		24	1	0	<1 %	60 0.0	2 years
23	S S	42		x	5	5				48	1			x		12	0	0	1%	25 4.8	4 years
	Total		6	17			1	2	6	1164	101	6	11	13	4	616	13.44	2		55 32.6	107
Average values		31.9 years			3.2	3.2				50,8	4/case	26.10%	47.80%	56.50%	17.40%	26	0.58	8.7 0%		24 1.0	4,6 years

TABLE 2. Distribution of the cases according to age, environment, number of affected joints and associated diseases

Nr. Of cases	Age (years)					Environment		No. of affected joints					Associated diseases		
	< 18 years	18 - 30	31 - 40	41 - 50	> 50	urban	rural	0	1	2	3	>3	Hepatitis B	Hepatitis C	Bronchial asthma
	4	7	5	6	1	6	17	2	4	3	5	9	2	7	1

Graph 1

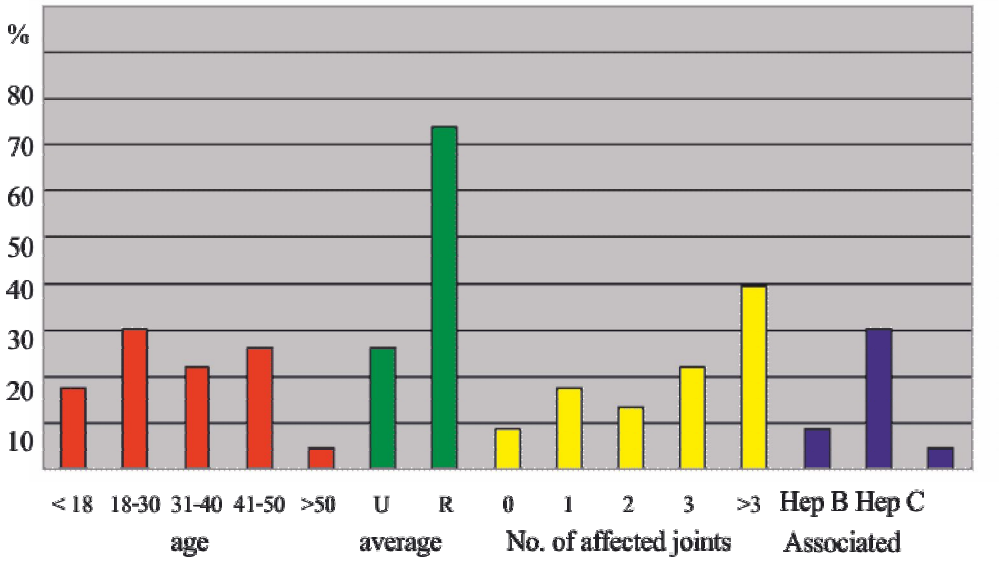


Table 3. Initial level of Factor VIII, administered quantity and F VIII inhibitor antibodies

	Initial level of F VIII			Administered F VIII units/ kg body weight/ year					Inhibitor F VIII antibodies	
	0.35-0.50%	0.51-0.99%	1-1.3%	182	218.4	254.8	291.2	600	<5 u B	>5 u B
---	5	16	2	5	8	5	3	2	1	1
%	22.1	69.6	8.3	21.7	34.8	21.7	13	8.3	4.4	4.4
average				241u/kg body weight/year						

Graph 2

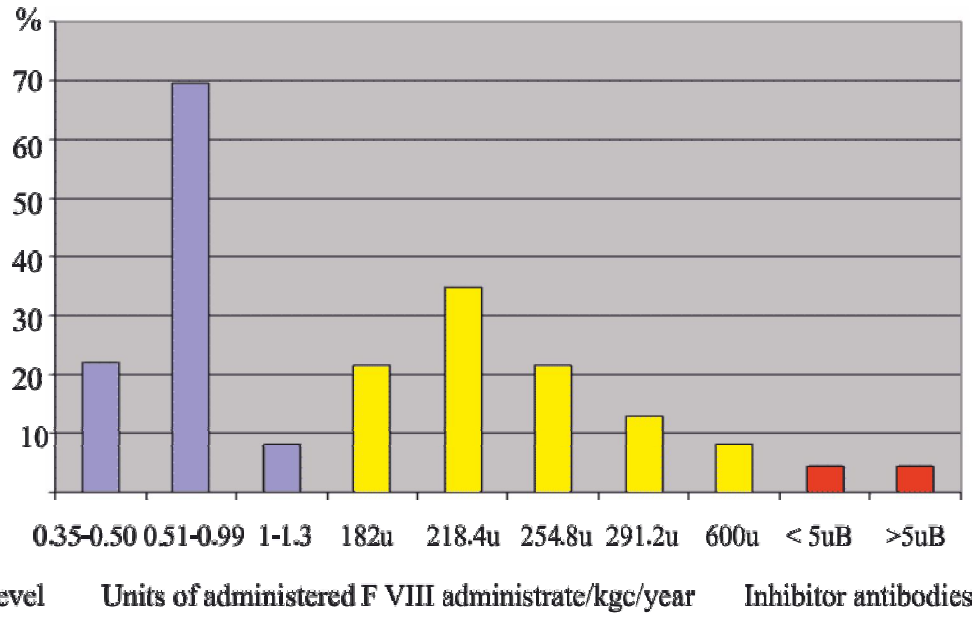
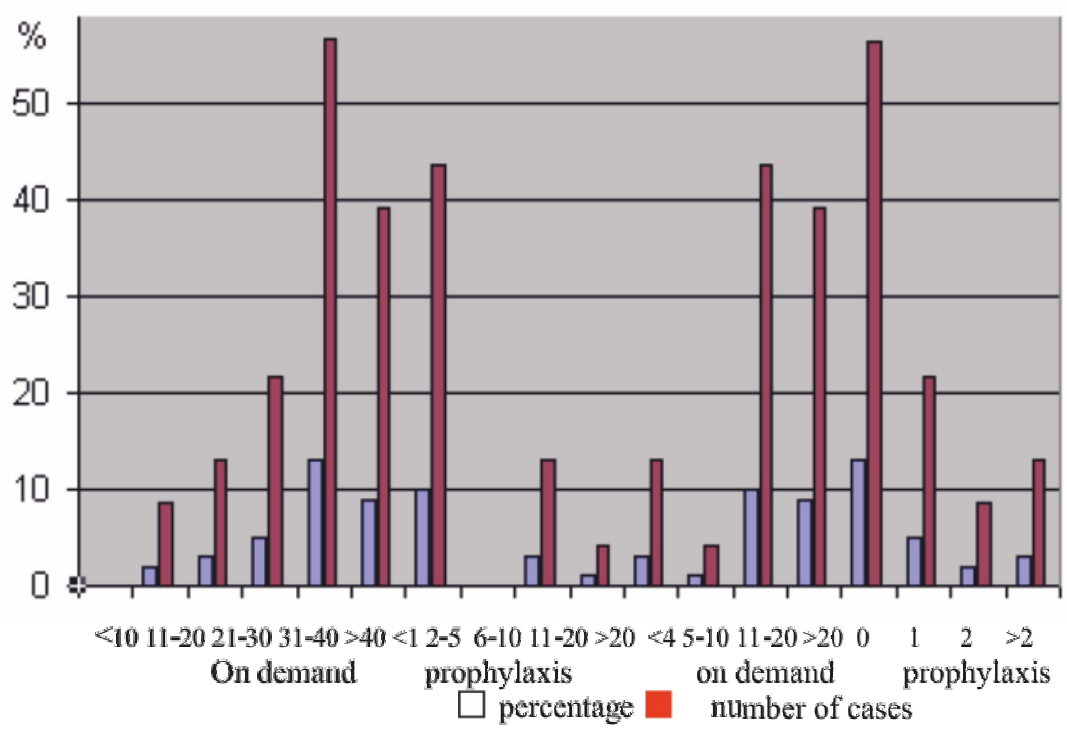


TABLE 4. Number of bleeding episodes and hospitalizations/year before and during prophylaxis

No. of cases	No of bleeding episodes/year										No. of hospitalizations / year								Bleeding during prophylaxis/no. of episodes			
	"on demand" therapy					during prophylaxis					"on demand" therapy				during prophylaxis							
	<10	11 - 20	21-30	31-40	>40	0-1	2 - 5	6 - 10	11 - 20	> 20	<4	5 - 10	11-20	> 20	0	1	2	> 2	Articular	Muscular and sub-dermal	Mucosa	Bowels hematuria
	0	2	3	5	13	9	10	0	3	1	3	1	10	9	13	5	2	3	6	9	8	3
%	0	8.7	13	21.7	56.6	39.1	43.5	0	13	4.3	13	4.3	43.5	39.1	56.5	21.7	8.7	13	26.1	39.1	34.8	13
average	50.8/ an						4/ year p < 0.01				26/year				0.58/year p < 0.01							

Graph 3



Implementing prophylaxis in the Romanian hemophiliac a dream come true

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Introduction

Hemophilia is the most commune hereditary hemorrhagic disease generated by the deficit of a coagulation factor. Determining hemophilia type A or type B is an essential stage of the biologic diagnosis. In hemophilia the hemorrhagic manifestations are present in all the patients having localizations that are more or less specific to the diseases and with a vital or functional prognosis, often reserved. The particular aspect of the hemorrhagic syndrome is that it is not always proportional to the degree of the trauma, therefore in severe forms bleeding events can occur without a cause that can be well determined.

Often the intra-articular hemorrhages can by reduced in quantity yet repeated, generate side effects like the perseverance of the water which "erodes the rock and crashes it from the crest".

The objectives of the therapy consist of preventing and fighting against hemorrhagic events, preventing and mitigating side effects in order to ensure a physical, psychic and social comfort to each patient. The basic elements of the therapy are knowing the type of hemophilia, the concentration and presence of inhibitors, the patient's weight, determining the severity of the bleed and perfectly knowing the singularities of the product. The concentrated products have numerous advantages amongst which the reduced antigenic profile and the viral inactivation, yet it has the disadvantage of not being available in sufficient quantities, due to higher costs.[1,2]

Prophylactic treatment

Professor Inga Marie Nilsson was the first one who in 1958 started administering prophylactic treatment with cold-precipitate to a hemophiliac child in order to reduce the number of hemorrhagic events. The idea was adopted by other doctors who treated hemophiliacs, especially during the production and development of concentrated products with coagulation factors. The more these concentrated products would become the more accessible the more adepts the prophylactic modality had and it was more expanded.

Considered as the most efficient form, the prophylactic treatment aims to achieve a better life as close as possible to normal and to prevent the risk of target articulation.

It is classified as:

- Primary prophylaxis defined as a regular and continuous treatment administered to patients under 2 years of age or after the first articular bleeding.
- Secondary prophylaxis:

- It represents the substitutive continuous long term treatment (long-term) administered after the age of 2 or after 2 intra-articular bleeding events

- Periodical short term treatment (short-term) following frequent bleeding events.

The prophylactic treatment varies from a national perspective and regional perspective, thus determining a reduction of the number of medical services but also of the patients' anxiety regarding the severity of potential bleeding events which occur during daily activities. Each nation has its own hemophilia treatment policy. Therefore the Netherlands administers prophylaxis with intermediary doses after the first case of hemarthrosis. Sweden was the initiator of the prophylactic treatment having a special tradition, practicing intensive treatment as of the age of 1 in doses of 25-40 ui/kgc three times a week in patients suffering from hemophilia A and the same dose twice a week in hemophilia B forms.

The evaluation of the results has shown the persons being administered prophylaxis have led a quasi-normal life and have a life expectancy similar to that of the other inhabitants. The purpose is that of mentioning a level superior to 1%, this

desiderate being obtained through the administration of 25 or 40 UI/kg 2-3 times a week.[4,5]

Continuous therapy for good efficiency is initiated at the age of 1-2 years for the severe forms of the disease before the first hemarthroses and is maintained until the age of 18. The doses must ensure levels of 1-2% factor through the administration 3 times per week, initially by the parents and after the age of 12 by the patient. A continuous prophylaxis is determined in children under the age of 5 with chronic synovitis. It is important to take into account the isoimmunization risk and the material cost.

The purpose of the hemophilia treatment is that of minimizing disabilities and prolonging life, maintaining a physical state as close as possible to normal and full social insertion so that each patient would be able to prove his/her potential. The therapeutic progress in the field of hemophilia has led to increasing life expectancy as well as its quality.

Quality of life

Numerous papers try to establish a method of quantifying the quality of life of hemophiliacs.[6,7,8,9] An European study recently published the data regarding the differences that exist between prophylactic treatment and on-demand treatment on the quality of life on a group of 1033 hemophiliacs in 16 centers. Several subjective and objective dimensions were evaluated. The physical state, the role of physical limitations, pain, and general state, vitality, social insertion, the role of emotional interference and mental health were monitored. The authors report differences between the two lots of patients under the aspect of physical status, somatic pain and general health index with a $p < 0.001$. The patients that were administered the prophylactic treatment have a higher quality of life as compared to the on-demand treatment and have reported physical pain having lower intensity, a better physical activity and in general a better state. Some physical dimensions like pain and functionality are more important in hemophiliacs.[11,12,13]

Conclusion

Administering prophylaxis to a child aims to maintain an osteoarticular system as close as possible to normal and when adulthood is reached to be able to be transferred to on demand treatment. The prophylaxis of the hemophiliac child requires a considerable financial effort from the state yet it also imposes for the family to comply with certain obligations: a permanent collaboration with the doctor that monitors the treatment, compliance with the treatment scheme, periodical clinical and biological examinations, physical therapy under specialized care, remitting to the Hemophilia center the utilized medication. Failure to comply with these obligations should lead to no longer administering to the patient the prophylaxis regime and moving him to on demand regimen, as the case is in other developed countries. Prophylaxis cannot be performed in a forced manner and with no rigorous control in regard to following instructions. The patient and the family must understand that it has rights and obligations.

The diagnosis and treatment protocol for hemophilia and the von Willebrand disease developed by a team of specialists in the field is based on the European guidelines on hemophilia management. The aim is to make it a useful work instrument for all doctors that treat these patients in order to standardize treatment at national level and develop therapy at the level of international standards.

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Analysis by means of high performing techniques in hematologic diagnosis. Recommendations for utilizing immunophenotyping for the diagnosis of acute leukemia

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Integrated Hematologic Diagnosis Group

Introduction

The diagnosis of acute leukemia relies on the simultaneous analysis of the leukemic cells by means of several laboratory methods. That is the reason why, modern diagnosis requires a team of specialists in morphology, immunophenotyping, cytogenetic and molecular biology.

The investigation starts from the clinical suspicion, following which the confirmation algorithm shall be applied in order to confirm the clinical suspicion, following which the algorithm for conforming the clinical suspicion through morphologic and cytochemical analysis shall allow the identification of the cellular line and morphologic subtype in 60-70% of the cases.

Imunophenotyping by means of flow cytometry represents the multi-parameter analysis method having an extremely high diagnosis power, which allows for the cellular markers (antigens) to be identified with the help of monoclonal antibodies. Defining the immunophenotype of the leukemic cell increases the degree of identifying the subtype of acute leukemia to more than 95%, and identifies certain subtypes of immuno-phenotypic acute leukemia, like leukemia with dendritic cells, entities which are introduced in the OMS 2008 classification of hematopoietic tissue tumors.

At the same time, immunophenotyping permits the identification of certain cytogenetic subtypes by detecting certain associations of abnormal expressions at the level of the leukemic cell, which helps orient the case from diagnosis to a prognostic subgroup.

In ore than 25% of the cases we can identify molecular/genetic markers in order to monitor the minimum residual disease (MRD), yet in the other cases, multi-parameter immunophenotyping represents the only method that has a significant resolution in the BMR analysis.

Criteria indicating the immunophenotyping analysis

Immunophenotyping is mandatory within the diagnosis of all cases of acute leukemia. The analysis is performed at the same time with the morphocytochemical analysis, from the bone marrow aspirate or peripheral blood, in accordance with the OMS 2008 diagnosis criteria.

1. Immunophenotyping of cases with clinical and morphologic suspicion of acute leukemia

When suspecting a new case of acute leukemia, take samples for the morphocytochemical diagnosis, and at the same time, take the sample for the immunophenotyping analisys. The sample for the immunophenotyping analysis comprises the bone marrow aspirate or peripheral blood in the event in which the number of blasts exceeds 20% in the peripheral blood and bone marrow aspirate cannot be sampled, in accordance with OMS 2008. It is recommended to purchase a minimum number of 100,000 cells per tube.

The patients suffering from acute leukemia following treatment, is monitored from the clinical and morphological perspective, and, depending on the treatment protocol, it might be required to perform a MRD evaluation, and in such a situation it is recommended to perform a morphologic, immuniphenotypical and genetic/molecular analysis.

2. Immunophenotyping of cases with acute leukemia for MRD monitoring

MRD monitoring during acute leukemia is performed when evaluating the response during (usually on day 15 and 35 and acute lymphoblastic leukemia - LAL) and after the induction treatments – optimum after the 2nd cycle of induction in the case of acute myeloblastoma induction acute leukemia (LAM) – in order to improve the prognostic layering and guide the post-remission guiding therapy, and to confirm the complete

response (full remission) after the consolidation treatments and in order to confirm the eligibility for the stem cell transplant.

At the same time, the MRD analysis performed after the allogeneic transplant is extremely important in order to guide the immunosuppressing treatment and chose the optimum moment for the infusion with lymphocytes from the donor.

Immuphonotyping can be applied to the MRD analysis in all cases of acute leukemia (over 90%), yet in approximately 25% of the LAM cases and more than 70% of LAL the utilization of molecular biology techniques has a higher level of sensitiveness. At present, only the MRD analysis in acute leukemia for acute progranulocytic leukemia is standardized through molecular biology analysis.

It is recommended to purchase a minimum number of 1,000,000 cells per tube, and the resolution of the analysis should be 10-4 - 10-5 similar to that of the molecular biology techniques and should reach the level of 10-6 if the used analysis is that of stem cell markers and more than 5,000,000 events per tube.

3. Immunophenotyping of acute leukemia relapse cases

The analysis of acute leukemia relapse cases requires an approach similar to the one of diagnosis, in order to be able to highlight the immuniphenotype changes, useful during the prognosis and monitoring activities of the analysis.

The multi-parameter analysis recommended for the diagnosis of acute leukemia. Panels.

At present there are several immunophenotype analyses generally accepted, developed especially on platforms with digital acquisition on 8 and 10 colors. The advantage of simultaneous analysis of 8-10 markers per tube consists in the possibility to perform a multi-parameter real analysis of markers, which permits to sub-type the cellular populations of the sample.

The expression criteria of the markers are generally established to the threshold of 20% as expression level for the cellular population, yet it decreases to the level of 10% for the following markers: cCD3, MPO, TdT, CD34, CD117.

The quantitative determination of the number of blastemas by means of flow-cytometry does not substitute the morphologic evaluation, quantification criteria of the number of blastemas, thus being left with the morphologic ones, in accordance with OMS 2008.

The platform with the highest standard is that developed by the Euroflow group, with analysis on panels with 8 colors, and strict procedures for calibrating the devices and reactants that are strictly utilized in optimized mixtures recommended on clones and fluorochromes. The analysis strategy relies on the Infinicyt software, that utilizes the PCA (Principal Component Analysis) type of analysis, which allows to have a simultaneous multi-dimensional analysis, and compare it with a joint data base, which contains patterns of types of leukemia and normal hemathopietic maturation.

Euroflow Panel

Screening

CyCD3 CD45, CyMPO CyCD79a CD34 CD19 CD7 SmCD3, to which is shall be added depending on the cellular line A or B:

A. LAM Standardization (acute myeloblastoma/non-myeloblastoma leukemia)

HLA-DR, CD45, CD16, CD13, CD34, CD117, CD11b, CD10

HLA-DR, CD45, CD35, CD64, CD34, CD117, CD300e (IREM2), CD14

HLA-DR, CD45, CD36, CD105, CD34, CD117, CD33, CD71

HLADR CD45 cTdT CD56 CD34 CD117 CD7 CD19

HLADR CD45 CD15 NG2 (7.1) CD34 CD117 CD22 CD38

HLADR CD45 CD42a+CD61 CD203c (Glycophorin A) CD34 CD117 CD123 CD4

HLADR CD45 CD41 CD25 CD34 CD117 CD42b CD9

B. LAL Standardization (lymphoblastic acute leukemia) B.1 or B.2

B.1. With B cell

CD20 CD45 CD58 CD66c (KOR-SA) CD34 CD19 CD10 CD38

SmIgk CD45 CyIgm CD33 CD34 CD19 SmIgM+CD117 SmIglc

CD9 CD45 cTdT CD13 CD34 CD19 CD22 CD24

CD21 CD45 CD15+CD65 NG2 (7.1.) CD34 CD19 CD123 CD81

B.2. With T cell

CyCD3 CD45 cTdT CD99 CD5 CD10 CD1a SmCD3
CyCD3 CD45 CD2 CD117 CD4 CD8 CD7 SmCD3
CyCD3 CD45 TCRgd TCRab CD33 CD56 CyTCRb SmCD3
CyCD3 CD45 CD44 CD13 HLADR CD45RA/CD45RO CD123 SmCD3

The platform developed by the European Leukemia Net (ELN) relies on the analysis of the immune-phenotype of the leukemic cell and on identifying the specific combinations, without imposing certain clones and fluorine-chromes. The analysis strategy is a classical one, yet it is applied on highly performing devices, of 8-10 colors. There is the possibility to perform a comparative analysis with an own data base or with inter-laboratory data bases, and it is oriented on the cellular line, by means of multi-dimensional analysis, as well as through automated compensation (Kaluza software).

European Leukemia Net (ELN) Panel

Screening
MPO cCD13 cCD79a cCD22 CD19 cCD3 CD45 to which is shall be added depending on the cellular line A or B

A. LAM Standardization
CD14 CD13 CD33 CD34 CD117 CD11b CD16 CD45
CD4 CD10 CD33 CD34 CD56 CD19 CD38 CD45
CD65 CD7 CD33 CD34 CD2 CD64 HLA-DR CD45
CD36 CD61 CD33 CD34 CD123 CD45

B. LAL B.1. or B.2. and PLUS for abnormal expressions
B.1. LAL with B cell standardization
CD58 CD10 CD33 CD34 CD123 CD19 CD38 CD45
CD81 CD13 CD34 CD22 CD19 CD20 CD45

B.2. LAL with T cell standardization
CD1a CD4 CD5 CD7 CD8 CD3 cCD3 CD45
TdT CD99 CD33 CD34 CD10 CD3 cCD3 CD45
PLUS CD13 CD34 CD2 CD117 cCD3 CD45

Both analysis strategies rely on identifying certain marker combinations which define the cellular and the maturing line, as well as the expressions specific to the leukemic stem cell (LAIP), which makes it possible to analyze with a very high level of sensitivity of the minimum residual disease in all the cases of acute leukemia.

Therefore, it is recommended to identify the following characteristics, extremely useful also during the MRD analysis:

1. Identification of the defining markers for acute leukemia like:
1. M 0, 1, 2: CD13/CD33/CD15/CD11b/CD7/CD19/CD56/CD117
2. M 3: HLA-DR/CD9/CD11c/CD2/CD56
3. M 4, 5: CD14/CD64/CD11b/IREM2
4. M 6, 7: Cd36 /glycophorin A/CD71/CD41/CD61
5. LAL: CD19/cCD79a/CD10/CD7/cCD3/sCD3/CD5/CD2,TdT

2. Abnormal expressions in LAM: expression of markers CD2/D5/CD7/CD22/CD56/CD19 and LAL: expression of markers CD15/CD65/CD33/CD13/CD66c
3. Asynchrony expressions in LAM: CD34+/CD56+, CD34+/CD11b+, CD34+/CD14+, CD117+/CD15+, CD33-/CD13+, CD13-/CD15+, HLA-DR+/CD15++, HLA-DR-/CD14+, CD11b+/CD4+ and in LAL CD19+/CD34+ CD10-, CD20+/CD34+, cCD3+/TdT-
4. Overexpression of markers: CD13, CD33, CD15, CD14 in LAM and CD10, CD7 in LAL
5. Markers' lack of expression: HLA-DR, CD33, CD13 in LAM and CD38, CD2, CD5 in LAL

MRD Analysis
For the MRD analysis we shall utilize markers which were identified during diagnosis, as well as markers which are specific to the leukemic stem cell, which provide the possibility to separate the abnormal immune-phenotype from the residual leukemic cell.

For LAM they are: CD34 CD22 CD117 CD15 CD13 CD14 HLA-DR CD33 CD11b CD2 CD56 CD7 CD36 CD133 CD19.

For LAL B it is recommended to combine at least the following markers: CD20 CD45 CD58 CD66c (KOR-SA) CD34 CD19 CD10 CD38 in order to identify MRD.

For LAL T it is recommended to combine at least the following markers: CD1a CD4 CD5 CD7 CD8 CD3 cCD3 CD45 TdT CD99 CD2 CD34 CD10 CD3 in order to identify MRD.

Conclusion.

The immunophenotyping analysis is necessary during the modern diagnosis of acute leukemia and it is absolutely necessary in the analysis of the minimum residual disease and in monitoring acute leukemia, for the analysis on performant platforms with 8-10 colors representing the most powerful modality of analysis during all the treatment and post- treatment stages.

By applying this analysis method in accordance with the international approach it was proven that it is extremely useful in the optimum management of acute leukemia. These analyses require expertise in the field of immunophenotyping diagnosis of acute leukemia, and the experience and skills of the analyst shall play an extremely important role in the correct use of the recommended strategies.

The Group for Integrate Hematologic Diagnosis recommends these strategies of using immunophenotyping in acute leukemia.

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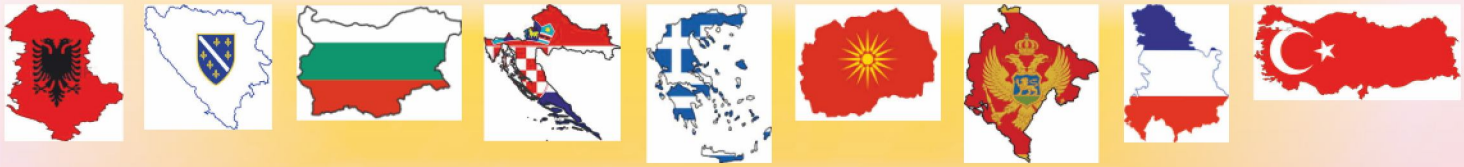

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ORAL PRESENTATIONS

FIRST LINE TREATMENT OF CLL.

Teoman Soysal
Istanbul University Cerrahpasa Medical Faculty Hematology Department, İstanbul-Turkey

Abstract not available

TREATMENT OF RELAPSED/RESISTANT AND HIGH RISK CLL PATIENTS.

Ayse Salihoğlu
Istanbul University Cerrahpasa Medical Faculty Hematology Department, İstanbul-Turkey

Abstract not available

ACUTE MYELOID LEUKEMIAS.

Oliver Karanfilski
University Clinic for Hematology, Skopje, Macedonia

Abstract not available

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THALASSEMIA.

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SCT-Unit, Aghia Sophia Children's Hospital, Thivon and Papadiamadopoulou, Athens, Greece

Abstract not available

SYSTEMIC LIGHT CHAIN AMYLOIDOSIS: MANAGEMENT.

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Systemic light chain amyloidosis (AL amyloidosis or primary amyloidosis) is a very severe disease caused by tissue deposition of immunoglobulin light chain produced by a small population of monoclonal plasma cells. This deposits of amyloid is done perivascular / intercellular in all body tissues, notable exception the brain, in a very uneven manner and will cause suffering of predominantly affected organs (heart, kidneys, gastrointestinal tract, liver, spleen, nerves ...)

The estimated incidence of the disease is around 10 patients / million inhabitants / year. This means in Romania about 200 new cases of AL amyloidosis / year. Unfortunately, the actual incidence and prevalence of the disease is much, much smaller than expected. The cause is lack of proper diagnosis of the disease, non-recognition of specific symptoms. There are several reasons for this:

- very heterogeneous pattern of clinical presentation, the patient shows symptoms related to predominant involvement of disease (neuropathy, restrictive cardiomyopathy, kidney disease, digestive disorders transit ...) and rarely the specialist (neurologist, cardiologist, nephrologist, gastroenterologist ..) thinks of this disease.
- lack of clear changes in laboratory tests: bone marrow usually indicates a slight plasma cells (60% of patients have bone marrow plasma cells less than 10%), serum protein electrophoresis is normal in 40 to 50% of cases, and serum protein immunofixation is normal 20 to 30% of the cases.
- difficulties in the histological diagnosis - only few pathologists have experience in Congo red staining and examination in polarized light. We encounter frequently false negative, and false positive results.

In addition, even if the diagnosis of amyloidosis was achieved, sometimes we have problems in differential diagnosis of light chain amyloidosis with hereditary amyloidosis - eg type transthyretin amyloidosis Gln 54 Glu specific population of Romania, the age of onset and clinical presentation (restrictive cardiomyopathy, neuropathy) are similar with AL amyloidosis, but completely different treatment and prognosis. Hereditary amyloidosis is suspected on family history and the

absence of signs of monoclonal gammopathy - need to do immunohistochemistry, sequencing amyloid fibril and molecular testing.

Correct diagnosis, determining prognosis and careful monitoring of AL amyloidosis require using some of new tools: serum free light chain assay (FLC) and NT-proBNP.

The prognosis of these patients depends on: the time of diagnosis - an early intervention gives excellent results; the number and type of affected organs predominantly - multiorgan involvement or severe cardiac disease (ventricular septum over 15 mm) indicates very severe prognosis.

Etiological therapy in light chain amyloidosis is aimed, similar to that of multiple myeloma, to destroy plasma cell clonal population producing amyloid precursor: bortezomib, melphalan, cyclophosphamide, dexamethasone, autologous bone marrow transplantation. Since the time of diagnosis we have to do risk stratification and therapy will be personalized according to risk group. An important role is supportive therapy that requires a multidisciplinary team trained in managing these patients.

This work was supported by the grant CEEX 74/2006 from the Romanian Ministry of Research and Technology. The authors express their gratitude to Professor Alan Solomon and his team from HICP, University of Tennessee, Knoxville, TN, USA for their permanent support in the diagnosis of difficult cases.

LEUKEMIA STEM CELL AND THE BONE MARROW NICHE: WHAT IS MORE IMPORTANT, THE SEED OR THE SOIL?

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Abstract not available

KINASE INHIBITORS IN HEMATOLOGICAL MALIGNANCIES. BEYOND CML.

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Abstract not available

CLOSED COLLABORATION BETWEEN CLINICIANS AND TRANSFUSION MEDICINE SPECIALIST IN BLOOD ESTABLISHMENT: BASIS TO MAKE PERSONALIZED TRANSFUSION THERAPY BECOME REALITY.

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Introduction: The effect of transfusion therapy is directly influenced both by prescription accuracy, compatibility with patient and quality of blood components administered. The difference between a prescription based on general indication of a blood component and one based on a review of the patient profile (age, pathology, history, antigenic phenotype, prognosis, etc.) marks the transition to modern transfusion medicine. To practice "personalized" transfusion medicine requires sustained availability for a large panel blood components types .Production of the whole list of blood components authorised is conditioned by the development of blood establishments, in correlation with the specificity and development of medical services in the region. Management commitment and institutions orientation towards patients' interests ensure the background for a functional clinical interface: a collaborative platform "transfusion medicine specialist – HBB Coordinator – treating physician", designed to increase patients' chance to a personalized treatment. Compromising the opportunities and risks arising from inappropriate treatment can be avoided by building a direct collaboration on principles of respect and ethics, between the transfusion medicine specialist and treating physician to identify optimal therapeutic solutions.
Material and method: The presentation brings into question the need to develop collaboration between treating doctors and blood establishments specialist, to capitalize the investment in blood processing and testing by increasing the quality of transfusion treatment.

The solutions proposed are illustrated with 15 years of experience in Constanta county. Working

with several treating physicians made it possible to provide a personalized treatment for patients with indications for long term transfusion therapy,

Results: : After a sustained information on the responsiveness capacity of Constanta blood establishment to complex transfusion needs of different categories of patients , basis for collaboration with clinicians from several departments were established (Pediatric Oncology, Hematology, Nephrology) allowing practice of a modern transfusion therapy for selected patients. In time, a database of antigenic patients' profile has been developed; by applying this approach, collection of compatible blood components for programmed transfusions in advance, administration at appropriate moment and reduction of transfusion risk became possible.

Conclusion: Currently, Blood Establishments in Romania have the potential to meet the complex transfusion needs of different categories of patientS. Feedack from treating physicians is essential to assess the responsiveness capacity, efficacy of blood components supplied and development needs.

THE ADDED VALUE OF TOP MANAGEMENT COMMITMENT TO ENSURE QUALITY AND SAFETY OF TRANSFUSION THERAPY IN A PRIVATE HOSPITAL: CONSIDERATION BASED ON A 5 YEARS EXPERIENCE.

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Introduction: Accession to EU was preceeded by the adoption of a set of laws and by-laws regulating the transfusion field starting in 2006. Although similar measures have been implemented since 1972, with the passing of generations specialized knowledge and fair practices disappeared in many hospitals, replaced by a considerable resistance to implement and follow the current rules.

Material and method: This paper presents the experience of a private hospital(obstetrics gynecology) in designing the management plan, organizing and implementing the standards for hospital transfusion activity. Being still under the influence of working in a public institution, the process was initially approached as an unimportant formality. In short time, ensuring compliance with legal requirements appeared to be a challenge whose importance was recognized in time .

Results: : Being the first private hospital in the county, the evaluation and authorization process was a challenge for county public health authorities and blood establishment, too. This experience has been used in time to create a model of organization, training and collaboration to make modern transfusion practice possible. Obtained results demonstrated the value of investing in quality for transfusion safety and the need for proactive measures to reduce the transfusion risk is a must. After 5 years, a complex process to review and develop applied documentation has been initiated, involving the whole team in order to integrate into a unified concept various activities regulated by specific rules. As a natural consequence, the first training program for clinicians in the field of blood transfusion was organized.

Conclusion: Experience highlights the real issues challenging the hospital practice, marked by the risk to transfer malpractice from public hospitals to the private health system. Adopting a supportive but firm attitude by the public health authorities and blood establishments, to assist the responsible approach on the hospital management side may be the key to achieve quality and safety by a private hospital transfusion service.

POSTERS

PROGNOSTIC FACTORS IN MYELODYSPLASTIC SYNDROMES.

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Introduction: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic disorders, characterized by peripheral cytopenias and risk of progression to acute myeloid leukemia (AML). Accurate prediction of a patient's prognosis is useful to define

disease risk and treatment options. Several factors have been shown to predict prognosis: cytopenias, age, sex, bone marrow (BM) blast proportion, performance status, co-morbidities, transfusion dependence, lactate dehydrogenase (LDH), albumin, serum ferritin (SF), karyotype and mutations.

Aim: The aim of this study was to see the implication of several factors such as age, sex, cytopenias, BM blasts, transfusion dependence, SF, LDH and albumin on overall survival and progression to AML, thus enabling us to predict disease prognosis.

Material and methods: we analyzed 120 patients, 67 men, 53 women, with MDS (primary and secondary), diagnosed at the University Clinic of Hematology, Skopje, Macedonia, in the period of January 2011 to December 2013. Several factors were taken into account at presentation: age, gender, cytopenias, BM blasts, transfusion dependence, SF, LDH and albumin. Patients were distributed according to FAB classification. Patients were followed 12 to 44 months from diagnosis, with two end points - leukemic transformation or death.

Results: analysis showed that of 120 patients with MDS, 116 were with primary, and 4 with secondary MDS. According to FAB classification, distribution of patients was as follows: RA - 85, RARS - 1, RAEB - 18, RAEB-t - 3, CMML - 9. Men to women ratio was 1,26. Mean age was 66,3 (range 17-89). Mean blast percentage in BM was 6,1 (range 2-30). Cytopenias: 1 - 27 pts, 2 - 50 pts, 3 - 43 pts. Mean blood transfusions were 12,9 (range 1-87). Mean SF level was 934,1 (range 9,5-3940). Mean LDH level was 830,9 (range 217-5790). Mean albumin was 39 (range 21-49). Patients were treated mostly with transfusion as a key supportive therapy. Some patients received stimulating agents (16 pts – granulocyte colony stimulating factor (GCSF), 11 pts - erythropoietin (EPO), 3 pts - GCSF + EPO. Iron overload in 7 patients was managed with chelation therapy. 3 pts underwent transplantation. 24 patients were followed without treatment. After statistical processing we found that older age, male gender, increased blast percentage (>5), more cytopenias (>1), blood transfusion dosage (>20), increased levels of SF (>500), increased levels LDH (>1000) and decreased level of albumin (<40) showed negative impact on survival and shortened the time to progression to AML.

Conclusion: Factors like older age, male gender, increased BM blast percentage, more cytopenias, blood transfusion dosage, increased levels of serum ferritin and LDH and decreased levels of albumin have negative prognostic value on survival and leukemic transformation and are predictors of poor prognosis in MDS.

BEACOPP IS A POWERFUL REGIMEN FOR POOR RISK HD PATIENTS.

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In 1998 the German Hodgkin Study Group reported the trial results of a newly constructed chemotherapy regimen for Hodgkin's disease, carrying the acronym BEACOPP. Many years of investigation have led to modifications in the protocol, finally establishing the originally escalated regimen as the single efficient and applicable schedule. It was originally designed for patients with advanced stage disease, but it widened the indications to all unfavorable categories, and eventually to all stages and types of the disease.

At our institution, we accepted the regimen gradually and with careful monitoring. Since in that period there were no quality alternatives available, our initial experiences were drawn from the administration of this regimen to patients who failed the standard chemotherapy regimens used.
Following the encouraging results, obtained in quite a few refractory/relapsed patients, we continued using BEACOPP in such cases, but also introduced the regimen as initial choice in patients with HD who carried 3 or more adverse prognostic factors, according to the Hasenclever IPI score.

The experience is a single-center one, therefore not carrying a statistical significance or impact. Nevertheless, the treatment results are instructive and provide an encouraging option for patients who are otherwise labeled as progressively and irreversibly deteriorating.

We present our overall results with this regimen, regardless of disease stage or risk factors,

regardless of previous treatment(s), as well as with different schemes and dosing alterations of the protocol, for the period of roughly 15 years in which we have implemented it.
As for the adverse effects of this regimen, widely discussed and reported, our observations are still not unsettling, since we have not observed any cases with a developing acute leukemia following the protocol administration. The period of observation may be too short for drawing conclusions on the secondary malignancies rate, but we have not observed any excessive occurrence rate. Regarding this issue, it is necessary to consider the relatively short period of utilization, small number of patients, the variability in dosing and length of treatment, as well as the heterogeneity of the patient population.

YOUNGER PATIENTS WITH MULTIPLE MYELOMA.

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BACKGROUND: While the majority of patients with multiple myeloma (MM) are diagnosed during their 7th decade of life, a small percentage of them belong to a much younger age group. With current treatment modalities, survival has been prolonged to 8 years or even longer. However, despite considerable progress in the pathophysiology and treatment of MM, eradication of the disease remains most of the times not feasible. It is still uncertain whether younger patients with MM have a better outcome and a superior response to therapy.

PATIENTS AND METHODS: We conducted a retrospective descriptive study of relatively young patients diagnosed with MM. Between 2003 and 2012, 275 patients were diagnosed with MM at our Center, 10 (3.6%) of which were at most 40 years old. Our primary objective was to evaluate the quality and duration of response to treatment, as well as overall survival.

RESULTS: Seven patients were men and 3 were women. The patients' age ranged from 30 to 40 years old, with a median age at diagnosis of 35 years. Medical history was unremarkable for most of our patients (6/10), while 1 had a thyroid gland nodule, 1 had had multiple surgeries due to car accident and ruptured cruciate ligaments, and in 2 patients medical history was unknown.

Median percentage of bone marrow infiltration by plasma cells at diagnosis was 50%. Extramedullary disease (brain plasmacytomas, resulting in left facial, sublingual and lower laryngeal nerve palsy) was present in only 1 patient. In 7 patients a chromosome analysis by karyotyping was conducted while fluorescence in situ hybridization (FISH) was performed in 6 patients. Karyotype abnormalities were detected in only 1 patient (complex, non hyperdiploid). However, by FISH, chromosomal abnormalities were detected in 5 patients, 3 of whom had normal karyotype. The most frequent karyotype abnormalities revealed by FISH were del(13) (in 3 patients) and t(4;14) (in 2 patients).

Five patients were diagnosed with stage IIA disease, 4 with IIIA and 1 with IIIB according to Durie-Salmon staging system. Regarding IPS staging, 6 patients were diagnosed with stage I disease, 2 with stage III disease and for the remaining patients data was not available.

First-line chemotherapy consisted of various combinations including proteasome inhibitor (bortezomib), anthracycline and/or alkylating agent (doxorubicin and/or cyclophosphamide) in addition to corticosteroids (dexamethasone). Specifically, first-line treatment was VDPACE (bortezomib, dexamethasone, doxorubicin, cyclophosphamide, etoposide and cisplatin) in 3 patients, VCD (bortezomib, cyclophosphamide, dexamethasone) and PAD (bortezomib, doxorubicin, dexamethasone) were equally administered in 2 patients, and VAD (vincristine, doxorubicin, dexamethasone), a combination of VAD with liposomal doxorubicin, and thalidomide and dexamethasone, were each one given to 1 patient. Eight out of 10 patients underwent autologous hematopoietic stem cell transplantation (ASCT) while 2 of them received tandem transplantation. One patient could not be submitted to ASCT due to rapid disease progression and death, and 1 patient proceeded directly to allogeneic hematopoietic stem cell transplantation

(alloSCT), after completion of 8 cycles of PAD and 4 cycles of lenalidomide/dexamethasone. The patient is still alive seven years from diagnosis and six years after allogeneic transplantation. In another patient alloSCT was performed after late 2nd ASCT, due to disease relapse, but the patient died because of progressive disease 8 months after alloSCT.

The median duration of response to first-line therapy is 25.3 months and overall survival is 50% at 5 years. Three patients are in complete remission 57 to 135 months after diagnosis (2 of which have received a tandem ASCT), 2 in near complete remission and 1 in partial remission. Four patients have died due to progressive disease.

CONCLUSION: Younger (less than or equal to 40 years old) patients with MM may have long-term survival, should they be offered hematopoietic stem cell transplantation, since their performance status usually allows even for alloSCT which remains the only true curative option. These patients require vigorous treatment with the aim of complete remission and hematopoietic stem cell transplantation in order to achieve the optimal outcome. In this age group, it seems reasonable to pursue even the cure of MM by appropriate therapy.

Poster

Enkeleida Trajce
Greece

Abstract not available

CLINICAL HAEMATOLOGY SECTION – EDUCATIONAL SESSION

E1. CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) OF 2014.

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The 2008 Word Health Organization (WHO) classification of myeloid neoplasms defines CMML as a clonal hematopoietic stem cell disorder that is characterized by the presence of an absolute monocytosis (> 1x109/L) in the peripheral blood and the presence of myelodysplastic and myeloproliferative features in the bone marrow (Orazi A et al.2008; Vardiman JW et al, 2009).

Because the clinical phenotyp of CMML displays features of both myelodisplastic syndromes (MDSs) and myeloproliferative neoplasms (MPNs), the classification of CMML has historicaly been a dynamic proces. CMML was included in the MDS category in the original French-American-British (FAB) classification in 1982 (Bennett JM et al, 1982) and there was considerable controversy as to whether it truly repesented MDS or a myeloproliferative neoplasm. The patients with CMML were divided into two separate categories depending on the degree of leukocytosis: CMML-MDS type (leucotyte count ≤ 13 x 109/L) and CMML-MPN type (leucocyte count > 13 x 109/L). This distinction was arbitrary bat the leucocyte count represents an important prognostic factor (Cazzola et al, 2011). Patients with CMML-MPN had a shorter survival and higher risk of AML that patients with CMML-MDS (Such et al, 2013). In 2001, the WHO classification assigned CMML to a new category known the "myelodysplastic/myeloproliferative (MDS/MPN) overlap diseases". Other disorders included in this category are juvenile myelomonocytic leukemia (JMML), atypical chronic myeloid leukemia (a CML) BCR-ABL1 negative, MDS/MPN unclasificable (MDS/MPN-U). The 2008 WHO revision classification changed the name of the general category of the "MDS/MPN overlap diseases" to "MDS/MPN neoplasms" to reflect the neoplastic nature of the diseases.

The 2008 WHO criteria of CMML are: 1) persistent peripheral blood monocytosis (>1 x 109/L); 2) no Philadelphia chromosome or BCR-ABL1 fusion gene; 3) no arrangement of PDGFRA or PDGFRB (should be excluded in cases with eosinophilia); 4) <20% blast (myeloblasts, monoblasts and promonocytes) in the peripheral blood and bone mrrow (BM); 5) at least one of the following: a) dysplasia in one or more cell lines; b) an acquired clonal cytogenetic abnormality or molecular genetic abnormality present in hematopoietic cells or c) persistence of monocytosis for at least 3 months and no evidence of other causes of monocytosis (infection, inflammation, malignancy). CMML is divided in two prognostic categories: a) CMML-1: blasts including promonocytes < 5% in the peripheral blood and < 10% in the BM and b) CMML-2: blasts including promonocytes 5-19% in peripheral blood or 10-19% BM blasts including promonocytes or presence of Auer rods. The distinction of CMML into CMML-1 and CMML-2 was shown to have prognostic significance (Germing et al, 2007).

CMML is the most aggressive chronic myeloid neoplasms with 3 year survival on the order of 20% (Rollison DE et al. 2008). Due to frequent changes in nomenclature and classification the exact incidence and prevalence of CMML remains unknown. Large population-based studies estimate that CMML constitues ~ 10% of all cases of MDS (Onida F et al, 2002). The median age at diagnosis ranges beetwen 65 and 75 years and there is an 2:1 male predominance. The clinical presentation of patients is variable. Those with a CMML-MDS like phenotype tend to present with peripheral blood cytopenias, effort intolerance, easy bruising and transfusion dependence. Those with a MPN like phenotype tend to present with leucocytosis, monocytosis, hepato-splenomegaly, pleural effusions, skin infiltration and symptoms of hypercatabolic state (night sweats, weight loss and cachexia)(Patiu M et al, 2000, Gologan R et al, 2002, Coliţă A et al, 2007) . CMML can directly present with blast phase disease (transformation to AML). The occurance of CMML following exposure to cytotoxic chemotherapy has been reported (Patnaik et al, 2013).

The diagnosis of CMML includes examination of peripheral blood, BM aspiration and biopsy, along with conventional metaphase cytogenetics and either molecular or fluorescent in situ hybridization (FISH) testing for BCR-ABL1. PDGFRA evaluation by FISH is best reserved for those patients that also have a concurent eosinophilia; the exclusion of PDGFRB rearrangement can be made by a satisfactory chromosomal study. Useful diagnostic information gained from peripheral blood smears examination includes: monocyte count, presence of dysgranulopoiesis, presence of promonocytes, blasts and neutrophil precursors (<10% in CMML and ≥ 10%

in a CML). The diagnosis of CMML requires absolute monocytosis (>1x109/L) in peripheral blood that persist for at least 3 months after carful exclusion of other condition that can cause monocytosis. Reactive monocytosis is common and is often seen in association with viral infection (arboviruses, varicella zoster) and chronic infections/inflamatory conditions such as tuberculosis, brucellosis, leishmaniasis, subacute bacterial endocarditis, sarcoidosis and connective tissue disorders. Monocytosis is also an early sign of a recovering BM following myelosupression. Clonal monocytosis is persistent and is associated with hematopoietic stem cell disorders such as CMML, JMML, AML with monocytic differentiation (Patnaik MM et al, 2013). Peripheral blood monocytosis, the hallmark of CMML, are always >1x109/L and usually range from 2 to 5x109/L, but may exceed 80x109/L (Orazi A et al, 2008). Monocytes are almost always >10% of leucocytes. The monocytes generally are mature but can exhibit abnormal granulation or unusual nuclear lobation or chromatin patern. The letter cells are termed "abnormal monocytes"- a designation used to describe monocytes that are immature but in comparison to promonocytes have denser chromatin, nuclear convulutions and folds, and a more greyish cytoplasm. Promonocytes have finely dispersed chromatin, subtle nuclear folding, fine cytoplasmic granules and usually, indistinct nucleoli (Goasguen JE et al, 2009). The WBC may be normal, decreased wih neutropenia or increased due not only to monocytosis but also to neutrophilia. Neutrophil precursors (promyelocytes, myelocite) usually account for <10% of the leucocytes. It may be difficult to distinguish between hypogranular neutrophils and dysplastic monocytes. Mild anemia normocytic or macrocytic is common. Platelet counts vary but moderate thrombocytopenia is often present. Atypcal, large platelet may be observed. Bone marrow is hypercellular in over 75% of cases, but normocellular and even hypocellular specimens also occur. Granulocytic proliferation is often the most striking finding in the BM biopsy. Monocytic proliferation is typically present but is often very difficult to appreciate in the biopsy or on BM aspirate smears. Cytochemical and immunohistochemical studies that aid in the identification of monocytes and their less mature forms are recommended. Monocytic derived cells are almost always positive for the cytochemical non-specific esterases (e.g. butyrate esterase), while normal granulocytic precursors are positive for chloroacetate esterase. In CMML, it is very common to have a "hybrid" cytochemical staining pattern with cells expressing both chloroacetate and butyrate esterases simultaneously. A predominant staining pattern of just butyrate esterase with out a dual esterase staining pattern is a very uncommon occurrence in CMML and should prompt a consideration of a de novo or emerging acute monocytic leukemia. Disgranulopoiesis is present in the BM of most patients. Erythropoiesis is generaly decreased and there may be accompanying abnormal nuclear contours, megaloblastoid changes and ring sideroblasts. Megakaryocytes are generally small and may have hypolobulated nuclei. A mild to moderate increase in the amount of reticulin fibres may be present in 30% of patients with CMML. Nodules composed of mature plasmocytoid dendritic cells (plasmocytoid monocytes) in the BM biopsy have been reported in 20% of cases. These nodules typically stain with CD123 (monocytic/dendritic cell marker)(Orazi A et al, 2008). The ultrastructural peroxidase patern of bone marrow and blood cells can help to classify the stage of maturity of proliferated cells (Mandache E et al, 1983).

In the immunophenotyping, the peripheral blood and BM monocytes usually express the myelomonocytic antigens CD33 and CD13. There may be variable expression of CD68 and CD64 and frequently decreased expression of CD14. Occasionaly may be observed aberrant expression of CD2. Aberrant coexpression of CD56 combined with underexpression of a myeloid marker (e.g. HLA-DR, CD13, CD15 or CD36) was unique for the monopoietic cells in CMML. With CD45/SSC analysis, significantly higher proportions of granulopoietic cells and lower percentage of monopoietic cells were detectable in CMML when compared to monocytic AML (Gorczyca W, 2004). Increase of CD34+ cell with aberrant phenotype may be associated with early transformation to AML.

Conventional metaphase karyotyping of bone marrow mononucleated cells is normal in two thirds of patients. By definition CMML cases do not show the Philadelphia chromosome. Cases associated with eosinophilia and rearrangements that fuse the platelet-derived growth factor receptor (PDGFRB) to another gene such as TEL in t(5;12)(q33;p13) are excluded from the CMML group by the WHO classification as this separate entity is sensitive to tyrosine kinase inhibitors such as Imatinib mesylate. The recurrent aberrations observed in CMML include loss of the Y chromosome, monosomy 7, trisomy 8, and interstitial deletions of chromosomes 20q, 11q, and 12p, all of which may be seen in other myelodysplastic and myeloproliferative disorders. Cytogenetic abnormalities were more frequent in patients with increased peripheral blood and BM blasts and those that demonstrated dyserythropoiesis and dysgranulopoiesis. Based on these findings a cytogenetic risk stratification system was developed categorizing patients in to three groups: high risk (trisomy 8, chromosome 7 abnormalities, complex karyotype abnormalities), intermediar risk (all

chromosomal abnormalities, encept for those in the high and low risk categories) and low risk (normal karyotype or – Y) with 5 year OS rates of 4%, 26%, and 35% (Such E et al, 2011).

In addition to cytogenetic abnormalities the next generation sequencing has identified molecular aberration in ~ 90% of CMML patients but none is specific of this entity (Itzykson R et al, 2013). The recurrently mutated genes encode signaling molecules (NRAS, KRAS, CBL, JAK2, FLT3, CSF3R, NOTCH, NCSTN, MAML1), epigenetic regulators (TET2, ASXL1, EZH2, UTX, IDH1, IDH2, DNMT3A, SETBP1), splicing factors (SF3B1, SRSF2, ZRSF2, U2AF1), and cohesins (STAG1, STAG2, RAD21, SMC1A, SMC3, PDS5B). Mutations in the transcription regulators RUNX1, NPM1 and TP53 have also been reported in CMML. The most frequently mutated genes are TET2 (50-60%), SRSF2 (40-50%), and ASXL1 (30-40%). TET2 and SRSF2 mutations are often combined. Most of the studies consistently report the poor prognosis of ASXL1 mutations. Although recurent mutations in CMML, such as those in TET2, ASXL1 and SRSF2 are not disease specific, it is the high frequency of these mutations that gives CMML its "unique genomic identity" (Itzykson R et al, 2013).

The prognosis of patients with CMML is unfavorable with a median survival of only 24-36 months and 20% - 30% risk of transformation to acute myeloid leukemia. Numerous prognostic systems have attempted to better define and stratify the natural history of CMML. The International Prognostic Scoring Systems (IPSS) is designed for patients with MDS and excluded patients with CMML that had a proliferative phenotype (WBC > 13 x109/L).

The MD Anderson Prognostic Scoring System (MDAPS) was developed in 2002 on a cohort of 213 CMML patients and identified a hemoglobin level < 120g/L, presence of circulating immature myeloid cells (IMC), absolute lymphocyte count (ALC) >2,5x109/L and ≥10% BM blasts as independent predictors for inferior survival (Onida F et al, 2002). This model identified four subgroups of patients with median survival of 24, 15, 8 and 5 months for low, intermediate 1 and 2 and high risk categories.

The association of higher lymphocyte counts with shorter survival has been confirmed by the MDS Düsseldorf Registry (Germing U et al, 2002).

In 2013, three prognostic scores have been developed and validated in CMML. The first reported prognostic scor was developed by Spanish MDS Group. The CMML Specific Prognostic Scoring System (CPSS) was developed in 558 patients and validated in 274 patients (Such E et al, 2013). The four prognostic variables predincting for OS and LFS were: CMML FAB subtype, CMML WHO subtype, cytogenetic risk stratification and red cell transfusion dependence. One point was allocated for each variable. Four risk groups were obtained: low (0), intermediate 1 (1), intermediate 2 (2-3) and high risk (4-5) with mediane survival of 72, 31, 13 and 5 months. The CSSP confirms the prognostic impact of FAB and WHO subtypes, recognizez the importance of RBC transfusion dependency and cytogenetics and offer a simple and powerful scor for accurately assesing prognosis and planning therapy in CMML.

Mayo Prognostic model was proposed in the last year (Patnaik MM et al, 2013). One point each was assigned to the following four independent prognostic variables: absolute monocyte count >10x109/L, presence of IMC, hemoglobin <100g/L and platelet count <100x109/L. This model stratified patients in to three risk groups: low (0), intermediate (1) and high (≥ 2) translating to median OS of 32, 18 and 10 months.

The Groupe Francophone des Myelodysplasia (GFM) (Itzykson R et al, 2013) analysed clinical and molecular parameter in 312 CMML patients. A multivariate model identified independent prognostic association with WBC >15x109/L (three points), ASXL1 mutations (two points), age >65 years (two points), platelet count <100x109/L (two points) and hemoglobin<100g/L in females and <110g/L in males (two points). This model stratified patients into three groups: low (0-4), intermediate (5-7) and high risk (8-12), with median OS of not reached, 38,5 and 14,4 months respectivelly. In this study, gene mutation that had a prognostic impact an a univariate analysis included: ASXL1, SRSF2, CBL and IDH2.

Date from Spanich MDS Group demonstrate that ringed sideroblast may also have prognostic significance in CMML (Such E et al, 2009). Patient with CMML-RS (>15% ringed sideroblast in BM at presentation) had better OS than patients with classical CMML and a lower risk of evolution to acute leukemia.

The treatment for CMML is broadly divided into supportive care and directed, targeted therapies [hypomethylanting agents, allogeneic hematopoietic stem cell transplantation (HSCT)]. Therapy should be started when the disease is symptomatic or progressive, and, in particular, when one of these events occurs: a) severe anemia (Hb less than 10 g/dL); b) percentage of blasts in peripheral blood >5% (including myeloblasts, monoblasts and promonocytes); c) platelet count ≤ 50x109/L; d) WBC count ≥20x109/L; e) immature granulocytes ≥10% in peripheral blood; f) extramedullary manifestations of the disease, such as cutaneous or lymphnodal; g) symptomatic splenomegaly.

The treatment strategy should be decided first

according to the disease hematologic phenotype, in particular whether it is an MDS phenotype or a MPN phenotype and to the number of blasts in BM. (Onida F et al, 2013; Padron E et al, 2013; Parikh SD et al, 2012; Patnaik MM et al, 2013).

Patients with MDS-CMML and less than 10% blasts in BM should be managed with supportive therapy aimed at correcting cytopenias. Patients with severe anemia (Hb<10g/dL) and with serum erythropoietin ≤500mU/dL should be treated with erythropoietic stimulating agents. Granulocyte-colony stimulating factors are indicated only for frequent neutropenic infections. Iron chelation are recomanded for patients with frequent transfusions and risk for haemocromatosis. Consideration for allogeneic HSCT should be a therapeutic option in younger patients with matched sibling donors. In patients with MDS-CMML with high number of blasts (≥10% in BM and >5% in the blood), the supportive therapy should be integrated with the use of hypomethylating agents (5-azacytidine or decitabine). In selected patients, allo-HSCT may be offered as an option. Patients with MDS-CMML with higher number of blasts, resistent or intolerant to 5-azacytidine and not eligible for transplant, should be treated with supportive therapy and enrolled in clinical trials.

The patients with MPN-CMML with a low number of blasts (<10% blasts in BM) should be treated with cytoreductive therapy. Hydroxyurea is the drug of choice to control proliferative myelomonocytic cells and to reduce organomegaly. In patients resistant or intolerant to Hydroxyurea, cytolitic therapy should be given to control the disease and avoid a rapid increase in WBL count. Etoposid, low dose ARA-C, thioguanine as single agents are reported to be efficacious. Hypomethylating agents should be used in the context of clinical trials. Patients, with MPN-CMML and high number of blasts (>10% in BM) should receive polychemotherapy followed when possible by allo-HSCT. Patients with a high blast count resistant to conventional blastolytic therapies should be treated with new and experimental therapies.

Other agents that have been tried alone or in conjunction with hypomethylating agents include histone deacetylase inhibitors (panobinostat, vorinostat), immunomodulatory drugs (lenalidomide), farnesyl transferase inhibitors (tipifarnib, lornafarnib).

Allogeneic stem cell transplantation (allo-SCT) remains the only curativ option for patients with CMML. For young patients with high-risk disease, poor prognostic scores, high- risk karyotip and increased BM blasts, early stem cell transplantation strategies should pursued.

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E2. CLL PATIENT PROFILE NEEDING TO BE TREATED.

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world with an incidence of 4.2/100.000/year. The incidence increases to >30/100.000 year at an age of >80 years. The median age at diagnosis is 72 years. About 10% of CLL patients are reported to be younger than 55 years.[1]
The median survival from diagnosis varies between 18 months and >10 years. With the new treatment options available, the overall survival of patients with advanced stages has improved. Additional prognostic markers are available to predict the prognosis of patients with CLL, in particular at early stages[2].

Treatment should only be given to patients with active, symptomatic disease. The following conditions define active disease: significant B-symptoms, cytopenias not caused by autoimmune phenomena and symptoms or complications from lymphadenopathy, splenomegaly or hepatomegaly, lymphocyte doubling time of <6 months (only in patients with >30.000 lymphocytes/l) as well as autoimmune anemia and/or thrombocytopenia poorly responsive to conventional therapy.

Treatment of early, stable disease (Binet stage A and B without active disease; Rai 0, I and II without active disease): previous studies have shown that early treatment with alkylating agents does not translate into a survival advantage in patients with early stage CLL.[3] The standard treatment of patients with early disease is a "watch and wait" strategy. Blood cell counts and clinical examinations should be performed every 3-12 months.
Treatment of advanced, active disease (Binet stage A and B with active disease, Binet stage C; Rai 0-II with symptoms, Rai III-IV): the fitness and co-morbidity of patients need to be evaluated for the choice of the treatment. For assessing the co-morbidity burden, the Cumulative Illness Rating Scale (CIRS) represents a helpful tool.[4]

An improved survival has been demonstrated following first line chemoimmunotherapy with FCR in physically fit patients with CLL.[5] Therefore, in this patient group (physically active, no major health problems, normal renal function) FCR is the standard first-line therapy.
In patients with relevant co-morbidity, chlorambucil seems to be the standard therapy.[6]
Alternatives are dose-reduced purine analog-based therapies (FC, PCR – pentostatin, cyclophosphamide and rituximab) or bendamustine.[7]

New targeted therapies, more active, and a

better tolerability profile are expected to get regulatory authorities approval mainly for those unfit patients, elderly and, usually, with co-morbidities.

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E3. **LATE COMPLICATIONS OF HEMATOLOGIC DISEASES AND THEIR TREATMENT.**

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There has been a marked improvement in survival for patients with hematologic malignancies over the past three decades, and the population of long-term cancer survivors continues to grow.

The disease- or treatment-specific subgroups of long-term survivors are at risk for developing adverse outcomes, including premature death, second neoplasms, organ dysfunction (cardiac, pulmonary, gonadal), reduced growth, decreased fertility, impaired intellectual function, difficulties obtaining employment and insurance, and overall reduced quality of life.

Complications observed after hematopoietic cell transplantation (HCT) have a multifactorial origin related to prior cancer therapy, intensity of the preparative regimen, graft-versus-host disease (GVHD), and other posttransplantation complications.

Cardiac Effects

Anthracyclines are causes of late-onset cardiomyopathy, characterized by increased afterload followed by development of a dilated, thin-walled left ventricle, which becomes poorly compliant. Among anthracycline-exposed patients, the risk for cardiotoxicity can be increased by mediastinal irradiation, uncontrolled hypertension, underlying cardiac abnormalities, exposure to chemotherapeutic agents other than anthracyclines and electrolyte imbalances. Risk is increased for survivors who are female, and those who were very young (<5 years old) at the time of therapy.

Chronic cardiac toxicity associated with radiation alone most often manifests as valvular abnormalities, coronary artery disease, pericardial effusions, or constrictive pericarditis, sometimes in association with pancarditis.

Late cardiac dysfunction after HCT is multifactorial in origin. The presence of the conventional cardiovascular risk factors (hypertension, diabetes, dyslipidemia, increased body mass index, physical inactivity, and smoking) could increase the risk for cardiac toxicity in patients already exposed to cardiotoxic agents.

The prevention of cardiotoxicity is a focus of active investigation. Liposome-encapsulated anthracyclines have been explored for their propensity to result in a lower incidence of cardiotoxicity and biopsy results have confirmed a low early cardiotoxicity and the relative safety in clinical use.

Agents such as dexrazoxane, which remove iron from anthracyclines, have been investigated as cardioprotectants. The cardioprotective effects appear to be sex specific, with females showing the greatest protective effect.

Joint recommendations for monitoring long-term survivors of HCT by the European Group for Blood and Marrow Transplantation/Center for International Blood and Marrow Transplant Research/American Society for Blood and Marrow Transplantation (EBMT/CIBMTR/ ASBMT) suggest that cholesterol and high-density-lipoprotein cholesterol (HDL-C) should be checked at least every 5 years for men starting by age 35 years and women starting at age 45. The screening for dyslipidemia should start at age 20 for smokers, patients with diabetes, or patients with a family history of heart disease.

Pulmonary Effects

Compromise of pulmonary function among survivors of hematologic malignancies has been reported after conventional therapy for Hodgkin lymphoma (HL) and leukemia and after HCT. Risk factors include exposure to certain chemotherapeutic agents (bleomycin), radiation to the chest, underlying

lung disease, and a younger age at exposure to the pulmonary-toxic therapeutic agents. The toxicities involving the airway and lung parenchyma, including restrictive and chronic obstructive lung disease and bronchiolitis obliterans, are observed after HCT.

The Children Oncology Group Long Term Follow-Up guidelines (COG LTFU) recommend monitoring for pulmonary dysfunction in childhood cancer survivors that includes assessment of symptoms such as chronic cough or dyspnea on annual follow-up and respecting cumulative dosage restrictions of bleomycin and alkylators, limiting radiation dosage and port sizes, and avoidance of primary or secondhand smoke. Pulmonary function tests and chest x-ray examination are recommended for patients at risk.

Joint recommendations for monitoring long-term survivors of HCT by the EBMT/CIBMTR/ASBMT suggest routine clinical assessment at 6 months, 1 year, and annually thereafter; institution of active smoking cessation programs; and pulmonary function tests and focused radiologic assessment at 1 year after allogeneic HCT for patients with signs or symptoms of lung compromise.

Endocrinologic Effects

Thyroid – Patients with hematologic malignancies treated with cranial, craniospinal, or mantle irradiation are at increased risk for thyroid complications. Abnormalities including hypothyroidism, hyperthyroidism, and thyroid neoplasms, have been reported to occur at rates higher than those found in the general population.

Growth – Poor linear growth and short adult stature are complications after successful treatment of hematologic malignancies in childhood. The adverse impact of central nervous system (CNS) irradiation on adult final height among childhood leukemia patients, appear to be related to age and sex, with females and children younger than 8 years at the time of therapy being more susceptible.

Obesity – An increased prevalence of obesity has been reported among survivors of childhood acute lymphoblastic leukemia (ALL). Obesity adversely impacts the overall health status in survivors and is associated with insulin resistance, diabetes mellitus, hypertension, and dyslipidemia. Growth hormone deficiency related to cranial radiation may predispose adult survivors of childhood ALL, particularly females, to abdominal obesity and metabolic syndrome.

Gonadal Dysfunction – Treatment-related gonadal dysfunction has been documented in male and female patients after therapy for hematologic malignancies. Radiation effects on the ovary are age and dose dependent. Reduced sperm production has been observed after testicular doses of 1 to 6 Gy and follows a dose-dependent pattern. Azoospermia has been reported among HL patients with calculated testicular irradiation exposures ranging from 1 to 3 Gy.

Ovarian and testicular damage can also result from chemotherapeutic agents, with alkylating agents showing the strongest association.

Pregnancy Outcomes – Offspring of survivors of childhood hematologic malignancies do not appear to be at increased risks for cancer or congenital malformations. The frequency of premature birth was not related to prior maternal exposure to alkylating agents, but prior exposure to doxorubicin or daunorubicin increased the risk for low birth weight independent of pelvic irradiation history.

Musculoskeletal Effects

Osteonecrosis is a painful and debilitating condition that develops when the blood supply to the bone is disrupted, usually in areas of terminal circulation; with resultant death of bone and cell tissues or disruption of bone repair mechanisms. Osteonecrosis has been reported after conventional therapy for hematologic malignancies, after exposure to dexamethasone between the ages of 10 and 20 years. Osteonecrosis is reported among HCT recipients. The hip joint was the most involved joint (80%); the knee, wrist, and ankle joints were also affected.

Osteopenia or osteoporosis is seen in survivors of hematologic malignancies. Risk factors include therapy with corticosteroids, methotrexate (at higher doses), and cranial irradiation with resultant pituitary insufficiency or gonadal dysfunction. Lifestyle factors that increase the risk for osteopenia include lack of regular weight-bearing exercise, inadequate calcium and vitamin D intake, smoking, and excessive alcohol consumption. Pain or a history of fractures may be the only indication of osteonecrosis or osteoporosis. The COG LTFU guidelines recommend a baseline dual-energy x-ray absorptiometry (DEXA) or quantitative CT scan for survivors 2 or more years following completion of treatment, with repeat studies as clinically indicated.

Neurocognitive Effects

Among survivors of childhood leukemia, neurocognitive late effects represent one of the more studied topics. These patients are prone to problems with receptive and expressive language, attention, and visual and perceptual motor skills, most often manifested as academic difficulties in the areas of reading, language, and mathematics.

The neuropathologic syndromes related to leukoencephalopathy may occur in survivors of childhood hematologic malignancies, including radionecrosis, necrotizing leukoencephalopathy, mineralizing microangiopathy and dystrophic calcification, cerebellar sclerosis, and spinal cord dysfunction, manifesting clinically as ataxia, spasticity, dysarthria, hemiparesis, or seizures.

Many survivors of adult-onset hematologic malignancies also experience impairments of neurocognitive function, including memory loss, distractibility, and difficulty performing multiple tasks. These patients may suffer from mood disturbances and symptoms that compromise their ability to function adequately, including fatigue and pain. HCT survivors are also at risk for neurocognitive late effects.

The adults patients are at risk for developing adverse sequelae related to neuropsychologic functioning, such as slowed reaction time, reduced attention and concentration, and difficulties in reasoning and problem solving; memory impairment; problems with executive functioning and processing spee; and cognitive impairment. Reduced memory function is associated with older age, longer interval since HCT, chronic graft-versus-host disease, and long-term cyclosporine use. Lower education level and poorer social functioning appear to impact cognitive performance.

Joint recommendations for monitoring long-term survivors of HCT by the EBMT/CIBMTR/ASBMT suggest that all recipients of HCT should undergo clinical evaluation for symptoms or signs of neurologic dysfunction at 1 year after HCT.

Other Toxicities

Ocular Effects – Survivors of hematologic malignancies are at risk for the development of cataracts as a consequence of therapy with corticosteroids, cranial irradiation TBI, or busulfan. Factors independently associated with an increased risk for cataract formation were older age (>23 years), allogeneic bone marrow transplantation, higher dose rate, and steroid administration for longer than 100 days. Xerophthalmia may also occur as a late complication because of decreased lacrimation resulting from damage to the lacrimal gland during radiation or, in HCT patients, from chronic GVHD.

Audiologic Effects – Survivors of hematologic malignancies who received platinum chemotherapy, those who had cranial irradiation at a young age, and those who required supportive therapy with amino-glycoside antibiotic are at risk for therapy-related hearing loss. Hearing loss associated with ototoxic agents is sensorineural in origin and is irreversible.

Dental Effects – Children whose teeth have not completely developed at the time of cancer treatment are vulnerable to dental complications, and treatment with chemotherapy during early childhood may result in qualitative problems with enamel and root development. The patients who received radiation therapy involving the head or neck are susceptible to dental complications, manifesting as increased susceptibility to dental caries and gingivitis as a result of diminished salivary gland function.

Hepatic Effects – Acute hepatic dysfunction may be seen with certain chemotherapeutic agents, including antimetabolites and anthracyclines, there has been a low reported incidence of delayed hepatotoxicity in patients receiving these agents.

Second and Subsequent Malignancies

Second or subsequent malignancies are defined as histologically distinct cancers developing after the occurrence of a first cancer. Second malignant neoplasms are one of the most devastating consequences of cancer therapy. Subsequent malignancies are categorized into two major types: therapy-related myelodysplastic syndrome and acute myeloid leukemia (t-MDS/AML) or solid tumors. The latency between diagnosis and treatment of the primary cancer and the development of t-MDS/AML is short, whereas nonhematopoietic malignancies or solid tumors seem to have a longer latency. Female sex, older age at diagnosis, earlier treatment era, HL, and treatment with radiation were identified to increase the risk for subsequent malignancies.

Several host and clinical factors are associated with an increased risk for subsequent malignant neoplasms after HCT. These include age at HCT, pre-HCT exposure to chemotherapy and radiation, exposure to TBI as part of conditioning, infection with oncogenic viruses, prolonged immunosuppression after HCT, autologous versus allogeneic HCT, and original cancer. t-MDS/AML is the major cause of nonrelapse mortality in patients undergoing autologous HCT for patients with a primary diagnosis of HL or NHL.

CNS tumors, the most common second malignancy observed among survivors of childhood ALL, are associated with exposure to cranial irradiation. Secondary thyroid malignancies, typically papillary carcinoma, are associated with radiation exposure to the thyroid gland as part of CNS irradiation, either prophylactic or for treatment of CNS leukemia.

Survivors of HL represent one of the subgroups of cancer survivors who are at a very high risk for secondary cancer, especially for patients who received earlier regimens with predominantly radiation-based therapies.

Breast Cancer – Breast cancer is the most commonly reported second malignancy among female survivors of childhood HL treated with mantle field irradiation, and the risk remains elevated for many decades after exposure.

Thyroid Cancer – Secondary thyroid cancer, the second most common solid tumor reported among survivors of childhood HL, is strongly associated with radiation therapy, occurs more frequently in females. Sex, age at exposure, and time since exposure were identified to be significant modifiers of the radiation-related risk for thyroid cancer.

Central Nervous System Tumors – Radiation is the

most important risk factor for the development of a new CNS tumor. There is the dose-response relationship between radiation exposure and development of new primary neoplasms of the CNS. Radiation exposure was associated with increased risk for subsequent glioma and meningiomas.

t-MDS/AML – Several studies have described an increased risk for t-MDS/AML with older age at HCT; Thus t-MDS/AML after autologous HCT is the result of cumulative toxicity that includes pre-HCT chemotherapy (alkylators and topoisomerase II inhibitors), topoisomerase II inhibitors used for stem cell mobilization, and transplantation-related conditioning. The COG LTFU guidelines recommend monitoring for t-MDS/AML with annual complete blood cell count for 10 years after exposure to alkylating agents or topoisomerase II inhibitors.

Most other subsequent malignancies are associated with radiation exposure. Screening recommendations include annual physical examination of the skin and underlying tissues in the radiation field. Screening for early-onset colorectal cancer (radiation doses of 30 Gy or higher to the abdomen, pelvis, or spine) should include colonoscopy every 5 years beginning at age 35 years or 10 years following radiation.

Psychosocial Effects

Survivors of hematopoietic malignancies are at risk for adverse psychosocial outcomes that may affect the overall quality of life, including anxiety, depression, posttraumatic stress disorder, and barriers to accessing the health care system due to problems obtaining health insurance coverage. The impact of cancer therapy on psychosocial functioning is dependent on many variables: intensity and duration of therapy, treatment-related complications, family functioning, developmental processes, and treatment-specific sequelae such as altered cognitive or physical functioning.

Evaluating Survivors for Potential Late Effects

The long-term complications of treatment for which an individual survivor is at risk are determined by several factors: the patient's diagnosis, age at treatment, specific chemotherapeutic agents received, specific radiation fields and doses, therapy-related complications, degree of psychosocial support received, genetic predisposition, and current health-related behaviors (diet, physical activity, tobacco, and alcohol use).

Therapeutic approaches to hematologic malignancies vary widely depending on the patient's age at diagnosis, biologic subtype and staging of disease, year (era) of diagnosis, initial response to therapy, and physician/institutional preference.

Conclusions.

As treatment for hematopoietic malignancies continues to improve, follow-up care for survivors of these diseases must be provided in a comprehensive manner. To minimize treatment-related sequelae and provide early intervention for identified late effects, the risks of long-term complications for each individual survivor must be evaluated.

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E4. RECENT ADVANCES IN PATHOPHYSIOLOGY AND TREATMENT OF APLASTIC ANEMIA.

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Aplastic anemia (AA) is most simply defined as peripheral blood pancitopenia and a hypocellular bone marrow (BM). The diagnosis requires excluding other causes of pancytopenia.

AA is most often idiopathic or apparently result from various causes: ionising radiations, chemicals, drugs, viruses. In childrens and young adults aquired AA should be disingned from the inherited forms of BM failure, Fanconi anemia (FAK) and dyskeratosis congenita (DKC). Patients with DKC can lack typical phisical anomalies and the pancitopenia can develop long after childhood miming idiopathic AA. Although AA is characterized by a severe diminution of BM function that affects all the hematopoietic lineages, granulocyte, platelet and red blood cells, levels may not be depressed uniformly.

A few histocompatibility types have also been associated with AA (HLA-DR2). HLA-DR subtypes have proved useful in predicting response to immunosuppressive therapy. Genetic predisposition may be responsable for some idiosyncratic reactions to drugs and chemicals leading to developpement of AA. Polimorphisms in cytokine genes, associated with an increased immune response also are more prevalent in AA.

A consistent laboratory finding for patients with AA is very low number of hematopoietic stem cells. At clinical presentation the stem cell number is estimated to be reduced to 1% or less than normal. The BM is not truly empty but is replaced by fat cells.

Sugestions from murine models that adypocytes inhibits hematopoiesis require further studies and validation in humans. The most powerfull evidence that aquired AA in adults is secondary to imune destruction coming from the clinic response to immunosuppressive therapy. There are abundant laboratory data supporting an immune pathophysiology but detailed mecanisms are lacking. A few antigens have been detected from peptide screens of sera but their relationship to the T-cell response is unclear. Cytotoxic lymphocytes and type I cytokines appear to be proximate effector cells. Why aberant cellular immunity persists is unclear. T-regulatory cells appeared to be deficient in quantity and function. Genomic applied to oligoclones in BM failure states might reveal an acquired genetic basis for abnormal persistence of an initially appropiate immune response.

First line treatment is determined by the severity of bone marrow faillure, the age of the pacient, the avaiability of a suitable donor and presence of comorbidities.

For patients under age 20 allogenic hematopoietic cell transplant is the treatment of choice. Transfusions of platelets and red cells should be minimized and family blood donors should be avoided to minimize sensitization. Aproximately 60 – 70% of such patients may be cured. Unfortunately only 25 to 30% of potential recipients with AA have HLA matched siblings. The remaining 70-75 of patients should receive immunosuppressive therapy, although matched unrelated transplants have been tried in younger patients with resistant disease. The approach of patients aged 20 – 45 years is changing, because current transplantation programs reduces incidence and severity of GVHD. If the pacient is in othervise excellent health and has a fully HLA matched sibling donor allogenic HCT could be the first choice.

Immunosuppressive therapy with antitymocyte globuline and cyclosporine should be given to patients

without such donor. For patients over age 45 immunosuppressive treatment is recomended. The response rates with ATG/CSA ranged between 60 – 80% with a 5 year survival comparable to BMT. Persistant cytopenia is common and many patients relapse, become dependent on cyclosporine or develop secondary clonal disease (PNH or MDS). There are no standard treatment for managing refractory and relapsed patients after immunosuppressive treatment. Therapeutic options includes matched, unrelated donor BMT, umbilical cord blood transplantation, high dose cyclophosphamide, other immunosuppressive drugs: allemtuzumab (alone or with cyclosporine) or other agents: trombopoietin mimetics, androgenes.

E5. **THE MYTH OF SISYPHUS IN ACUTE LEUKEMIA. NEW AND OLD THERAPIES IN ACUTE MYELOID LEUKEMIA.**

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Acute myeloid leukemia (AML) is one of the most aggressive forms of hematologic malignancies. More than 40 years away from establishing standard induction therapy - cure "3 + 7", although there is progress in understanding pathogenesis of acute myelogenous leukemia and in establishing new therapeutic targets, so far no significant results appeared in the current therapy of AML with new therapeutic agents. Complete remission can be achieved in most patients under 60 years, however only 30-40% of them will survive long term. Long-term survival in patients over 60 years of age is 10-15%.

In this context it is necessary to develop new treatments that target the mechanisms involved in proliferation and cell survival in AML. In this paper we review the epigenetic therapy, immunomodulatory, hipomethylating drugs and the role of monoclonal antibodies in the therapy of AML.

In conclusion, although there are promising results of these continually growing in number new therapies, individualized treatment of AML based on patient-specific molecular abnormalities seems to be the best option for patients with this condition, yet not becoming a real available option in the present .

E6. **MULTIPLE MYELOMA FROM BIOLOGY TO THERAPY.**

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Multiple myloma is a malign plasma cell dyscrasia whose origin lies in a germinal center B lymphocyte.
This germinal center cells undergo somatic hypermutation and izotypic switch.
Malignant transformation is sequential multistep process involving the progressive accumulation of several abnormalities starting with an aberrant immune answer initiated by a B cell.
Cytogenetic changes are: numeric - trisomies of impar chromosomes, or structural - translocations which are not random.

The resultant clone, malign by its biology, may rest asymptomatic (MGUS).
About 1% of those cases per year, progress to clinical certain malignancy.
The final transformation is provided by a new genetic abnormality of the malignant cell or/and changes in bone marrow microenvironment concerning immune surveillance, angiogenesis, etc.

MM is a heterogeneous disease by pathogenesis and not only. The biology of malignant process is reflected in disease aggressively, prognostic, treatment response and overall survival.

Prognostic stratification is primary based on:
- presence/absence of genetic abnormalities (t(14;16), t(14;20) or del 17p13 by FISH) or
- molecular biology (molecular expression of the genetic abnormalities).

MM high risk (with the abnormalities mentioned above) is characterized by short overall survival (2-3 years) with conventional treatment, including ASCT (autologus stem cell transplant).
In conclusion: the molecular biology of the malignant clone has a major role in choosing a personalized chemotherapy for the patient with MM.

E7. **MANAGEMENT OF HEREDITARY THROMBOPHILIA IN PREGNANCY.**

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So far, in the scientific literature were reported a significant number of mutations and polymorphisms potentially thrombophilic. There are also published data showing the involvement of these mutations / polymorphisms in the development of thrombotic accidents of pregnancy and pregnancy-related complications: recurrent pregnancy loss, severe eclampsia without apparent cause, "abruptio placentae", intrauterine growth restriction, intrauterine death.

According to international guidelines (ACOG, CHEST, BJH guidline) screening for heritable

thrombophilia in pregnancy is indicated for patients with a personal history of VTE (especially unprovoked thrombotic events or in the presence of a minor risk factor); patients with a family history of VTE or hereditary thrombophilia (especially relative to grade 1). It is not recommended to perform screening in unselected population. Depending on the type of hereditary thrombophilia there are two risk groups: 1. low risk of hereditary thrombophilia: factor V Leiden - heterozygous form, prothrombin gene mutation 20120 - form heterozygous deficiency of protein C or protein S deficiency hereditary thrombophilia; 2. high risk of hereditary thrombophilia: antithrombin deficiency, factor V Leiden - homozygous, prothrombin gene mutation 20120 - homozygous double heterozygous for factor V Leiden and prothrombin gene mutation 20120. Molecular testing is not recommended for other polymorphisms with thrombophilic risk. When we indicate the molecular testing for hereditary thrombophilia we must be sure that this result will influence the management of patients. Also, we need to ensure that these tests are performed in experienced laboratories accredited for these tests and the results are interpreted by experienced clinicians.

Each patient must be individually analyzed and therapeutic decision will be taken depending on the type of hereditary thrombophilia and the presence of additional risk factors, including family history of thrombosis installed before 50 years. Thrombophilia prophylaxis in Pregnancy Study (TIPPS) is the only randomized trial aimed to determine whether prophylactic administration of dalteparin reduces the risk of venous thromboembolism (VTE) and the risk of complications related to pregnancy in pregnant women at high risk of developing these complications. The results were recently published (Lacet, July 2014) and indicates the absence of a benefit to pregnant women receiving prophylactic anticoagulation. There are situations in which prophylaxis is mandatory - ie. women with previous VTE.

Administration of prophylactic antithrombotic therapy in pregnant women has to be a decision assumed by the obstetrician in collaboration with haematologist. This therapy should not be recommended solely on the basis of hereditary thrombophilia testing and, apart from the cost of treatment (about 8000 U.S. $ / pregnancy), we must consider the psychological impact of diagnosis, and management of anticoagulant (approx 400 shots / pregnancy).

This work was supported by the grant PN 42-099 from the Romanian Ministry of Research and Technology.

E8. DIFFUSE LARGE B-CELL LYMPHOMA – UNITY IN HETEROGENEITY: CLINICO-PATHOLOGIC ENTITIES, ETIOPATOGENETIC, DIAGNOSTIC AND THERAPEUTIC ASPECTS.

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Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL, reprezenting up to 40% of cases, with an increasing occurrence. The incidence increases with age. It has an aggressive natural history, and it may present in lymph nodes or in extranodal sites (including bone, skin, gastro-intestinal tract, thyroid, lung etc). The etiology of DLBCL is unknown. **Factors that confer increased risk** include immunosuppression (including AIDS, autoimmune diseases or iatrogenic etiologies in the setting of transplantation), radiation, diet, pesticides, hair dyes. A subset of DLBCL, including immunoblastic and primary CNS lymphoma, is highly associated with the EBV virus; the concept of antigen-driven lymphomagenesis is less developed in DLBCL. DLBCL usually develops de novo, but can also arise from transformation of an indolent lymphoma.

DLBCL represents one of the most **heterogeneous** categories in WHO classification. The disease, that is seen as a „unity" in the aggressive lymphoma group, is clearly heterogeneous at a clinical, pathological, cytogenetic and molecular level. Morphologically, DLBCL is composed of large B cells with a high proliferation index resembling germinal centroblasts. There are several **morphologic variants** that include centroblastic, immunoblastic, plasmablastic, T-cell/histiocyte-rich, and anaplastic (usually ALK+) subtypes. The neoplastic **cells of DLBCL express** pan B-cell markers, including CD19, CD20, CD79a, CD45RA, and the nuclear transcription factor PAX5. Germinal centre-associated markers CD10 and Bcl-6 are expressed in aproximately 30-40% and 60%, respectively. Translocation of BCL2 gene (a hallmark of follicular lymphoma) is present in 20-30% of cases; CD5 is expressed only in 10% of DLBCL. Aproximately 10% of DLBCL cases harbor a t(8;14) MYC translocation (that confers a worse prognosis).

On the basis of **gene expression profiling (GEP)** and genes signatures, DLBCL can be divided into at least three different subtypes: 1. germinal centre B-cell (GCB) – like; 2. activated B-cell (ABC)-like; 3. primary mediastinal B-cell lymphoma (PMBL), each with significant differences in terms of prognosis, PFS, and OS following immunochemotherapy. Distinct **cytogenetic abnormalities** have been described in DLBCL subtypes. In GCB-DLBCL the most common

are t(14;18) with rearrangements of BCL2 and IGH chain genes, and translocation leading to rearrangement of MYC gene. In ABC-DLBCL the most common are translocation involving BCL6 gene, and trisomy 3; deletion of tumor suppressor gene P53 is observed in 15-20% of cases. In PMBL a gain of long arm of chromosome 9 is reported (50%), with up-regulation of the JAK2 gene.

Subtypes of DLBCL arise from **genetic alterations occurring during the proces of B-cell differentiation /maturation** and, in general, are characterized by a blockage of the programmed cell death process, an increase in cell profiferation, or impaired terminal differentiation. Several oncogenic pathways have been identified in DLBCL: B-cell receptor signaling pathway, constitutive activation of NFkB activity pathways, and deregulation of the Bcl-6/apoptosis pathway.

Diagnosis of DLBCL should be made on the basis of a surgical specimen/excisional lymph node or extranodale tissue biopsy. Minimal immuno-histochemistry (CD45, CD20, and CD3) is mandatory. Molecular characterization is recommended although GEP remains investigational. The staging is established according to the Ann Arbor system. For prognostic purposes, IPI and age-adjusted IPI (aa-IPI) should be calculated. For staging are required: a complete blood count, routine blood chemistry including LDH and uric acid as well as a screening for HIV and hepatitis B and C; CT scan of the chest and abdomen; a bone marrow aspirate and biopsy; performance status and cardiac function (ejection fraction). A diagnostic spinal tap should be considered in high-risk patients.

The **clinical presentation** of DLBCL is variable and depends on histology, age, and immune status.Tipically presents with lymphadenopaty, from asymptomatic to causing pain or organ compression (ureteral, spinal cord). The involvement of bone marrow is present in approximately 20% of cases. Constitutional manifestations or „B" symptoms (weight loss, malaise, fevers, night sweats, loss of appetite) may be present as a consequance of production, by the lymphoma cells or host tissue, of inflammatory molecules and of other cytokines and chemokines.

DLBCL incorporates clinical and/or pathological distinct **subtypes and variants**, and also new entities based on unique clinical features, age or anatomic site, viral pathogenesis, or distinctive pathological features:

1. **DLBCL, not otherwise specified**; morphologic: centroblastic, immunoblastic, anaplastic; molecular subgroups: GCB, ABC; immunohistochemical subgroups: CD5+, GCB, non-GCB.
2. **T-cell or histiocyte-rich LBCL**; often presents in younger patients, with advanced stage, BM, liver and spleen involvement, and aggressive clinical behavior.
3. **Primary mediastinal B-cell Lymphoma (PMBCL)**. Commonly presents in young women and usually remains localized to the mediastinum with frequent superior vena caval syndrome. Regional lymph nodes may be involved, but spread to distant nodal sites is uncommon. Frequent extranodal sites, particularly at relapse, include the liver, kidneys, adrenal glands, ovaries, gastrointestinal tract, and CNS. An origin from medullary thymic B-cells has been proposed. GEP studies have found that PMBL shares features of classical Hodgkin lymphoma.
4. **Primary DLBCL of the CNS**. Less than1% of NHL and 2-3% of brain tumours; may be present in immunocompetent or immunodeficient (HIV) patients. Clinical features: neurological deficits (50-80%), neuropschiatric symptoms, headache, asymmetric cranial neuropathies, blurred vision and floaters. It has some distinctive features on GEP and shares some similarities with DLBCL arising in other immune privileged sites such as the testis. Poor prognosis ameliorated by novel chemotherapeutic protocols.
5. **Primary cutaneous DLBCL, leg type (PCLBCL)**. Tipically occurs in elderly patiens, in particular in women, preferentially affects the lower legs, with red or bluish-red tumours; frequently disseminates to extracutaneous sites. PCLBCL has a GEP resembling the ABC type of DLBCL, and it has an unfavourable prognosis.
6. **EBV positive DLBCL of the elderly**. Occurs in patients >50 years and without any known immunodeficiency or prior lymphoma, as a consequence of decreased immune surveillance as a part of the aging process. 70% of patients present with extranodal disease (skin, lung, tonsil, stomach), with or without lymph node involvement. The clinical course is aggressive, with a median survival of about two years. Lymphomatoid granulomatosis may progress to an EBV+ DLBCL.
7. **DLBCL associated with chronic inflammation**. It occurs in the context of long-standing chronic inflammation (over 10 years), it is associated with EBV-driven large B-cell proliferation. Most cases involve body cavities or narrow spaces (most often pleural cavity, but also bone, joint and periarticular soft tissue). Pyothorax-associated lymphoma (PAL) develops in the pleural cavity of patients with long-standing phyotorax, predominantly in men, with a median age around 65-70 years. The tumour mass (in the pleura and/or lung) is larger than 10 cm; patients present with chest pain, back pain, fever, cough, hemopthysis or dyspnea. Good prognosis if successfully resected.
8. **Intravascular large B-cell lymphoma (IVLBCL)**. It is a rare type of extranodal DLBCL, with selective growth of lymphoma cells within the lumina of vessels, particularly capillaries. IVLBCL occurs in

adults, it is usually widely disseminated in extranodal sites. The symptoms are predominantly neurological or cutaneous (Western form), or patients present with multiorgan failure, hepatosplenomegaly, pancytopenia and hemophagocytic syndrome (Asian form). This is an aggressive lymphoma which responds poorly to chemotherapy.

9. **ALK-positive large B-cell lymphoma.** It is a neoplasm of ALK+ monomorphic large immunoblast-like B cells, sometimes with plasmablastic differentiation. It involves lymph nodes or presents as a mediastinal mass, affect older individuals, most patients present with advanced stages; the prognosis is very poor.

10. **Plasmablastic lymphoma (PBL)**. PBL is a diffuse proliferation of cells with immunophenotype of plasma cells (CD138, CD38), and high incidence of MYC translocations. It has higher incidence in HIV-positive patients, is usually positive for EBV; most often presents extranodal (especially oral cavity). Clinical course is very aggressive.

11. **Primary effusion lymphoma (PEL)**. PEL arises in HIV-infected patients, it is universally associated with HHV8, the cells are coinfected with EBV, with immunophenotype resembling plasmablastic cells. Usually presents with serous effusions (pleural, pericardial, peritoneal), in the absence of lymphadenopathy or organomegaly. Clinical course is extremely unfavourable (survival < 6 months).

12. **Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease (MCD)**. Profiferation of HHV8-infected lymphoid cells resembling plasmablasts expressing IgM, and arising in HIV-infected patients who have developed HHV8 MCD. Characteristically involved lymph nodes and spleen, but can disseminate (including leukemic aspect). Very aggressive entity, survival a few months.

13. **Borderline lymphomas**: **a**. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; **b**. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Hodgkin lymphoma.

Treatment of DLBCL. The mainstay of treatment is systemic chemotherapy (current standard is R-CHOP); other therapies include radiation (with no proven clear benefit), stem cell transplantants (ASCT), and other chemotherapy. Treatment strategies should be stratified according to age, aa-IPI and feasibility of dose-intensified approaches. In cases with high tumor load, precautions (prednison administering) are required to avoid tumor lysis syndrome. Prophylactic use of hematopoietic growth factors to prevent febrile neutropenia is justified in patients treated with curative intent and in all elderly patients.

1. In young patients (<60 years), ***IPI low-risk without bulky disease***, R-CHOP 21 x 6 is the current standard; consolidation by radiotherapy to initial sites has proven no clear benefit. In cases ***IPI low-risk with bulk or IPI low-intermediate-risk*** the treatment is R-CHOP 21 x 6 with radiotherapy to the site of previous bulky disease, or the intensified regimen R-ACVBP. In ***IPI intermediate-high risk or IPI high-risk*** patients, 6-8 cycles R-CHOP are most frequent applied; intensified treatment with R-ACVBP or R-CHOEP; high-dose chemotherapy (HDC) with ASCT as consolidation treatment after immunochemotherapy has shown promising results; HDC with ASCT may be suggested for selective high-risk patients.

2. In elderly patients (>60 years), 8 cycles of R-CHOP 21 is the current standard. In patients with localized disease, consolidation by radiotherapy has proven no benefit. In patients aged >80 years without cardiac dysfunction, R-miniCHOP combination could be used; in patients with cardiac dysfunction can be considered doxorubicin substitution with etoposide, mitoxantrone or liposomal doxorubicin – R-C(X)OP 21 x 6, or palliative care.

3. CNS profilaxis should be recommended for patients with high-intermediate and high-risk IPI, especially those with more than one extranodal site or elevated LDH, who are at higher risk of CNS relapse. Can be used intrathecal methrotexate or intravenous high-dose methrotexate associate with efficient disease control. Testicular lymphoma and other specific involvement sites (paranasal sinus, upper neck or bone marrow) must receive CNS prophylaxis.

4. Some extranodal DLBCL require special consideration. Treatment of **primary DLBCL of the CNS** must contain high-dose of methotrexate and of cytarabine; CNS irradiation is usually administered as consolidation. **Primary DLBCL of the testis (PTL)** is characterized by an increased risk of extranodal, CNS, and contralateral testis recurrence with poor outcome. The standard treatment of PTL is R-CHOP21 with CNS prophylaxis (intrathecal and intravenous) and contralateral testis irradiation. In **PMBCL** the R-CHOP 21 is not established as the definitive treatment option and radiotherapy remains controversial.

5. **Response evaluation and follow-up**. Abnormal radiological tests at baseline should be repeted after 3-4 cycles and after the last cycle of treatment. Bone marrow aspirate and biopsy should be only repeted at the end of treatment if initially involved. PET is highly recommended for the post-treatment assessment. A CT scan is recommended at 6, 12, and 24 months after the end of treatment; a blood count and LDH at 3, 6, 12, and 24 months need to be evaluated.

6. **Relapsed and refractory DLBCL**. Histological verification should be obtained whenever possible, and is mandatory in relapse >12 months after the initial

diagnosis, especially in order to ensure CD20 positivity. ***In suitable patients*** with adequate performance status, salvage regimen (R-DHAP, R-ICE) followed in responsive patients by high-dose regimen (BEAM) with stem-cell support is recommended. ASCT following chemotherapy should be considered in patients with refractory disease, early relapse or relapse after ASCT. **Patients not suitable** for HDC may be treated with the same or other salvage regimens (R-GEMOX), which may be combined with involved-field radiotherapy or preferentially be enrolled in clinical trials.

Conclusions. Despite its initial appreciation as a single entity, DLBCL is in reality very heterogeneous at a clinical, pathological, cytogenetic and molecular level. DLBCL incorporates distinct subtypes and variants, based on unique clinical feature, age or anatomic site, viral pathogenesis, or distinctive pathological features. DLBCL are aggressive but potentialy curable with multi-agent chemotherapy. R-CHOP regimen remains a standard therapeutic approach for most patients with DLBCL. The future optimal therapy will incorpotare molecular information (particularly from gene espression analyses) for appropriate risk-adapted therapy, and novel agents (including epigenetic targets) will be included in treatment regimens.

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E9. FANCONI ANEMIA. DIAGNOSIS AND MANAGEMENT GUIDELINES.

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Until now, at least 13 types of Fanconi anemia (FA) have been described, each of them characterised by a complex molecular pathology. Determining the genotypes of the patients and mutational analysis are advisable in every case as they play an important role in choosing therapy. FA can be described as a multiple stage affliction. The hematological manifestations do not represent the main symptoms of a patient with FA and are not the only clinical manifestations either. The genetic instability of the patient with FA is caused by the exposure to ionising radiation, carcinogenic agents of environmental nature and chemotherapy. Thus, the exposure to X-rays is an undesirable risk.

Cerebral tumours (before the age of five), Wilms tumour (highest incidence at the age of four) and other renal cancers (at patients with BRCA2 idiotype multiple studies are in progress) can develop.

As stem cell transplant from related donors or skin grafts "in mismatch" are nowadays a challenge, this option becomes valid for an increasing number of patients with FA. Because the prevalence of squamous cancers especially of the integument, head, neck and also genitals is high, the evaluation of adult patients before the selection of therapy is essential. After the primary exam, an endocrinologic and gastrointestinal evaluation is required. The patient and his family must be psychologically observed due to the possible traumata.

Tests for diagnosis

The suspicion of FA requires the hospitalisation and exploration of the patient in specialised clinics with experience in the diagnosis and treatment of this illness.

Classification of clinical manifestations of FA	
Hematological	cytopenia (can precede the installment of bone marrow failure), increased risk of developing myelodysplastic syndromes and AML (beginning from the age of 13, the risk of AML increases 800 times in patients with FA)
Dermatological	hyperpigmentation, ,,café au lait" skin, hypopig mented areas, squamous cancers with different sites of localisation (skin, cervix, vagina, vulva, anal canal)
Gastrointestinal and hepatic	about 7% of the patients with FA have anomalies of the gastrointestinal tract (ozena, nausea, vomitus, abdominal pain). Esophageal, duodenal or jejunal atresias, eso -tracheal fistulas, anus imperforatum can exist. Hepatic tumours may appear in the absence of alcohol consumption or of an acute viral hepatitis in the past
Cardio-pulmonary	closely associated with cardio-pulmonary malformations
Locomotory	absent/bifid/vestigial/triphalangeal fingers, syndactyly, digital hypoplasia; absent or hypoplastic radius, dysplastic ulna, hypoplasia of the hypothenar eminence or the absence of the 1 st metacarpal; micrognathia, microcephaly, triangular facies. The patient may present spina bifida, scoliosis. Sprengel or Klippel -Feil syndromes may be associated with the locomotory manifestations
Ocular	microphtalmia, strabismus, epicanthus, cataract, astigmatism
Renal	Ectopic kidneys, renal hypoplasia/dysplasia/atresia, hydronephrosis, hydroureter
Endocrinologic	hypothyroidism, late puberty, diabetes mellitus, osteopenia/osteoporosis
ORL	atresia of the auricle of the ear, deafness or hypoacusia
Gynecologic	hypogenitalism, ectopic testes, hypospadias, micropenis in men; hypogenitalism, bicornuate uterus, infertility, abnormal menstruation in women

The exploration begins with cytogenetic tests which reflect the fragility of chromosomes: DEB (Diepoxybutane) and MMC (Mitomycin) tests conducted on lymphocytes from peripheral blood. All children suspected of FA, once hospitalised, will be evaluated: familial history, consanguinity, FA AHC, physical abnormalities, neoplasms, medullary aspiration and biopsy, imaging techniques (ultrasonography) for patients with renal dysplasias and hydronephrosis. After the peripheral blood exam has been conducted, medication should be administered. The hepatic (bilirubin, ASAT, ALAT) and renal (serum electrolytes, creatinine) functions will be evaluated. The urologic exam is useful in the diagnosis of genitourinary reflux, urinary infection and genitourinary malformations. Then, endocrinologic evaluation should target the: thyroid function, glycemia, OGTT, lipid profile, osteo-medullary density, discovery of osseous abnormalities. The ophthalmologic exam is mandatory.

The risk of cancer will be identified to both pacient and relatives. Through genomic analysis, FANCD1/BRCA2, D2, I, M, N will be identified. Mutational analysis is required to classify the patient into one of the five genomic groups. The most advanced genetic and prenatal tests will be conducted, but only in specialised centres. The severity of FA is determined by the specific gene FANC. The patients with FANCA do not present homozygotic mutations which account for the sinthesis of proteins able of inducing anemia or a high susceptibility for the development of AML. The type of homomorphic mutation FANCA produces proteins with an abnormal structure. FANCC IV S4+4 A>T, common in Ashkenazi Jews, is associated with severe and aplastic anemia. VACTERL-H is frequent and is associated with vertebral/anal malformations, eso-tracheal fistulas and renal abnormalities. At Japanese patients with FA, FANCC IV S4+ A>T is prevalent, but is linked with a better phenotype than the one of Ashkenazi Jews. The FANCD1/BRCA2 and FANCN/PALB2 mutations are associated with an increased predisposition of AML and an abnormal physical phenotype. The abnormalities of the VACTERL-H cluster are correlated with the biallelic mutations FANCD1/BRCA2 type. The FANCD 2 and FANCI mutations are associated with severe

abnormalities and FANCI1 with the development of an early anemic syndrome.

The majority of patients with FA develop aplastic anemia, but the age of development is variable. In case of AML or SMD development, cytogenetic abnormalities should be detected. The HLA typing will be conducted to both patient and family to discover possible donors. The transfusional treatment will be cautiously administered. The patient who has undergone multiple transfusions is at high risk of secondary hemochromatosis. Iron can accumulate in the liver, heart or endocrine organs. In time, hemochromatotic cirrhosis, cardiomyopathy, endocrine disorders can evolve. After the transplant, iron metabolism will be supervised and iron chelators will be used only after one year after the transplant. Sometimes, phlebotomies will be necessary. No drug will be given to the patient without notifying the therapist. Radiological exams will be avoided or cautiously performed. All abnormalities of the skeleto-muscular system will be detected and an orthopedic exam will be methodically performed. All finger abnormalities impose orthopedic interventions. The psychologic support of the patient will not be neglected.

Ocular and ORL malformations will be registered and ophthalmologic and ORL exams will be conducted starting with the age of four months as surgical procedures meant to correct defects are recommended. The patient will be monitored by a handicap commission, verbal therapy being required. If the patient has received ototoxic medication, antibiotics IV, iron chelators, CT, after the transplant audiograms are necessary. Gastroenterologic control and treatment are also mandatory. Hepatic complicantions are sometimes caused by the treatment with androgens. Every six months, ASAT, ALAT and ultrasonographic exams should be repeated. Due to the risk of developing squamous cancers of the genital tract (cervix, vagina, vulva, anal canal), gynecologic exams in patients over 13 are advisable. External genital organs will be inspected. After 18, cervical cytologic exams (Papanicolau test) will be conducted. Oral contraceptives will be administered. Colposcopy is required if lesions of the cervix or cytologic abnormalities are present. Since the age of nine, the HPV test is obligatory to prevent neoplasms. Abnormalities of the genital tract are more frequent in patients with renal malformations. Breast cancer is also frequent in women with FA who should be monitored since the age of 20 and in women over 25 “screening” mamographies are to be made. Each woman who has undergone a transplant will be monitored in case of pregnancy. Administration of androgens is forbidden in this case. Women with FA can have risky pregnancies, premature menopause, major cardiovascular risk, squamous cancer risk of the head and neck (HNSCC). Each patient with HNSCC will be investigated: hematologic abnormalities (anemia, monocitosis, and thrombocitopenia), finger abnormalities; in case of cancer, an unusual response to CT will be investigated. ORL exam will be conducted since the first year of life (nasopharynx, oropharyns, and larynx). Oral hygiene will be cautiously supervised and alcohol consumption and smoking will be interdicted. After TMO, a major risk of infections and bacteremias exists.

Each cutaneous lesion will be supervised (pigmented warts especially, those with with mutations of the FANCD1/BRCA2 having the highest risk of developing a cutaneous squamous cancer). The risk of developing medulloblastoma is higher in these patients and annual cerebral MRI ought to be performed. Patients with FA may develop esophageal and genital neoplasms at about 20 years.

In conclusion, the presence of FA should be suspected in the following cases: the existence of one or more cases of FA in the patient's family, abnormalities of the skeleto-muscular system (abnormal/dysplastic radius or ulna, abnormal fingers, renal abnormalities, microphtalmia, microcephalia, microcephaly), development of aplastic anemia, ,,café au lait” skin, eso-tracheal fistulas, anus imperforatum, primary AML or myelodysplastic syndrome diagnosis, abnormal sensitivity to CT. FA can be suspected if anal/vulvar neoplasms develop under 40 years. The physician can think of this diagnosis in case of cytopenia, unexplainable macrocytosis (serum B12 vitamin, folates – normal levels), hepatic tumours, ovarian insufficiency before the age of 30, infertility, cerebral tumours before the age of five and Wilms tumours in children under four years.

Cytogenetic exam (monosomy 7 syndrome & 1,3,4,7 pairs of chromosomes are observed) will be conducted. Frequently, the 3q26q29 endoreduplication (trisomy or partial tetrasomy) appears. Monosomy 7 syndrome is also prevalent (the 3q clone) and one third of the affected patients develop myelodysplastic or primary AML. All cases in which chromosome 3 is amplified will be investigated (the chromosomal material of the G band will be used for genomic exams). Amplifications of the 3q chromosome will be confirmed by FISH or SKY technique. Comparative genomic hybridisation will also be performed. As FA patients with abnormalities of the 3q clone present a higher risk of developing AML, they will be cautiously evaluated as they can undergo marrow transplants.

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E10. **MANTLE CELL LYMPHOMA- DIAGNOSIS AND TREATMENT ACTUALITIES**

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Mantle cell lymphoma (MCL) is a rare type of non-Hodgkin Lymphoma (NHL), representing about 7% of adult NHL. The median age of patients at presentation is around 65 years, with a male: female ratio of 3:1. The majority of MCL patients are diagnosed on advanced stages and about 1/3 have malignant lymphocytes in peripheral blood. Central nervous system involvement is rare and usually associates leukemic phase of disease. More than 1/3 of the patients have B symptoms at diagnosis and gastrointestinal tract involvement. From a histological point of view, malignant cells are small or medium monomorph B lymphocytes with irregular nuclei. Cells morphology could vary from small lymphocytes (centrocitic-like) to lymphoblast-like cells (blastoid variant). Mitotic index is higher than indolent lymphomas. The cells presents B cell immuno-phenotype: CD 19+, CD 20+, CD 5+, FMC 7+. Rarely, the cells culd be CD 5 – and CD 23+, associating a high surface IgM and IgD level. In 95% of the cases, D1 Cycline is positive, even in CD5 – cells. D1 Cycline could be used in differential diagnosis between MCL and indolent lymphomas like lymphocytic lymphoma, splenic marginal cell lymphoma, etc. D1 Cycline is negative in 5% of cases. 50-60% of cases are positive for t(11;14). Other chromosomal alterations (that associates c-myc mutation) are present in blastoid variant, frequently associated with poor prognosis.

Currently, there is no general consensus on the MCL treatment. Considering that some MCL patients, especially those with low International Prognostic Index (MIPI) score have an indolent clinical course for months to years, a watch-and-wait approach has been advocated for selected patients. In general, fit younger than 65 years patiernts without comorbidities are candidates for intensive chemotherapy (regimens based on high dose ARA-C) followed or not by stem cell transplant as consolidation. This strategy evolved from the fact that less intensive regimens (as CHOP or R-CHOP) produced only a modest benefit with median progression free survival of 16 months. All the studies proved a better outcome in patients treated with high doses ARA-C. As expected, intensive regimens were associated with significant toxicities, especially in older patients. The studies reported a complete remission (CR) rate of 87% using R-HyperCVAD/R-MTX-ARA-C regimen and of 61% using R-CHOP+ 3R-DHAP followed by autologus stem cell transplantation (ASCT). It is still not clear weather all patients should be offered ASCT because CR rates (after R-HyperCVAD and no ASCT) are similar with those who were transplanted.

In elderly patients who are not candidates for intensive therapy, the choice of therapy should take into account balancing clinical benefit with treatment related toxicities. R-HyperCVAD/R-MTX-ARA-C regimen is less beneficial in patients older than 65 years and also more toxic. Otherwise, R-CHOP regimen is relatively safe, but ineffective. Therefore, these patients are candidates for testing new regimens more efficient than CHOP. To improve progression free survival, maintenance strategy was explored, using Rituximab or α-Interferon. Rituximab is less toxic and more efficient than Interferon. Other treatment option was (1) R-HyperCVAD/R-MTX-ARA-C regimen without R-MTX/ARA-C alternance, followed by Rituximab maintenance. (2) R-Bendamustine associating Lenalidomida and Ofatumumab. Regimens containing Fludarabine were not efficient and hematologic toxicities were more frequent. Elderly unfit patients with comorbidities are candidates for palliative treatment such as R-Chlorambucil.

Two new agents were approved for the treatment of relapsed MCL, Bortezomib and Temsirolismus. Furthermore, Bendamustine and Lenalidomide demonstrated promising clinical activity. Allogenic stem cell transplantation was performed in selected

cases of relapsed lymphomas, but large studies should be performed to define the role of stem cell transplantation in MCL management.

Because failure and relapses are inevitable, novel agents targeting pathways implicated in cell growth and survival regulation have been developed. One of those is the phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway implicated in cell proliferation, grow and survival. Other research centered on targeting B-cell antigen receptor (BCR) signaling pathway. Bruton tyrosine-kinase (Btk) is required for BCR signaling and place an important role in B cell maturation and it is overexpressed in B-cell malignancies. Ibrutinib is a potent selected and oral inhibitor of Btk. An international, multicentric, phase 2 study of Btk inhibitor, Ibrutinib in relapsed or refractory MCL showed a 68% overall response rate.

Future clinical trials are needed for creating specific therapy targeting tumor biology markers and genetic abnormalities.

E11. **LANGERHANS CELL HISTIOCYTOSIS: DIAGNOSIS AND CLINICAL APPROACH.**

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Langerhans cell histiocytosis (HCL) is a rare, polymorphic disorder, which can affect any organ or system. Formerly known as "Histiocytosis X" , the disease is due to clonal proliferation of CD1 + histiocytes.

Malignant clone expresses the morphology and phenotype of Langerhans cells, which are specialized dendritic cells found in the skin and mucosa (CD1a, MHC II, Birbeck granules) and also presents markers of activated Langerhans cell (CD54, CD58).

The Working Group of the Histiocyte Society has divided histiocytic disorders in three groups: (1) dendritic cell histiocytosis (2) macrophage-related disorders and (3) malignant histiocytosis. LCH belongs to the first group and includes several forms of the disease (Letterer-Siwe disease - acute disseminated form, Hand-Schüller-Christian disease - intermediate clinical form, eosinophilic granuloma - chronic, indolent disease, Hashimoto-Pritzker disease - congenital self-limiting form).

The pathogenesis of the disease remains unknown - neoplastic process versus reactive process.

In some cases, the diagnosis is purely random, but sometimes the disease begins dramatically , in the early months of life, with leukemia-like image (fever, hepatosplenomegaly, lymphadenopathies, cytopenia) and high mortality.

Clinical presentation of LCH is essentially influenced by the interested organ, the degree of functional involvement and the number of involved sites (unifocal or multisystemic disease). Favorite headquarters are bone, skin and pituitary gland. Lymph nodes, liver, spleen, hematopoietic bone marrow and central nervous system are rarely affected. Pulmonary LCH is common in adults and may announce multisystemic damage.

The diagnosis is based on histologic and immunopnenotypic examination of a lesional biopsy. Accepted criteria for diagnosis include CD1a positivity and / or CD 207 (Langerin) and the presence of Birbeck granules on electronic microscopy.

So far, there is no standard therapeutic protocol accepted. Treatment options depend mainly on the extent and severity of the disease at diagnosis. The Working Group of the Histiocyte Society proposed a risk stratification according to the number of affected systems. Unifocal involvement may benefit from surgical treatment. First-line systemic therapy is indicated in forms with multisystemic involvement, and in cases of unifocal disease unresponsive to treatment.

Current recommendations for systemic therapy (Histiocyte Society / 2008) propose the combination of cytotoxic medication (vinblastine, methotrexate) and corticosteroids. The role of allogeneic stem cell transplantation in LCH has not yet been elucidated; further trials are needed.

Evolution of single system LCH (unifocal) is generally favorable, but if there is multisystemic involvement, the prognosis is variable (60% chronic evolution, 30% complete remission, 10% deaths). Response to chemotherapy in the first 6 weeks of treatment (induction therapy) is the most important prognostic marker for multifocal LCH.

E12. **PAROXYSMAL NOCTURNAL HEMOGLOBINURIA.**

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Paroxysmal nocturnal hemoglobinuria (PNH) is a condition characterized by a defect in the receptor glycosylphosphatidyl-inositol (GPI), due to a defect in the PIG-A gene, causing a partial or complete absence of GPI-linked proteins, particularly CD59 (membrane inhibitor of reactive lysis, or protectin) and CD55 (decay accelerating factor), resulting in increased

sensitivity of erythrocytes to the hemolytic action of complement.
The disease has a mean age of onset at 32 years, and a slight predominance of females (53.2%), with the predominant clinical manifestations of damage hematopoietic function: aplastic or hypoplastic anemia (44%), myelodysplastic syndrome (5.8%), myelofibrosis (0.4%), and acute myeloid leukemia (0.4%).
Common symptoms are: fatigue (80%), dyspnea (64%), headache (63%), and hemoglobinuria (62%). In men, erectile dysfunction is described in 38% of men with this condition.

Other common complications are: history of thrombotic events in 15.5%, impaired renal function in 13.9%, 31.1% in anticoagulant therapy.

Survival is closely related to the severity of complications, of which the most common are: pancytopenia (15%), thrombosis (28%) and myelodysplastic syndrome (5%). It described a considerable mortality in PNH, with a median survival of 14.6 years and a risk of death by 35% in five years.
Diagnosis is difficult because the polymorphic clinical picture and should be suspected in the following situations: evidence of non-immune acquired hemolytic anemia with evidence of intravascular hemolysis, cytopenias, venous thrombosis, aplastic anemia, myelodysplasia and episodic dysphagia or abdominal pain.

Confirmation of diagnosis is based on identification of PNH clone. In the past, was indirectly diagnosed by showing sensitivity of erythrocytes to lysis by complement or by the sucrose lysis test and the Ham acid hemolysis test.

Currently, the identification of PNH clone by flowcytometry is a direct method for identifying defects in the granulocyte membrane receptors (CD24, CD16, flyers, CD157), monocytes (CD14, flyers) and erythrocytes (CD59, CD55) and quantification clone sensitivity of 0.01%.
Identification of PNH clone in other hematopoietic failure disease has important prognostic and response to treatment in aplastic anemia indicating a good prognosis immunosuppressive therapy. In patients with PNH clone periodic monitoring PNH every 6-12 months is indicated.

PNH treatment was based (in the absence of a specific treatment) on supportive therapy: recovery of iron and folate, red blood cell transfusions, immunosuppression and stimulation of hematopoiesis.
Treatment with monoclonal antibodies (eculizumab) brings a dramatic improvement in response to treatment, and significantly reduces the stem-cell transplant indication.

Allogeneic stem cell transplantation is the only curative treatment option, but there was described a survival advantage of PNH clone in transplanted patients.
In future, it is expected the possibility of gene therapy.

E13. **TREATMENT OPTIONS IN PRIMARY MYELOFIBROSIS.**

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Myeloproliferative neoplasms (MPN) are clonal diseases originating in pluripotent hematopoietic stem cells. Clonal expansion leads to increased and abnormal hematopoiesis that produces a group of interrelated syndromes classified according to the predominant phenotypic expression of myeloproliferative clone.

The main entities that are part of NPM are:
- Chronic Myeloid Leukemia
- Polycythemia vera (PV)
- Essential thrombocythemia (ET)
- Myeloid metaplasia with myelofibrosis / Idiopathic; Post PV; post TE

Myeloid metaplasia with myelofibrosis or primary myelofibrosis (MF) is characterized by the association of nonclonale bone marrow stromal reaction (proliferation and fibroblast activation) that causes collagen fibrosis, osteosclerosis, and angiogenesis (processes that are targets of modern therapy) and age of occurrence - in most cases> 60 years. Incidence is 0,5 /100.000/year.

Characteristics of MF:
- Clinical: progressive anemia, splenomegaly, cachexia, extramedullary hematopoiesis (HEM)
- Hematological: blood leucoeritroblastic picture, dacriocytes
- Progressive alteration of the quality of life (need for transfusions, compressive signs related splenomegaly and signs of severe hipercatabolism)
- Life Expectancy: 3,5 5 years (> 10 – young patients with good prognosis)
- Causes of death: infection, bleeding, portal hypertension, organ failure, acute leukemia transformation (10-20%)

WHO diagnostic criteria (the 3 major and 2 minor criteria must be met)
Major criteria:

1.Presence of megakaryocyte proliferation and atypia, usually accompanied by reticulin fibrosis and / or collagen, in the absence of significant reticulin fibrosis, megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often reduced erythropoiesis (diseasein prefibrotic stage)

2.WHO criteria for PV, CML, MDS or other

myeloid neoplasm are not met

3.Demonstrating the existence JAK2617VF or other clonal marker (MPL515WL / K), or in the absence of a clonal marker, excluding secondary myelofibrosis (inflammatory or neoplastic)

Minor criteria:

1. Leucoeritroblastic picture

2.High LDH levels

3. Anemia

4. Palpable splenomegalia

A number of clinical and laboratory parameters were found to be independent prognostic factors that allowed risk stratification of patients with MF: Age> 65 years; Hb <10 g / dl; WBC> 30,000 / l; WBC <4,000 / l; Circulating blasts> 1%; Presence of constitutional signs; Cytogenetic abnormalities; Erythroblasts in peripheral blood > 2%; Platelets <300,000 / l; Splenomegaly ; JAK2 (V617F) / CALR; CD34 + circulating cells.

Based on these prognostic factors several prognostic scores were developed, the most used currently being DIPSS- plus taking into account age, the number of leukocytes and platelets, anemia, transfusion needs, simptimele constitutional karyotype and the percentage of circulating blasts. Based on this score patients are stratified into 4 risk categories (low, intermediate 1 SI2, high) with impact on survival and therapeutic strategy.

MF treatment possibilities are represented by:

-Conventional methods that primarily targets improvement of disease symptoms - Supportive and palliative in nature and have no impact on survival

-New methods represented by new agents that target the pathogenic mechanisms of disease and bone marrow transplantation - the only potentially curative method

Conventional Treatment has several components:

1.Substitution - blood products

2.Hipouricemiants + hidration + urine alcalinization

3.Antianemic treatment: Androgens, Corticosteroids, Erithropoietins ,

4.Antiproliferative treatment: Interpheron; citotoxic agentis (Hidroxiurea, Busulfan, 2-Clordezoxiadenosine, Melphalan, 6-Tioguanine, Citosine arabinozid), Anagrelide

5.Reduction of extramedullary hematopoiesis: cytotoxic agents, surgical ablation, radiotherapy

New Therapeutic Methods

1.IMIDs (antiangiogenic activity): Thalidomide; Lenalidomide (cases with 5q deletion)

2.Intracellular signal transduction inhibitors: Imatinib mesilate; JAK2 inhibitors – ruxolitinib, momelotinib, etc

3.Other molecules are under investigation:

- action on intracellular signaling pathways
- hipometilanting agents
- proteasome inhibitors
- new IMIDs
- Anti TNFα agents

4.Allogeneic stem cell transplantation (Allo SCT) - the only therapeutic method that can remove marrow fibrosis and has the potential to cure the disease.

Currently the management of patients with MF depends on prognostic stratification:

- Patients in the low risk category - if asymptomatic are kept under observation; if symptomatic receive conventional symptomatic treatment
- Intermediate-1 Risk - conventional symptomatic therapy or investigational therapy
- Intermediate-2 and high risk : <45 years - myeloablative Allo-SCT; 45-65 years - non-myeloablative Allo-SCT; :> 65 years - investigational therapy

Important notions:

- median survival is 5.5 years, but there are wide variations.
- Clinical and hematological presentation - varied according to disease stage
- The main prognostic factors: age> 65 years, presence of constitutional symptoms, Hb <10 g / dL, leukocytosis> 25 x109 / L, blasts in SP> 1%; Some cytogenetic abnormalities
- Based on prognostic factors patients can be stratified into four risk groups
- Risk group stratification is critical in adopting therapeutic strategy.

E14. **ACTUALITIES CONCERNING HODGKIN'S LYMPHOMA.**

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Introduction: Hodgkin's lymphoma is a malignant limfoproliferation in which Reed Sternberg cells are in a reactive inflammatory environment. The incidence of lymphoma is approximately 5/100,000 inhabitants/year (1) and represents about 1% of cancer cases that occur annually (2). Its incidence varies according to gender, age, geographic location, ethnic group and socioeconomic status (3). In developing countries the incidence depending of age is bimodal: prevail in the 3rd and 8th decade of life. The second peak may be due to the confusion with other clinical cases of lymphoproliferations which it resembles with (4). In young people, the incidence is equal between the genders (1). The disease occurs more often during the months of February and March. The highest rates of

mortality due to it are recorded in countries where the incidence of lymphoma is the smallest and vice versa (3). Classic Hodgkin's lymphoma has a particular histology, meaning that few lymphoma cells (less than 1% of the lymph node cell population) are surrounded by numerous inflammatory cells. It is considered that, in the affected lymph nodes, the interaction between stromal cells and reactive nonmalignant cells with Reed Sternberg cells plays an important role in the pathogenesis of the disease. The present inflammatory cells have an abnormal activity: there is an expansion of myeloid suppressor cells, a signaling dysregulation through regulatory T cells and HLA-G protein and a malfunction of the natural killer cells, which may have prognostic role (5). Hodgkin and Reed Sternberg cells would have originated in the germinal center cells (GC) centers or lymphocytes B post-GC, because they are carrying the mutated gene coding the variable region of immunglobulin (6).

Ethiopathogenesis: The possible role of infection with Epstein-Barr virus in the pathogenesis of this lymphoma is discussed, through long-lasting antigenic stimulation (at 40-60% of patients Sternberg-Reed cells are latent infected with Epstein Barr virus). It is believed that the virus may have a central pathogenic role, in an initial event, when B cells from GC affected by apoptosis would be saved (5). A genetic signature associated with Epstein-Barr virus status in the histopathological lymph node sections seems to be characteristic of TH1 antiviral immune response. Sustained activation of NF-kappaB supported is responsible for the proliferation of Hodgkin and Reed Sternberg cells and prevents their entrance into apoptosis. TNFAIP3 gene encodes a natural inhibitor of NF-kappaB (called A20). Cells bearing the TNFAIP3 mutation are negative for Epstein-Barr virus. So, the 2 transformer events, involved in the pathogenesis of classical Hodgkin's Lymphoma, are mutually exclusive (2). Infection with HIV virus favours the emergence of Hodgkin's lymphoma, especially with mixed celularity or lymphocyte-depleted forms (it is 15 times more common in people infected with HIV than in the general population). Comparative genomic hybridization studies have determined that 4 gene correlates with an increased risk of classical Hodgkin's lymphoma disease: COX2, IL10, ILR4, IL18, a fact which underlines the importance of pathological cytokine signalization in the pathogenesis of the disease. Genetic variants of the genes involved in DNA repair are also associated with an increased risk of the disease (7). Study of microarray profiles of Hodgkin's lymphoma cell lines showed FOXC1 and FOXD1 gene overexpression and the lowering transcription of FOXP1, FOXO1, and FOXN3 genes , involved in the differentiation of B lymphocytes (8). Additionally, there is a silencing of apoptosis inducing genes BIK and INPP5D, an inhibitor of PI3K-related oncogenic pathway. There were identified 2 molecular subgroups of classical Hodgkin's lymphoma, depending on the intensity of the activity of the transcription factors of proto-oncogenes IRF4, MYC, and NOTCH1 (6). Increased exposure to ultraviolet radiation may have a protective role against the occurrence of Hodgkin's lymphoma, especially of those Epstein Barr virus positive. Among the plausible mechanisms involved would be: induction of ultraviolet radiation on the T regulatory lymphocytes or cellular response to DNA damage (9).

Evolution and prognosis: The European Organisation for Research and Treatment of Cancer (EORTC) established that patients with early stages Hodgkin's lymphoma which have one of the following risk factors have poor prognosis: ≥ 50 years old, bulky mediastinal lymph nodes, ESR ≥ 50 mm/1 h (or ≥ 30 mm/1 h if they have B symptoms), ≥ 4 regions involved. According to the German Hodgkin Study Group, the risk factors for patients with early stages disease are: bulky mediastinal lymph nodes, ESR ≥ 50 mm/1 h (or ≥ 30 mm/1 h if they have B symptoms), ≥ 3 regions involved and the presence of extranodal disease. If there are no risk factors, disease is considered in limited stage, and if there are 1 or more risk factors – in intermediate stage (10). EORTC also established and the risk factors for the disease in its advanced stages: age over 45 years, stage IV, male, albuminemia less than 40 g/l, hemoglobinemia under 10,05 g/dl, over 15,000 leukocytes/mm3 and under 600 lymphocytes /mm3 or below 8% (International Prognostic Score 0-7) (11). The study of gene expression profile, based on ARN signature from frozen lymph node sections of the patients that have not responded to the first applied treatment, found an increase in gene expression signature of macrophages, adipocytes, angiogenic cells, Reed Sternberg cell, with an overexpression of matrix metalopetidases and an underexpression of lymphocytes B genes of germinal centers. Immunohistochemistry confirmed that the presence of less than 5% macrophages (CD68+ cells) was correlated with a longer progression-free survival; in patients in early stages disease (IA and IIA) the absence of macrophages in lymph node sections was correlated with a disease-specific survival rate of 100%. CD68 is a superior prognostic marker comparing to IPS score on the prediction of disease free survival, according to several studies, including multivariate analysis. CD163 is another marker specific for monocytes/macrophages and it has similar meaning to CD68, but is more specific than this and allows identification of macrophages with more certainty (7). A large proportion of CD20+ cells

dispersed on the background sections of classical Hodgkin's lymphoma lymph node seems to have a favourable prognosis regarding the progression-free survival and overall survival in this type of lymphoma, unlike the depletion of CD20+ cells on the background of histological preparations which coexist with an increased number of CD68+ tumor associated macrophages – a negative prognostic factor (12). The predictive value of PET-CT in examination after first course of polychemotherapy was studied in a group of 126 patients. It proved to have prognostic significance for progression-free survival and overall survival. Progression-free survival at 2 years for PET1-negative patients (made after the first chemotherapy cycle) was 98.3%, while for those PET1-positive – 40,8%. No other prognostic marker does identify a group of patients with a more favourable prognosis that the examination carried out after the first course of polychemotherapy (10).

Treatment

HL ESMO guide for 2014 recommandes for limited stage disease to make 2 ABVD cycles, followed by irradiation type ISRT with 20 Gy (10, 13). For the intermediate stages it recommandes 4 ABVD cycles, followed by radiotherapy type ISRT with 30 Gy; the young patients with good performance status can receive 2 escalated-dose BEACOPP cycles + 2 ABVD cycles, followed by irradiation type ISRT with 30 Gy. For advanced stages it is recommanded to make 6-8 ABVD cycles or 6 escalated BEACOPP cycles, followed by irradiation type ISRT with 30 Gy on PET-CT positives residual masses (and with dimensions greater than 2.5 cm after BEACOPP and greater than 2 cm after ABVD). The recommendations for patients with refractory or relapsed disease are: life-saving therapy (DHAP, ICE, IGEV) followed by high-dose chemotherapy and peripheral stem cell transplantation; brentuximab vedotin (BV) can be used in patients who releapse after high-dose chemotherapy and peripheral stem cell transplantation and in those noneligible for aggressive chemotherapy; some combinations which include gemcitabine can be used to increase the quality of life and to prolong the survival; allogeneic peripheral blood stem cells transplantation after reduced intensity conditioning can be taken into consideration at the young subjects, chemosensitive, to which the disease has relapsed after high-dose chemotherapy and peripheral stem cell transplantation (10, 13, 14). PET-CT prior chemotherapy allowed to increase the estimation of prechemotherapy tumor mass volume on average 8.8% and the postchemotherapy clinical target volume with 7.1% and contributed to a better delineation of radiotherapy on the affected lymph nodes, without a necessary increase of the dose of irradiation (INRT) (15). A tiral of phase 2 made on 22 patients with supradiafragmatic Hodgkin's lymphoma established that radiation therapy with breathing locked in deep inspiration was able to reduce the lung dose irradiation in average with 2Gy, and those of the heart with 1,4 Gy, without lowering the target doses applied for mediastinal lymphoma (16). Some patients have atypical and extranodale determinations of Hodgkin's lymphoma, which creates difficulties in choosing therapy, especially to diagnosis, in particular when there is liver or renal failure. A useful scheme for those with abnormal liver biochemistry is: cyclophosphamide, etoposid, prednisone, and procarbazine (17). BV product is a solution for very high risk patients, which improves the risk/benefit ratio with increased efficacy and low toxicity. Patients with progressive disease or releapse after autologus peripheral stem cells transplantation can receive life-saving chemotherapy, followed by therapy with BV, product containing an antimicrotubular agent (monomethyl auristatin E), which will be selectively deliver in CD30+ B-lymphocytes (from Hodgkin's lymphoma and CD30+ nonHodgkin's lymphoma) due to its coupling with anti-CD30 monoclonal antibodies (18) and which lead to cell cycle arrest in G2/M phase. An estimated overall response rate would be about 75% (19). Even if it is administered alone in patients with relapsed or refractory disease, it leads to an overall response rate of 75% and complete answers to 33% of them, a superior result of any tested agent or drug combination (19). The patients who relapsed after allogeneic peripheral stem cells transplantation can also be treated with BV (20). The addition of BV to reduced intensity conditioning scheme to patients with relapsed Hodgkin's lymphoma before the allogeneic peripheral stem cells transplantation has been a success, after a median follow-up 14.4 months. BV treatment before reduced intensity conditioning (fludarabine/melphalan) for allogeneic transplantation, allowed the reduction of the graft-related complications, of peritransplant toxicity and resulted in an increased progression-free survival at two years of 59.3% compared with 26.1% (without BV) and reduced the cumulative incidence of disease progression and releapses to 23.8% from 56.5% (21). For patients who have no transplant indication, there are ongoing studies that associate BV with chemotherapy (replacement of bleomicine, that has known toxicity, in ABVD or BEACOPP schemes) in limited forms of Hodgkin's lymphoma.

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CLINICAL HAEMATOLOGY SECTION
ORAL PRESENTATION SESSION

C1. CALR AND JAK2 V617F MUTATIONS DELINEATE SUBGROUPS OF PATIENTS WITH ESSENTIAL THROMBOCYTHAEMIA AND PRIMARY MYELOFIBROSIS DISPLAYING DISTINCT BIOLOGICAL FEATURES. A MULTICENTRIC STUDY ON 199 PATIENTS

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Introduction

Essential thrombocythemia (ET) and primary myelofibrosis (PMF) represent non-BCR-ABL classical myeloproliferative neoplasms characterized in around half of cases by the somatic mutation JAK2 V617F. Around 5-10% of the JAK2-negative patients harbour somatic mutations within the c-MPL gene. Recently, the CALR gene (Calreticulin) has been shown to be mutated in 60-70% of the JAK2-negative patients.

Material and methods

This a multicentric study, including 199 patients (153 with ET and 46 with PMF), diagnosed and followed in haematology clinics and departments from Cluj-Napoca, Bucureşti, Tîrgu-Mureş, Baia-Mare and Sibiu. JAK2 V617F was analyzed by a tetra-primer PCR assay, the c-MPL W515L/K/A and S505N mutations were analyzed by a multiplex alle-specific PCR assay. In order to analyze the type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion) mutations of the CALR gene, making roughly 90% of all the CALR mutations described, we developed and validated by DNA sequencing an own simplex PCR assay.

Results

JAK2 V617F was the most frequent mutation, seen in 83 patients with ET (54.2%) and 20 patients with PMF (43.5%). The CALR mutations were seen in 43 patients with ET (28.1%) and 13 patients with PMF (28.3%). The c-MPL mutations were rare events, seen in 3 patients with ET (2%) and 2 patients with PMF (4.3%). Twenty-four patients with ET (15.7%) and 11 patients with PMF (23.9%) were triple-negative.

CALR-positive patients displayed: a more important thrombocytosis and a less important leucocytosis than their JAK2-positive or triple-negative counterparts, regardless of disease (ET or PMF). They also had less frequently thrombosis or splenomegaly, than those JAK2-positive or triple-negative ones.

Conclusions

CALR-mutated ET and PMF represent entitiies with a distinct p[henotype, compared to those JAK2-positive or triple-negative. This phenotype is „milder", probably conferring a better survival.

Key-words: essential thrombocythemia, primary myelofibrosis, JAK2 V617F, CALR, c-MPL

C2. AUTOLOGOUS TRANSPLANTATION IN A PATIENT WITH λ LIGHT CAIN MULTIPLE MYELOMA STAGE III WITH DIALYSIS FOR CHRONIC RENAL FAILURE.

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INTRODUCTION

The relative frequent association of myeloma multiplex with different types of renal complications are well known secondary as: tubular insufficiency, amyloidosis, deposits of calcium, hyperuricaemia etc.

MATERIAL AND METHODS

We present an efficient modality of treatment by autologous stem cell transplantation at a young patient with myeloma multiple and severe renal insufficiency who needed 3 dialysis sessions/week.
The best method of obtaining remission and good consolidation is autologous stem cell transplantation. The risk of the case was the renal toxicity of this treatment and severely impaired renal function with a serum creatinin of 9.2 mmol/l with 3 dialysis sessions/week.

We harvested stem cells only with G-CSF and for conditioning we administered HD meplhalan after a dialysis session.

RESULTS

We present the evolution and the obtained results including the relative good evolution of the renal functions. After autologous stem cell transplantation the value of serum creatinine decreased to 4.2 mmol/l and the patient needs dialysis only twice a week.

CONCLUSIONS

Although the nephrotoxic effect of the melphalan, in renal failure caused by the light chain MM the most effective treatment is the autologous stem cell transplantation. Using in time this method can impove the renal function and reduce the need for dialysis.

C3. THE ROLE OF FLOWCYTOMETRY IN MONITORING ACUTE MYELOID LEUKEMIA PACIENTS.

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INTRODUCTION

The measurement of minimal residual disease, which reflects an assessment of the biology of acute leukemia is a postreatment prognostic factor and is rapidly moving into the forefront in recent years, although the optimal timing of MRD for risk stratification and therefore treatment will probably be redefined in the near future. Detection of MRD by multiparametric flowcytometry is based on identifying leukemia associated immunophenotypes (LAIP) on the malignant cells. They are absent or extremely rare in healthy peripheral bood or bone marrow cells.
AIM

The purpose of this work was the study of LAIP and subsequent determination of BMR and evaluation of the prognostic value of BMR in patients with acute myeloid leukemia following induction treatment.

MATERIAL AND METHODS

The study included 164 adult patients hospitalized at the Clinical Hematology and Marrow Transplantation Unit Targu Mures with acute myeloid leukemia (AML). Immunophenotyping was performed from bone marrow or peripheral blood using 4 colors flowcytometry both at diagnosis and after induction treatment.

RESULTS

By using a large panel of combinations of monoclonal antibodies, leukemia associated immunophenotype could be detected in 90% of patients with AML. The most common LAIP were aberrant antigen expression (lineage infidelity) and asynchronous antigen expression.

After the 2nd course of induction treatment, out of the 164 cases diagnosed with AML, complete hematologic remission was achieved in 58 cases and incomplete hematologic remission (neutrophil count <1,0x109/l and/or platelets <100x109/l) in 44 cases. In these cases the presence of MRD in bone marrow samples was studied by immunophenotyping. Minimal residual disease was detected in 29 cases. In univariate analysis the presence BMR after induction treatment had a negative impact on overall survival ($p < 0.0001$). The multivariate analysis showed that the presence of BMR is an independent negative prognostic factor ($p < 0.0001$).

CONCLUSIONS

Identification of LAIP being present in a significant proportion of cases of acute myeloid leukemia is a feasible approach to detect minimal residual disease. The presence of BMR after induction treatment is an independent prognostic factor in AML and has implications for risk stratification and treatment decision in patients with AML.

C4. MILIARY TUBERCULOSIS AND HAIRY CELL LEUKEMIA IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA.

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The input of tyrosine - kinaze inhibitors (TKI) into the therapeutic arsenal of the chronic myeloid leukemia lead to a dramatic change of the natural course / prognostic of these diseases.
TKI produce both hematological (bone marrow suppression), immunological (immune suppression)

and non hematological side effects that are distinct from one drug to another.
The immunosuppressive effects may be caused by imatinib, but are especially due to dasatinib. Treatment induced granulocytopenia and immunosuppression are accountable for several infections, including for miliary tuberculosis.

The development of a myeloproliferative and a lymphoproliferative disorder in the same patient is uncommon. The association between chronic myeloid leukemia (CML) and hairy cell leukemia (HCL) is extremely infrequent. The undercause of this adjunction is not known. The concurrent or sequential association between CML and HCL raises various question marks about their joint pathogenesis: the cytoreductive drug's part, the involvement of a common stem cell with bi-lineage manifestations or merely a chance coincidence of the two.

We are going to portray the clinical case of a 51 years old man that has been diagnosed with chronic myeloid leukemia in 2005. From 2005 until 2012 the patient underwent treatment with Glivec, 400 mg/day and presented a sustained major molecular response.
The treatment with Glivec was interrupted in January 2012 and was substituted with Dasatinib due to sepsis caused by Candida glabrata and coagulase postitive staphylococcus; the antibiotics and antipyretics were inefficient and we were in the presence of a newly installed hepatosplenomegaly with jaundice. The lab works revealed pancytopenia, hepatocytolisis, cholestasis and altered coagulation tests, meaning a disseminated intravascular coagulation (DIC). The computer scan revealed micronodular lesions in the lungs, liver and spleen. We had negative blood cultures.
We arose the suspicion of a disseminated tuberculosis and we begun a trial treatment with antitubercular agents that had as a result the resolution of all clinical symptoms, the regression of the hepatosplenomegaly and the progressive normalization of the bioumoral markers. Subsequently the diagnosis is confirmed by sputum culture.

The treatment with Glivec 400 mg/day is reintroduced and after two months of treatment a progressive splenomegaly emerges accompanied by pancytopenia with lymphocytosis, presenting atypical lymphocytes with cytoplasmatic projections on the blood smear. The diagnosis of hairy cell leukemia is set by marrow biopsy, flowcytometry and molecular analysis of the BRAF mutation. The joint treatment with Glivec and α interferon is followed by the remission of the HCL and the persistence of the major molecular response of the CML.

We argue the part played by the immunosupression caused by TKI, possibly an underclinical expression of HCL, in the manifestation of a miliary tuberculosis, the significance of the CML-HCL association and the optimal treatment course of these morbid couplings.

C5. **SEEKING A SECOND MEDICAL OPINION- A PATIENT'S RIGHT.**

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During centuries, seeking for a second medical opinion was considered being a concession for a patient and after that, in the middle of the XXth century it becomes a patient's right. Second opinion may have a critical influence on the diagnosis, treatment and prognosis, especially for malignant patients. The patient can benefit from treatment optimization and avoid unnecessary risks. Seeking a second medical opinion or providing one can cause ethical or legal dilemma.

Second medical opinions can be recommended by doctors, asked by the patients or by the private or public insurance companies. Patients often search for a second opinion, for additional information on the diagnosis and/or treatment options and the potential prognosis, which will help the patient decide what to do or not to do, where, with whom and how. Cancer patients denying their diagnosis are asking for a second opinion hoping that the second doctor will find a benign diagnosis. Physician asking for a colleague's opinion may benefit from less exposure to legal claims. For the insurance companies, asking for second medical opinions by the patients can provoke unnecessary medical costs, but these companies themselves are asking for second medical opinion (especially in western countries) because are trying to control treatment costs (eliminate unnecessary surgeries or other expensive treatments).

In our country asking for a second medical opinion is protected by Patient's Right Law and by Medical Deontological Code.

In western medicine, asking for a second medical opinion represents a common practice and many hospitals have websites providing information asked by the external patients. In these countries implementation of practice guidelines was determined by the need for seeking a second medical opinion, but these guidelines will not eliminate the consultation for a second opinion because every patient and every disease have particularities.

In Eastern European countries, the medicine is now centered on patient's autonomy, but a paternalistic approach persists. That's why asking for a second medical opinion could produce first doctor's

disagreement.

Few studies were done in Romania based on knowing and observing patient's rights. As we know, there is no research about second medical opinion. In an European regular report "The Empowerment of the European Patient – Options and Implications", in the "patient's rights" category, out of 31 countries, Romania was situated in 22-27th position because our country failed to implement patient rights recognized by law and restricted or obstructed patients' rights to second medical opinion.

The presentation will reveal the results of a pilot study conducted in Cluj-Napoca Hematology Clinic that has the purpose of evaluating patient's view about seeking a second medical opinion. We have done semistructured interviews with 40 malignant patients. All of them had a suspicion on their medical care (investigations, diagnosis, treatment or prognosis accuracy), but only two patients asked for a second medical opinion, even if 20% of patients surveyed said they get less than half of doctor's explanation. Patients who did not request a second medical opinion motivated by the fact that it is not a common practice in the Romanian medical system, have not thought about it or did not know whom to ask. Asked to describe the attitude of their doctor if he would know that the patient asked another medical opinion, 32.5% of patients felt that their doctor would show empathy, 30% that the doctor would have a disapproving reaction and 37.5% could not answer. Regarding the characteristics of patients who would require a second opinion, there is a statistically significant association between level of education and the desire to seek a second medical opinion.

On the basis of this study, a number of seminars for physicians can be conceived to improve the knowledge and application of patient rights in practice and on the other hand informational campaigns for patients regarding their rights.

C6. **DEVELOPMENT IN CELLULAR ANALYSIS TECHNOLOGIES OFFERED IN ROUTINE HAEMATOLOGY SYSTEMS TO HELP IN BETTER IDENTIFICATION OF ABNORMAL CELLS**

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- Development in Technologies: multidimensional Flow Cytometry Analysis of Cells in DxH Systems
- Decision rules to help lab automate sample reviews and reduce manual steps
- HaematoFlow and CytoDiff

Flow Cytometric Digital Morphology: multiple direct measurements with a total of 29 measurements per Cellular Event, including: volumetric, radio frequency, 5 x angles of laser light scatter, nucleus to cytoplasmic ratio, cell complexity, granularity, nucleus structure

Advanced Algorithm Applications: snake algorithm, separation of defined cell populations, utilizes population coordinates, aids in 3D Cell Population Display.

Pre-Installed Decision Rules: in addition to the 41 ISLH Consensus rules that are installed on the DxH 800 V2.0, there are 16 pre-installed rules utilizing Cell Population Data (CPD) to help identify Anisocytosis as an example.

Decision Rules Work Bench: Decision Rules Workbench allows for generation/editing of inactive rules while on-line.

Rules are then enabled once offline

HematoFlow Cellular Analysis Solution:

The new solution for validation of abnormal WBC samples with CytoDiff

Brings flow cytometry in the routine hematology lab

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Soluții variate în oncologie – dedicate fiecărui pacient

RO/TBD/14/0007

CLINICAL HAEMATOLOGY SECTION
POSTERS

P1. **PRIMARY PLASMA CELL LEUKEMIA-CASE PRESENTATION.**

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Introduction: Primary plasma cell leukemia is one of the rarest form of the plasma cell malignant diseases. The prognostic of this disease is extremely poor with a medium survival by only several months. It is defined by the presence of over 20% plasma cells in peripheral blood. In most cases it is primary, but it could be secondary, in the multiple myeloma evolution.

Case presentation: An old woman, 76 years old, was presented in our clinic, complaining of nausea, vomiting, dizziness, fever and weight loss. In May this year the patient was admitted in Nephrology Clinic for an episode of acute renal failure, where she was found with high leukocytes count, anemia and she was guided to our clinic. Laboratory analyses and marrow puncture was found 38% plasma cells, with over 20% plasma cells in peripheral blood, with plasma cell immunophenotyp features: CD20+, CD38+, CD19-, CD56-. Laboratory analyses were showed a high white blood count 53.000/mmc, an anemia 8,3g/dl, a thrombocytopenia 77.000/mmc and renal failure, creatinine 2,84mg%. No evidence of osteolytic bone lesions was showed at skeletal survey. We administered first cycle VAD, day 1-4, with decreased of white blood count at 8870/mmc and a better renal function, creatinine 1,21mg%. After chemotherapy the patient made an infectious complication, Clostridium Difficile acute enterocolitis, so we had to stop the chemotherapy.

Conclusions: Compared with multiple myeloma, plasma cell leukemia presents more often acute leukemia clinical features: anemia and thrombocytopenia appears more often and there are more aggressive. Osteolytic lesions are less expressed but hypercalcemia as well as impaired renal function are often presented.

Keywords: plasma cell, leukemia, prognostic

P2. **EVOLUTION AND COMPLICATIONS OF THE MULTIPLE MYELOMA PATIENTS AT AN ADVANCED STAGE OF DISEASE.**

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Introduction. The multiple myeloma is a neoplasia characterized by the clonal proliferation of malignant plasma cells in the bone marrow, the presence of a monoclonal protein and the damage on some target organs.

Aim. The study proposes the evaluation of the complications and evolution of patients suffering of multiple myeloma at an advanced stage of disease.

Methods. We conducted a retrospective analytical study on 80 patients with multiple myeloma at an advanced stage of disease and who have been in the records of the Department of Haematology, Timisoara since January 2008 – December 2013. The diagnosis of multiple myeloma was established by means of showing the presence of the monclonal immunoglobulin in the serum , excretion of light chains in the urine (Kappa and Lambda), over 10% medular plasmocytosis, bone lytic lesions. All patients were clinically examined, including blood counts, biochemical tests, X-ray photographs (skeleton). The computer assited tomography was performed depending on the clinical doctor recommendation.

Results.The average age of the patients was 60 years old and the group included 61% male patients and 39% female patients.

Of the 80 patients, 52% showed IgG , 29% IgA , 2% IgD , 1% IgM and 16% free chains. 24% of the

patients were in stage II of disease and 76% were in stage III, and 34% of the patients showed the Beta2microglobulin increase over 3.5 mg/L lawhile 66% showed an increase above 5.5 mg/L. The review of the complications related to disease and therapy revealed that 70% of them developed anemia while 36% showed fractures with vertebral compaction, 30% chronic renal impairment , 12% neurological complications, 10% infections and 2% hyperviscosity syndrom.

Conclusions. Multiple myeloma remains a challange in terms of diagnosis and therapy, because of the multiple complications, whose management is an essential condition for a better evolution of this patients.
For most of the patients, particularly for those at an advanced stage of disease, the evolution of this neoplasia is still unfavorable.

P3. THE IMPORTANCE OF PANCYTOPENIA FOR THE EVOLUTION AND TREATMENT OF NON HODGKIN LYMPHOMA PATIENTS.

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Introduction. For Non-Hodgkin lymphoma (LNH) patients, polychemotherapy can affect the bone marrow throw the cytotoxic effects of the medication. In the medical research for the LNH evolution, regardless the hystological appearance, the pancytopenia is correlated with an unfavorable evolution. The application of a more aggressive chemotherapy, although it may favorably influence the diminution of the tumor mass, adds more severity to the global evolution of the disease through the secondary effects including also the toxicity on the bone marrow and consequently the pancytopenia enhancement.

Aim:The study proposes the evaluation of the survival rate at patients with pancytopenia and its role as prognostic factor in the evolution and treatment of patients with LNH.

Methods: We conducted a retrospective analytical study on 151 patients between May 2008 and April 2013 with the diagnosis of Non Hodgkin lymphoma in the Department of Haematology, Timisoara. The main method used for diagnosis consisted of biopsy, followed by histopathological and immunohistochemistry examination of the sampled tissue. The disease stage was determined using the computer tomography (CT) and bone marrow biopsy (BOM). The polychemiotherapy treatment and the number of cycles performed were established depending on the stage and histological grade of the disease. The patients data regarding their medical history, as well as the laboratory tests were taken from the patient medical records.

Results:The average age of the investigated patients was 49.69 years old , the youngest being 18 years old, while the oldest were 89 years old, of whom 37.7% were female and 62.3% were male. The monitoring period from the time of the diagnosis was 13.92±6.24 months up to complete remission for 33.7% of the patients and partial remission for 45.7% of the patients. 17.2% of the patients showed progressive disease , 0.6% showed recurrence and 2.5% died. The values of hemoglobin, plateletes, white blood cells and pancytopenia grade were monitored on the investigated patients. The survival curve was reviewed depending on the analyzed parameters, resulting an approximately 8 month survical at patients with severe pancytopenia.

Conclusions. The results of this study suggest that pancytopenia is associated to a low survival rate. The low values of the associated hemoglobin , white blood cells and plateletes could be a significant prognosis element for the evolution of these patients.

P4. TREATMENT WITH BORTEZOMIB IN MULTIPLE MYELOMA- EXPERIENCE OF MEDICAL CLINIC I DEPARTMENT OF HEMATOLOGY TÎRGU-MUREȘ

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Introduction: Multiple myeloma is a disease having an unpredictable evolution, which has

recently presented an increase in the number of newly diagnosed cases. Bortezomib treatment has significantly changed the survival and prognosis at these patients.

Material and methods: The study is observational and prospective. It has been performed on 120 patients treated between January 2009 and June 2014 in the Medical Clinic I, Department of Hematology Tirgu-Mures. The group has been divided into two subgroups: one consisting of 41 patients treated with Bortezomib (Bortezomib + Dexamethasone, Bortezomib + Cyclophosphamide or Melphalan + Prednisone) and the other one of 79 patients treated with standard chemotherapy. We have performed a descriptive analysis, the Chi-square test, survival analysis has been estimated using Kaplan Meier curves and compared with the Logrank test.

Results: The subgroup of patients treated with bortezomib had an average age of 63 years, 53.65% were IgG secretory, 46.34% were in stage III of disease. A number of 23 (56.09%) patients have received Bortezomib as first-line treatment, the average number of administered cures being 6.68. A percentage of 21.95% had a complete response to therapy, 41.46% a partial response and 36.58% a minimal response/ non-response. There has not been any statistically significant difference in terms of CR + PR rate among patients treated as first line with Bortezomib and those with relapsed disease (63.41% versus 60.86%, p=0.754). The median survival of patients treated with Bortezomib is significantly higher, 51 months versus 22 months in case of standard chemotherapy (p = 0.0484). A number of 4 patients who had responded to the treatment performed bone marrow transplantation. The most common adverse effects from the treatment were polyneuropathy(14.63%), infections(9.75%), thrombocytopenia(4.86%) and pancreatic reaction (1 patient).

Conclusions: There is a superior survival among the patients treated with Bortezomib, associating an acceptable tolerance to the treatment.

Keywords: multiple myeloma, Bortezomib, survival, response to therapy

P5. MANAGEMENT OF INFECTIOUS COMPLICATIONS IN HAIRY CELL LEUKEMIA – CASE REPORT – POSTER

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Hairy cell leukemia (HCL) was first described in 1958 under the name of leukemic reticuloendotheliosis, and in 1970 was recognized as well defined clinical entity. HCL is a malign lymphoproliferation characterized by pancytopenia existing on the periphery smear and bone marrow and the occurrence of untypical lymphocytes with cytoplasma extensions which associate an enhanced infection risk.

Between 2004 -2014, at the Clinics of Haematology - Timisoara, there were diagnosed 30 cases of HCL, 22 male patients (73,33%) and 8 female patients (26,67%), of whom 21 (70%) patients developed infectious complications.

It is hereby presented the case of a 36 years old female patient diagnosed with HCL in May 2013 and treated with Litak (Cladribina) 0.1 mg/kgb/day for 7 days. The treatment was well tolerated, post –chemotherapy reconstituted the red cells and platelets series, but with the persistence of neutropenia. The control bone marrow biopsy revealed a reduced tumoral infiltrate, considering it a partial remission, a new cycle of chemotherapy was initiated which led to marrow aplasia. At the end of the treatment, the patient showed prolonged feverish syndrome, dry irritating cough, dyspnea with orthopnea, tachycardia and the patient's general condition became progressively worse. The inter-disciplinary investigations corroborated with the radio-imagistic and computed tomography scan established the diagnosis of acute broncho-pneumonia with acute breathing deficiency. The patient's progress under therapy was slowly favorable, the therapeutic scheme being repeatedly adjusted. The chest X-ray investigation performed at the discharge did not show any sequellary lesions. A new bone marrow biopsy was performed

and it showed a minimum lymphoid infiltration. The patient is periodically re-evaluated and she did not develop any other infections.

The infectious complications occurred at patients with HCL are life threatening and require a multi-disciplinary approach and a complex treatment administrated in very early stage.

P6. **EVOLUTION OF CLL PATIENTS IN ADVANCED STAGES OF DISEASE IN MONOCLONAL ANTIBODIES ERA**

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Introduction. The chronic lymphocytic leukemia (CLL) represents the most frequent lymphoproliferation of the grown up patients. It is characterized by the accumulation of malign lymphocytes at the level of bone marrow, blood and lymph nodes with the occurrence of adenopathy and hepatosplenomegaly and the incidence peak is around the age of 72.. From clinical point of view, its evolution implies two stages: a non-aggressive stage which does not require any treatment and an aggressive stage with modest response to conventional therapies. The addition of monoclonal antibodies to the classic treatment schemes at advanced disease stages led to the prognosis amelioration .

Goal. The study goal consists of the evaluation of the prognosis of patients in advanced stages of disease (stage C) as well as the efficiency of the modern treatment schemes.

Material and methods. I conducted a retrospective study on 40 patients with diagnosed with stage C of CLL who were undergoing chemotherapy in association with monclonal antibodies (Rituximab) at the Clinics of Haematology Timisoara between January 2009-December 2013. The treatment efficiency was evaluated based on the following criteria: remission of general clinic symptomatology , diminish of organomegaly , decrease of lymphocytosis , evaluation of the minimal residual disease and the prognosis was estimated based on the blood cell counts , bone marrow aspirate, immunophenotype and cytogenetic investigation.

Results. 85% of the patients with ages ranging between 60-80 years old. At the end of the treatment, 37.5% of the patients showed complete remission, 20% partial remission 22.5% showed disease progress and 20% deaths were recorded.The adverse reactions developed after the administration of Rituximab at 2 patients (5%) required the treatment interruption. 67.5 % of the patients did not show any adverse reactions. Organomegaly disappeared at 62.5% of the patients and the adenopathy at 72.5% of the patients at the end of the treatment.

Conclusion. The association of monoclonal antibodies to the different chemotherapy schemes applied to patients diagnosed with stage C of CLL is well tolerated even at older ages , their evolution being positive.

P7. **PRIMARY CUTANEOUS LARGE B-CELL LYMPHOMA, LEG TYPE- CASE PRESENTATION.**

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Introduction: Primary cutaneous large B-cell lymphoma, leg type represents a rare and aggressive type of large B-cell Non-Hodgkin lymphomas. It affects especially old women and it's characteristic traits are elevated, red or bluish red lesions on the skin, localized on the lower half of the lower limbs.

Clinical case: We present the case of an 85 years old pacient, ago 10 months attending the Department of Surgery of the Oncological Institute "Ion Chiricuta" Cluj-Napoca, due to the occurrence of an elevated, bleeding lesion on the right calf, associated with increased intensity pain during walking. The radiological examination of the right calf showed a fracture zone on the tibia, an opacity with several lobes, 7,7/6,2 cm in the adjacent soft parts and other opacitys with the same features in the lower right leg. The biopsy of the biger lesion was performed and the histopathological and the Immunohistochemistry examination established the diagnosis- primary cutaneous large B-cell

lymphoma, leg type.Later, the pacient is hospitalized in the Hematology Clinic for specialized treatment, for chemotherapy. At the presentation, on the right calf were localized several elevated lesions, between 1,5 and 5 cm diameter and one larger, bleeding, suppurative lesion, with deposits of fibrin, of 6 cm diameter. Biological examination revealed anemia, normal leukocytes and platelets count; elevated LDH level. It was administered chemotherapy- CEOP (Etoposide instead of Doxorubicin, due to the rising age), every 3 weeks, with good clinical tolerance and favorable evolution. After 4 sessions of chemotherapy, the lesions disappear. To the chemotherapy it was associated a monoclonal antibody-Rituximab and it were administraded 2 sessions R-CEOP and 1 R. After the last one with R, the leasions on the right calf reappear, up to 2-3 cm, construed as relapse, with the decision of a second-line of treatment- R-COP sessions with good clinical tolerance. So far have been administered 3 sessions R-COP, the pacient presenting relaps on the lower right leg, reason why has been initiated a new line of therapy.

Conclusions: This case highlights typical features of the primary cutaneous large B-cell lymphoma, leg type: the old age, feminine sex, the aggressive nature of the disease and the tendency to relapse, the necessity of multiple lines of chemotherapy.

P8. PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY IN A PATIENT WITH NON-HODGKIN'S LYMPHOMA FOLLOWING TREATMENT WITH RITUXIMAB.

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Progressive multifocal leukoencephalopathy is a demyelinating disease of the central nervous system caused by the polyomavirus JC. It is considered a fatal infectious complication that occurs in patients with HIV/AIDS and in patients on immunosuppressive or new biological therapy.

We present the case of a patient with splenic marginal zone non-Hodgkin's lymphoma that developed progressive multifocal leukoencephalopathy after the treatment with rituximab plus chemotherapy.

The 64 years old patient was diagnosed with stage IV splenic marginal zone non-Hodgkin lymphoma by histopathologic examination and immunohistochemistry of the bone marrow. The patient was also known with chronic hepatitis B. CHOP chemotherapy was initiated followed by splenectomy and after that the patient underwent 4 cycles of R-CHOP. Due to the worsening of the hepatic disease the treatment with rituximab was halted. 3 months after stopping the treatment the patient developed progressive multifocal leukoencephalopathy, demonstrated by brain MRI and PCR for polyomavirus JC from the cerebrospinal fluid.

Severe immunosuppression allows viral reactivation leading to multifocal leukoencephalopathy, which can occur in patients with hematologic malignancies following chemotherapy and new biological therapies such as monoclinic antibodies. Imaging investigations, demonstration of virus by PCR in CSF or brain biopsy are necessary for the diagnostic. This case and those described in the literature aim to sensitize the clinicians to the possible diagnosis of progressive leukoencephalopathy in hematological patients who undergo immunochemotherapy and present neurological phenomena.

P9. MUSCULOCUTANEOUS BLEEDING AND TISSULAR NECROSIS IN AN ELDERLY PATIENT WITH ACQUIRED HEMOPHILIAA. CASE REPORT.

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BACKGROUND
Acquired hemophilia A is a rare bleeding disorder caused by the appearance of autoantibodies against coagulation factor VIII. A condition that occurs more frequently in the elderly and is associated with malignancy, autoimmune diseases or pregnancy. In 50% of cases remain idiopathic.

AIM
We want to present the case of a female patient 83

years old diagnosed in 2011 with severe acquired hemophilia A (FVIII activity <1%, the inhibitors titer 166 U Bethesda) and who developed a giant hematoma in the left forearm with musculocutaneous necrosis with favorable evolution under treatment with bypass agents.

MATERIAL AND METHOD

An elderly patient, diagnosed for about 2 years with severe acquired idiopathic hemophilia A with recurrent skin and mucosal bleeding. The patient followed immunosuppressive therapy but failing to eradicate the inhibitors. In Oct.2013 she is hospitalized in the Oradea Hematology Clinic with bleeding and important swelling of the left forearm with skin infection which evolved with necrosis of the muscle, skin and the soft tissue of the anterior-medial face of the forarm. Treatment consisted of intensive local dermatologic and antibiotic therapy and rFVIIa (Novoseven). Evolution was favorable with detachment of the necrotic tissue and re-epithelization, without significant bleeding.

RESULTS

Favorable development of a serious bleeding episode complicated by infection and musculocutaneous necrosis in an elderly patients with Hemophilia A severe acquired.

CONCLUSIONS AND DISCUSSIONS

Acquired hemophilia A is a coagulopathy with a high degree of complications and mortality that requires prompt diagnosis and treatment. The principles of treatment are bleeding control and inhibitor eradication therapy. The treatment for elderly patients may pose many problems because of their comorbidities and the adverse effects of treatment. For this patient, for whom the eradication therapy failed is very important to control the bleeding using hemostatic and anti-hemophilic by-pass agents.

P10. MANAGEMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA OF ELDERLY. SINGLE CENTRE EXPERIENCE.

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Background. Acute Lymphoblastic Leukemia is uncommon and less curable in patients over 60 years of age because of a greater resistance to chemotherapy, a relative inability of elderly patients to face the toxic effects and complications of therapy and influence of co-morbidities.

Aims. We presented our experience of 48 consecutive cases of ALL of elderly age collected in the last twelve years. Median age was 65 years (range 61-84).

Methods. L2/L1 FAB classification: 39/9; Median WBC was 18xl09L (range 2-179); Male/Female ratio was: 17/31. Forty-two (87,5%) belonged to B cell lineage (pre-pre-B 10, common 27, pre B-5) and 6 (12,5%) to T cell lineage (pre-T staged). Philadelphia chromosome was present in 12 patients (25%).

Out of the 48 revisited patients, 35 patients (median age 64 years, range 61-74, good performance status and without co-morbidity factors), received an intensive treatment such as ALL protocols. In the remaining 13 older patients (median age 76 years (range 65-86) and those with severe coexisting cardiac, pulmonary, renal and hepatic disease, a gentle chemotherapy including prednisone and vincristine, 6-mercaptopurine and methotrexat was utilised.

Results. Ten patients (20,8 %) of the group treated with curative intent died during the induction phase; 23 patients (47,9%) achieved complet remission (CR) and, at present, 4 patients are alive at 12 and 42 months Out of 13 patients receiving less intensive and supportive treatment only 4 (8,33%) achieved a short CR: other patients had an early relapse and died.

Conclusion. Our data demonstrated that immunophenotypic patterns of patients is very important for survival and prognosis. In addition in our experience emerged that to the younger patients who can well tolerate an aggressive tratment could benefit of this approach, because of it is possible to achieve longer survivals.

P11. **THROMBOTIC AND HEMORRHAGIC COMPLICATIONS IN POLICITEMIA VERA.**

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Introduction. Polycythemia vera (PV) belongs to the bcr-abl negative group of myeloproliferative disorders, characterizated throw the JAK2V617F mutation. Thromboembolism and bleeding events are complications with a negative impact on overall survival.

Aim. We studied thrombotic and hemorrhagic complications for a group of patients diagnosticated with PV in accordance with the WHO 2008 and the possible correlations between the thrombotic/bleeding events and clinical and laboratory variables of PV.

Method. We retrospectively analized the incidence of arterial and venous trombosis and hemorrhagic events and their connection with the clinical and laboratory variables and overall survival.

Results. We evaluated 132 patients: 78 patients were female and 54 patients were male. At diagnosis 38% were asymptomatic, 24% had splenomegaly anf 15% hepatomegaly. A thrombotic episode was reported at 35 patients (26,5%) during the evolution of the disease, and 11% patients were with bleeding episods. The most frequent were the arterial episods. Median time and the median curve between diagnosis tratament and the thrombotic event was 190 and 149 days. Median values of Ht, Hb, MCV, lWBC and PLT were 53,8%, 17,5g/dl, 82, 8fl, 1105x 109/L si 620x109/L. Also LDH level was elevated in 41% patients. Median value of serum erytropoetin level was 6,75µ/ml. Bone marrow biopsy revealed panmyelosis in 58%, fibrosis in 28%. Antiplatelet and anticoagulant therapy was administrated in 69% and 7% respectively.

Platelets, leukocytes and hematocrit level at diagnosis was not statistically significant between patients who presented a thrombotic/bleeding event and the patients without this events. Values of the hemogram parametres presented significant differences between thrombotic/bleeding events and their level at diagnosis. Median fallow-up time was 40 months (0,5-148). 16 deaths were recorded and the survival at 5 and 10 years wae 87%, 68% resectively. Age >65 years and the presence of a thrombotic / bleeding event were unfavorable prognostic factors for overall survival.

Conclusions. It was revealed a high incidence of thrombotic/bleeding complications in PV patients. Leukocytosis has been suggested as a risk factor for thrombosis, but in our study no parameters analyzed did not influence significantly the occurrence of bleeding/ thrombotic event.

P12. **RELAPSED AND REFRACTORY MULTIPLE MYELOMA, EVOLUTION AND COMPLICATIONS.**

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Background. Multiple myeloma (MM) is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration or the bone marrow and is associated with an increased level of monoclonal protein in the blood and/or urine.

Aim. Retrospective evaluation of the therapeutic results of evolution and complications of therapy with bortezomib, doxorubicin and dexamethasone (PAD) in the treatment of relapsed/refractory myeloma patients.

Patients and Methods. 57 patients were treated for median of four 28-day PAD cycles (1-8). Bortezomib was given at 1.3 mg/m2 (days 1, 4, 8,11), doxorubicin at 9 mg/m2 (days 1-4) and dexamethasone 20 mg po (days 1-4, 8-11).

Results. 57 patients were evaluable for efficacy, 66% had refractory disease and 34% were relapsed. The median age was 62 years (37-76), 57% were male, 43% female. Serum protein electrophoresis revealed a localized band in 75% of patients, and immunoelectrophoresis or immunofixation showed a monoclonal protein in 84%. A

monoclonal light-chain was found in the urine in 62%. Non-secretory myeloma was recognized in 2% of patients, whereas light-chain myeloma was present in 17%. Serum albumin less than 3mg/dl was found in 61% of patients. Conventional radiographs showed an abnormality in 85%.

Median time from diagnosis was 17 months (2-115) and median number of prior therapy lines was 2 (1-5): 72% had undergone conventional chemotherapy, 15% Alkerane and Dexamethasone and 13% were autografted. Overall response rate of 60% was observed, 31% of patients achieved a complete response (CR), 24% a very good partial response(VGPR), 28% a partial response (PR). Stable disease (SD) was observed in 17%. The median progression free survival (PFS) was 15,9 months. The most common grade 3-4 toxic effects were neutropenia 15%, thrombocytopenia 18%, anemia 10%, infections 14%, peripheral neuropathy 6% and gastrointestinal disturbances 3%. One toxic death (1.1%) due to sepsis was noted.

Conclusion. The combination of bortezomib, doxorubicin and dexamethasone (PAD) is well tolerated and induced clinically signifiant responses and prolonged remission duration in patients with relapsed and refractory MM.

P13. **MANAGEMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA, SINGLE CENTRE EXPERIENCE.**

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Background. The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely.
Aims. To evaluate the treatment of ITP patients in Departament of Hematology, County Hospital, Timisoara during 15 years (I 1999-XII 2013).

Methods. A retrospective study for 325 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 12 months prior to their most recent visit.

Results. The mean age was 45.1 years, with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Prior to the observational period, 35% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 72% of all patients were treated. The most frequent reasons given for treatment were platelet count (73%), followed by bleeding symptoms (51%). Corticosteroids represented 62% of treatments, followed by IVIg (19%), azathioprine (11%) and rituximab (8%). Splenectomies (11% of patients) and platelet transfusions (32% of patients) were performed during the observational period. For monitoring the platelet levels, 82% of patients visited their hematologist 1 to 10 times during the observation. Main reasons for a visit were a low platelet count (42% of visits) and bleeding (34% of visits). Overall, 42% of patients required hospitalization. Mean duration of hospitalization was 13,2 days.

Conclusions. The retrospective study of 325 patients provides therapeutic outcomes resulting from treatment methods from our department. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids represent the most used treatment from our department.

P14. **INDOLENT LYMPHOMA IN YOUNG PEOPLE - DIAGNOSTIC, PROGNOSTIC AND THERAPEUTICAL IMPLICATIONS.**

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Abstract: Non-Hodgkin malignant lymphomas are tumors of the immune system cells. These chronic lymphoprolyferative disorders are neoplasms with varied histological aspects, clinical features, evolution, prognosis and aggressiveness.Follicular lymphomas are the most frequent form of indolent lymphomas and they represent ~ 25% of all malignant lymphomas in adults.

Patient analysis was done from the point of

view of prognostic factors: age, gender, Ann Arbor disease stage, value of hemoglobin and of LDH, number of affected node areas.

Method: On the account of available data in the medical literature, we have analyzed a group of 109 patients hospitalized in the Hematology Clinic Hospital Colțea Bucharest in January 2008 - December 2012.

In the stratification of patients included in the study, the following parameters were analyzed: patient data (sex, age), clinical balance (ECOG performance status, disease signs B, syndrome tumor, stage of disease at diagnosis), paraclinical balance (complete blood count, bone marrow cytology, renal and hepatic function, eg.histo-patological of lymph node and bone biopsy, molecular biology tests in selected cases, imaging tests - chest X-ray, ultrasound and computer tomography .

Conclusion: The following parameters did not have any prognostic value: age; sex; area of origin, stage of disease, presence of bone marrow damage onset, the value of Ki-67.

The following parameters had prognostic value: presence of extranodal determinations; size and location of lymph nodes, presence of B-signs of disease, presence of hepatosplenomegaly, serum levels of LDH, serum levels of ESR, serum levels of fibrinogen, platelet count at diagnosis, hemoglobin level at diagnosis, WBC count and the number of peripheral blood lymphocytes at diagnosis, histological type of indolent lymphoma, IPI value, type of response.

P15. **MANAGEMENT OF THE PATIENS WITH CHRONIC MYELOID LEUKEMIA IMPACT OF NEW DIAGNOSTIC AND THERAPEUTIC METHODS.**

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INTRODUCTION
Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder; the molecular hallmark of the disease is the BCR-ABL gene rearrangement which usually occurs as the result of a reciprocal translocation between chromosomes 9 and 22. Tyrosine kinase inhibitors (TKI) were the first drug that targeted the constitutively active BCR-ABL kinase and it has become the standard frontline therapy for CML. Monitoring the treatment of CML patients with detection of BCR-ABL transcript levels with real time qualitative polymerase chain reaction (RQ-PCR) is essential in evaluating the therapeutic response.

MATERIAL AND METHODS
At the Clinical Hematology and BMT Unit Tg-Mures between 2008-2014 we performed the molecular monitoring of bcr-abl transcript levels with RQ-PCR at 30 patients diagnosed with CML.

RESULTS
We have 16 patients on imatinib treatment who achieved major molecular response. One patient loosed the complete molecular response after 5 years of treatment. Five patients underwent allogeneic hematopoietic stem cell transplantation from identical sibling donors. One patient is in complete molecular remission after 9 years of the transplant. Six patients present positivity to Met351Thr mutation with increasing transcript levels. We performed the switch to the 2nd generation of TKI.

CONCLUSIONS
Because a rising level of BCR-ABL is an early indication of loss of response and thus the need to reassess therapeutic strategy, regular molecular monitoring of individual patients is clearly desirable.

P16. **THE INFLUENCE OF POLYMORPHISM ASSOCIATED WITH THROMBOPHILIC PREGNANCY STATUS**

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Introduction: Thrombophilia includes a series of hypercoagulable states, predisposing to intravasculare thromboses. The existence of a hereditary abnormalities in coagulation-fibrinolysis system associated with an additional risk factor (smoking, venous stasis, atherosclerosis, consumption of contraceptive pills), predisposes to triggering thrombotic process.

Material and methods: the study includes women of childbearing age who have had 2-3 miscarriages in their past and want to get a pregnancy, and multipare women that have not been investigated in terms of thrombophilic status. Clinical evaluation was done according to a specially elaborated observation sheets and paraclinical investigations have included blood tests, imagistic explorations , molecular techniques.

Results: causes of thrombophilia are either congenital (hyperactivity of clotting, deficiency of anticoagulant system) or acquired, who performed a procoagulant status; antiphospholipid syndrome is relatively frequently met, manifested by increased levels of antibodies against anionic phospholipids membrane and plasma protein associated, which determine the appearance of venous or arterial thrombosis or other complications that can lead to the interruption of pregnancy.

Conclusion: the gravity of the consequences of intravasculare thrombosis at pregnant woman justifies primary prevention measures, in conjunction with the necessity of finding and applying an algorithm for an early diagnosis.

P17. **THE CLINIC AGRESIVITY AND EVOLUTION OF A PACIENT DISEASE DIAGNOSTICATED WITH BLASTIC PLASMOCYTOID DENDRITIC CELL NEOPLASM**

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematologic malignancy characterized by the clonal proliferation of precursors of plasmocytoid dendritic cells. The clinical features an evolution of BPDCN consist of two main patterns, one, 90% of cases characterized by an indolent onset dominated by cutaneous lesion followed by tumor dissemination the other, 10% cases, showing features of an acute leukemia with systemic involvement from the beginning. Also in these cases multiple skin nodules are frequently present.

On flow cytometry the expression of CD4, CD45RA,CD56 and CD123 is considered to represent the pathognomic phenotype.
No specific chromosomal aberration have been identified until now.

Case is of a man who shows multiple tumor located the anterior and posterior thorax, scalp, legs and hands, ranging in size from 5/6 cm to 10/15 cm, with aspect of ulcerative necrotic burjonate some containing worms, painless. BPDCN was diagnosed based on AP and IHC examination of skin biopsy and chemotherapy was initiated with initial favorable trend but subsequently encumbered impaired consciousness due to solid tumor localized parieto-occipital right and left cerebellum.

Despite the apparently indolent clinical presentation, the course is aggressive and the median survival is approximately 12-14 months.

At present, there is no consensees for optimal treatment of BPDCN. With intensive therapy for acute leukemia the rate of remission increases, but only allogeneic bone marrow the first remission is a chance of long term survival.

P18. **CHRONIC MYELOID LEUKEMIA WITH BASOPHILIA AND EXTREME THROMBOCYTOSIS - CASE PRESENTATION.**

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Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm having as a trend mark the presence of the Philadelphia chromosome and/or the BCR-ABL1 rearrangement.

From a clinical point of view, LMC is characterized by splenomegaly, the overproduction of white blood cells, with a left deviation of the blood formula, a moderate basophilia and variable eosinophilia. Thrombocytosis is a common thread of the chronic myeloproliferative diseases, being asymptomatic in CML, having an incidence of 30-50%. Extreme thrombocytosis is defined as a platet

number higher than 1000 x 10 9 / L and, similar to an isolated thrombocytemia, without a high white blood cells count, is rarely encountered. Basophilia and extreme thrombocytosis are unusual findings in the onset of a chronic myeloid leukemia.

Basophilia and progressive thrombocytosis suggest the disease progression to a accelerated phase or they notify the impending of a blast crisis. The natural progression of the disease, without treatment, includes two or three phases: the chronic phase, the accelerated phase and the blast or acute phase. Usually the accelerated phase is defined, according to the WHO criteria, as: over 20% basophils in the blood, a platelet count of over 1000 x 109 / L or unresponsive to threatment, 10-19% blasts in the blood or bone marrow, progressive splenomegaly and an increase number of white blood cells, uninfluenced by treatment and various citogenetic abnormality. It is said that the presence of the e6a2 BCR-ABL transcript, with a very aggresive clinical course, is related with marked basophilia. There are authors that say that the same proangiogenetic molecules (such as HGF - hepatocyte growth factor) are reliable for both the disease acceleration and the blast transformation of CML and that furthter studies of the 9q deletion are required.

For the risk stratification of pacients, several scoring systems are available. Two of these systems are Sokal and Hasford, and they allow the stratification of patients into risk groups: low, intermediate and high. A major critiria of the Hasford score is the basophilia.

We are going to present the clinical case of a young woman, which presented asymptomatic extreme thrombocitosys, of 2.700.000/µL, discovered by chance, with a blood smear that revealed basophilia of 60%. The cytogenetic studies performed subsequently confirm the presence of the Philadelphia chromosome in 100 % of the metafases analysed and the BCR-ABL transcript is majorly present.

The evolution of the patient, under treatment with thyrosis-kinase inhibitors, Dasatinib 50 mg, is a favourable one, the pacient having at the last clinical check-up, a platelet number of 95.000 / µL and a basophilia of 1%.

We chose to present this case beacause of the uncommon association, in the onset of a chronic myeloid leukemia, of both a marked basophilia and extreme thrombocytosis in a completly symptom free patient, and for the extremely prompt favourable response, undergoing treatment.

P19. IMMUNOSUPRESIVE TREATMENT IN APLASTIC ANEMIA-EXPERIENCE OF HEMATOLOGY DEPARTMENT OF IOCN.

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Background. Aplastic anemia is a clonal disease of stem cell characterized by peripheral blood pancytopenia with hypocellular bone marrow. In most cases, acquired aplastic anemia is an autoimmune, T-cell mediated disease.

Material and methods. We have enrolled in the study all the patients which were diagnosed with aplastic anemia, during their admission in the Hematology Department of "Ion Chiricuta" Cancer Institute Cluj Napoca, between 2004-2014. We have analyzed the next parameters: the age, gender, clinical manifestation of the disease, haemogram, bone marrow biopsy, kind of treatment, answer of treatment, adverse events and evolution of patients.
Results. The studied group was formed by 85 patients. The average age for the investigated patients was 46 years and there were 47 women and 38 men. The treatment consisted in ATG in 28 patients and CSA in 34 patients, with the response rate of 70%.

Conclusion. The response rate in this study is good, correlate with young age, absolute reticulocyte and lymphocyte count.

P20. THE EXPERIENCE OF HEMATOLOGY DEPARTEMENT OF COLENTINA CLINICAL HOSPITAL IN THE TREATMENT WITH VIDAZA.

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Introduction: the clinical studies demonstrated that treatment with hypometilating agents, 5-azacytidinne and decitabine, in intermediate/high

risk MDS resulted in complete cytogenetic responses even in cases with a complex karyotype. On the other hand, patients with AML who do not qualify for aggressive chemotherapy and allogeneic medullary transplantation, treatment with 5-azacytidine lead to transfusional independence and increase of quality of life in half of treated patients.

Materials and methods: We present the cases of 5 patients who received 5-azacytidine (Vidaza) treatment in our clinic between 2009-2014. All 5 patients are males, with ages of 56-84 years, 3 of them diagnosed with intermediate/high risk MDS, one with RAEB-1 and two with RAEB-2, and the other two diagnosed with relapsed/refractory AML-M4 FAB subtype. Cytogenetic exam was performed in three patients and a normal karyotype was obtained in all cases. A total of 29 cycles were administered overall, with a medium of 5.8 cycles. The selected schedules were 75, respectevely 100 mg/m2/d, 7 days, repeted every 28 days and 100 mg/m2/d, 5 days, repeated every 28 days.

Results: The first patient with RAEB-2 received 6 cycles of Vidaza, followed by transfusional necessary reduction, but persistent severe thrombocytopenia, and after 12 month from the end of therapy, the transformation into AML was registered and the patient died. The second patient with RAEB-2 received 14 cycles of Vidaza overall; after 4 cycles the evaluation showed cytogenetic progression, with a complex karyotype, including del(5q), but with a reduction of the percent of medullary blasts, a minimal reduction of transfusional demand, although with persistent severe thrombocytopenia; after 14 cycles of Vidaza the transformation into AML was registered and the chemotherapy was initiated. In patient with RAEB-1 after 3 cycles of Vidaza the hematological picture was almost normal. In the case of patients with AML treated with Vidaza, the first one have received untill today 3 cycles and he is presently transfusion independend, with a normal thrombocyte count; the second patient received untill today a single cycle of Vidaza and he is persistently pancytopenic.

All patients have had a good tolerance to therapy, without significant non-hematological adverse events.

Conclusions: The presented data indicate similar results to that in the literature, the most important effect being on the quality of life by the reduction in the transfusional demand. The hypometilating agents are a less toxic alternative to classical cytotoxic/antimetabolites agents, and their main mechanism of action is enzymatic depletion of DNA-methyltransferase and cell cycle exit of the malignant cells, irrespective of the mutational status of p53.

P21. **MYELODYSPLASIC / MYELOPROLIFERTIVE NEOPLASIA – A DIAGNOSTIC CHALLANGE FOR THE CLINICIAN.**

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Introduction: Myelodysplastic/ myeloproliferative neoplasia display combined dysplastic and proliferative features. The 2008 WHO classification of myeloid neoplasia included four entities in this category: chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia, BCR/ABL1 negative atypical chronic myeloid leukemia, myelodysplastic/ myeloproliferative neoplasia – unclassified, and a provisional entity, refractory anemia with ringed sideroblast associated with marked thrombocytosis (RARS-T).

Materials and methods: We present three cases of patients diagnosed with RARS-T in our clinic between 2012-2014.

Results: The first patient, a female 77 years old, presented with anemia, erythroid dysplasia and ringed sideroblasts at morphologic evaluation of bone marrow (BM). The BM cytogenetics revealed del(11p) and t(3;6) and the first diagnosis was RARS. After 4 months of evolution she presented with myeloproliferative features, including hepatosplenomegaly, leukocytosis and left shift to myeloblast, thrombocytosis. This picture led to JAK2 V617F testing which resulted positive; the triphine bone marrow biopsy showed large atypical megakaryocytes proliferation. Based on these new elements the final diagnosis was RARS-T.

The second case, a 89 years old male patient, presented with anemia and marked thrombocytosis; BM morphology showed erythroid dysplasia and the presence of ringed sideroblast;

BM cytogenetics revealed a normal karyotype, JAK2 mutation was positive and triphine BM biopsy revealed large atypical megakaryocytes proliferation and erythroid hypoplasia, which was a particular feature. The diagnosis in this case was also RARS-T.

The third case, a male of 75 years old, presented with mild hepatosplenomegaly, anemia, marked leukocytosis and left shift to myeloblast, erythroid and magakaryocytic dysplasia and the presence of ringed sideroblasts at BM morphology. The testing for BCR/ABL1 fusion gene resulted negative and BM cytogenetics failed because of absence of metaphases. The diagnosis at this point was MDS/MPN – unclassified. Later on progresive increasing thrombocytosis apeared and, consecutively, JAK2 mutation was tested, yealding a negative result this time. Yet, the diagnosis was also RARS-T.

Conclusions: We want to highlight the clinical, morphological, genetical and molecular diversity of this MDS/MPN entity, which could be explained by different molecular pathogenic mechanisms. The testing of different markers could be helpfull, one example being SF3B1 mutations that were demonstrated to play a pathogenic role in the apearance of ringed sideroblasts. The therapeutic means in this disease are quite limited. Hydroxyurea is a good cytoreductive agent, but with deleterious effects on anemia, Lenalidomide proved beneficial in some cases, but it is inaccessible to patients from our country. The identification of new molecular markers could be the source of new targeted therapies.

P22. **THE FREQUENCY AND PATHOGENY OF RENAL DISEASE IN MALIGNANT HEMOPATHIES**

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Aim/Objectives:Research of the prevalence of renal impairment in patients with hematologic malignancies, identifying the main pathogenic mechanisms, assess the impact of renal impairment on the evolution and prognosis of the malignant hemopathy.

Material and method: We analyzed the results of evaluation of the renal function in a group represented of 424 subjects diagnosed with hematologic malignancies in our clinic between 2008-2014: 231(55,8%) males and 183 (44,2%) females, aged between 33 si 92 years, mean age 65,3 years.

We investigated:
<the frequency of renal impairment in patients with cu Multiple Myeloma (MM) (80 subjects), Non-Hodgkin Lymphoma (NHL) (140 subjects), Acute Leukemia (AL) (17 subjects), Myeloproliferative Neoplasms (MPN): Chronic Myeloid Leukemia (CML) (22 subjects), Essential Thrombocythemia (ET) (30 subjects), Primary myelofibrosis (PMF) and Polycythemia vera (PV) (28 subjects), Myelodysplastic Syndromes (MDS) (15 subjects), Chronic Lymphocytic Leukemia (CLL) and Hairy Cell Leukemia (HCL) (62 subjects), Hodgkin Lymphoma (HL) (20 subjects).
<the type of renal impairment: tubular nephropathy (TN), glomerular nephropathy (GN), kidney malignant invasion, compression of the urinary tract and kidney vessels.

The impact of renal impairment on the evolution, treatment and prognosis of the malignant disease.

Results: The frequency of the renal impairment in the 414 patients was: 29,47% (122 subjects); the highest frequency was identified in the patients with MM: 48,75% (39 subjects), followed by AL: 47% (8 subjects), MPN (PV, MMM): 35,71%, ET: 26,66% (8 subjects) and MDS: 26,66% (4 subjects), HL: 25% (5 subjects), CLL: 22,58% (14 subjects), NHL: 22,14% (31 subjects), CML: 13,6% (3 subjects).

Of the 122 of patients with renal impairment, NT were found in 68% of subjects (83), NG in 23% of subjects (28), complex mechanisms (kidney localization of the disease, renal vein thrombosis, compression/urinary tract invasion) in 9% of subjects (11). TN had the highest frequency in patients with MM: 41.25% (33 of the 80 subjects with MM) and NG had high frequency in subjects with BH: 15% (3 out of 20 subjects) and in subjects with NHL: 7.1% (10 of 140 subjects.) The main etiopathogenic mechanisms identified were hyperuricemia 42.6% of subjects (52 of 122), hypercalcemia in 16.39% of subjects (20), serum

concentration and urinary lambda light chains (present in 60% of subjects with MM and NT)) and kappa (present in 40% of subjects with MM and NT), recurrent infections in 14.1% of subjects (17), autoimmune induced phenomena in 6.5% of subjects (8), cryoglobulinemia in 1.64% of subjects (2), recurrent urinary tract infections, antibiotic therapy and proapoptotic tumor lysis syndrome in 3.2% of subjects (4), tumoral invasion in 1.64% of subjects (2), compression of the urinary tract and renal vessels 7.37% of the subjects (9).

Conclusions : Renal impairment in malignant haemopathies has a significant frequency of 29.47%. Etiopathogenic mechanisms are the major metabolic abnormalities: hyperuricemia, hypercalcemia, tumor lysis syndrome, the presence of pathological immunoglobulin, autoimmune mechanisms, and infections, complications of the anti-infection therapy, chemotherapy, tumoral involvement of the kidney, compression/invasion of the urinary tract and renal vessels. Also we note the pathology associated with diabetes mellitus, cardiovascular disease and a mean age of 65 of the subjects monitored.

Biological weakening the patient with underlying comorbid chronic kidney disease, medication used, dose adjusted chemotherapy, antibiotics antibacterial / antifungal / antiviral have an adverse effect on prognosis of the haematologic disease. This context requires immediate correction of all potentially agressive mecanisms on renal function.

P23. ALLOTRANSPLANTATION OF HEMATOPOIETIC STEM CELLS IN A CASE OF CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) MYELODYSPLASIA STAGE IN TRANSFORMATION TO ACUTE LEUKEMIA.

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Chronic myelomonocytic leukemia (CMML) is a clonal disorder of hematopoietic stem cell characterized by the presence of an absolute monocytosis (> 1000 / mmc) in peripheral blood and the presence of myelodysplastic aspects and myeloproliferative in the bone marrow. Framed by WHO in the category of myelodysplastic syndrome / myeloproliferative neoplasm is the most aggressive myeloid cancer with survival between 14-24 months and 30% transformation in acute leukemia.

Treatment strategy is determined by the phenotype of the disease together with the number of blasts in MO. The only curative option for CMML there is the hematopoietic stem allotransplantation. HSCT should be recommended as soon as possible for young patients with aggressive disease and increased number of blasts in MO.

We present you the case of a patient aged 46 years who was admitted to the Center of Hematology and Bone Marrow Transplantation IC Fundeni in May 2013 having asthenia.

Laboratory examinations revealed: Hb = 7.g / dl, Ht = 23%, MCV 100 fl, Plt 241,000/µl
WBC = 7,790 /µl N 1% S 35% E 1% L 52% M 10%.

Bone marrow examination: aspiration - Mbl 10-11%; presence of dysplastic features (hypogranular granulocyte, megaloblastoid forms to the erythroid series).

Bone marrow biopsy - moderate granulocytic hyperplasia, rare groups of erythroblasts, small Mk with hipolobulated nucleus.

Immunohistochemistry stains of CD 34 + cells 12-14%. Karyotype - 6 metaphases 3 cells with chromosome 1 inversion; hyperdiploid with 84 and 99 chromosomes.

Between March and June 2013 requiring repeated hospitalizations for anemia related and requires repeated transfusions of packed red blood cells. Repeated full blood count shows a decrease in neutrophils and an increase in the monocytes (1400 / mmc), which was maintained > 3 months. Bone marrow aspirate from May 2013: 6-7% myeloblasts + 5-6% monocytic cells atypical (promonocytic).

Bone marrow biopsy - 12-14%. CD34. The patient was initially framed as RA with excess blasts in transformation.

The appearance and maintenance of monocytosis in peripheral blood and later on in MO led to a change of framing in LMMC-myelodysplasia form (FAB classification) and CMML-2 (myeloblasts + monoblasti + promonocytes) in the blood and MO <20% (WHO classification).

Treatment performed: chemotherapy (two courses' 3 + 7 " and 2 courses EMA). Each course was followed by long periods (30 days) of severe cytopenia feverish. Restoration of cytopenia occurred after each treatment with increased numbers of monocytes followed by their decrease and the recovery of the neutrophil amount. Immunophenotyping MO- July 2013: a population of CD45 positive cells, mean internal complexity (40%) expressing monocytic cell markers in various stages pathological maturation follows: CD117 positive (15%) of these CD177 + and CD34 + (10%), CD64 + and CD14- (8%), CD64 + and CD14 + (18%) the most likely co-expressed CD36, CD11b, CD300, CD4, CD56.

Conclusion: The proliferation of monocytic cell line with high percentage of immature cells. Cytogenetic analysis- review highlights t (1, 3). Molecular biology tests: FLT3, NPM1, E2A-PBX1, PML-RAR alpha, MLL-AF4, CBFb- MYH 11, BCR-ABL1, SIS-TAL, MLL- AF9 negative.
Allotransplantation of hematopoietic stem cells from unrelated donor in April 2014. The last control with CSH 100 % chimerism donor cells.

P24. MOLECULAR DIAGNOSIS IN MYELOPROLIFERATIVE NEOPLASMS JAK2 POSITIVE: EFFICIENCY OF DETECTION METHODS.

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V617F mutation in Januss kinase 2 (JAK2) gene is detected in more than 50% of patients with myeloproliferative neoplasms (MPN), such as: polycythemia vera, essential trombocythemia and idiopathic myelofibrosis. This mutation results in self inhibition of the JAK2 activity from cytoplasm, an enzyme with a role of signal transduction from growth factor receptors, resulting in increased cell proliferation in hematopoietic bone marrow and periphereal blood.

Numerous new molecular genetics methods are presently used for V617F mutation detection in JAK2 gene. Our initial implemented method used, Amplification Refractory Mutation System (ARMS-PCR), described by Baxter et al., has been re-designed and optimized in our laboratory for the concomitant detection of the mutant allele and the wild type gene. The detection sensitivity by this method is 1%.

In our laboratory were analysed 620 samples of periphereal blood from the patients diagnosed and ambiguous for MPN. Half of the positive samples were evaluated as low level positives. Mutant allelic burden is expressed as ratio of JAK2 V617F / JAK 2 wild type. A ratio of 1.5% or less is considered as negative result; a ratio between 1.5% and 5% is considerred ambigous; a ratio more than 5% is considered as positive result.
In some individuals are pressent some positive clones with JAK2 V617F, but these clones remain as a subpopulation of cells, because such clones do not have sufficient advantaje for growth and hence do not develop further.
The ambiguous results from these cases may be caused by:

- Previous cytoreductive therapy
- Presence of two MPN clones
- Presence of an alternative mutation in exon 14
- Presence of an hereditary polymorphism which affects the binding of ARMS primers.

These cases should be interpreted in a clinical context, considering other diagnostic criteria also.

Comparatively, for some low level pozitive samples, there was performed the mutation detection with JAK1 ACE Genotyping kit (Seegene) (sensitivity up to 10%), the results being „negative".

For confirmation, some low level pozitive samples were sequenced by Next Generation Sequencing (MiSeq - illumina) method with

sensitivity between 1 to 5%. The results were confirmed the presence of V617F mutation in exon 14 of JAK2 gene.

For a semiquantitative evaluation that can be approached in molecular monitoring of therapy, a high accurate and sensible method of detection – High Resolution Melting temperature (HRM) based system (i-densy - Arkray) was performed. This method performs extraction, PCR amplification and melting curves analyzes in the same reaction cartridge within 2 hours.

The results of this study approach provides useful information for the most advantageous methods in JAK2 V617F mutation detection for a high professional therapeutic decision.

P25. HEALTH TECHNOLOGY ASSESSMENTS IN HSCT–CENTRE OF BONE MARROW TRANSPLANTATION TIMIȘOARA

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Introduction: Hematopoietic stem cell transplantation (HSCT) is a complex and demanding therapeutic procedure, with numerous early and late risks , but life-saving, whit very large costs. Profitability and feasibility is dependent of many factors (type of transplant, age of patients, pathology, evolutive risk factors, but, also ,the size of the center, the annual number of transplants, etc.) and the economic potential of the country.
Objectives:
-The primary objectives were assessing results and determining the financial resources of HSCT
-Secondary end points refers to assessment of cost-effectiveness (average Cost-Effectiveness Ratio)(ACER) , report of incremental cost-effectiveness (incremental cost-Effectiveness Ratio)(ICER) and to minimizing the cost analysis
-Tertiary objectives: predicting the impact of hypothetical medical and organizational measures of cost-effectiveness of HSCT

Methods: The study was conducted on a sample of 51 consecutive patients transplanted throughout 2013, with the following distribution by age: 7 patients under 18 years, 4 patients between 18-26 years and 40 patients over 26 years.45 patients received autologous HSCT, 4patients –matched related HSCT HLA compatible,1 patient matched unrelated allogeneic HSCT HLA compatible and 1 patient matched unrelated HSCT HLA partially compatible .The pathology was: 24 patients with multiple myeloma, 11 patients with Hodgkin lymphoma, 9 patients non-Hodgkin lymphoma, 1 patient with nephroblastoma , 4 patients with AML, 1 patient with ALL and 1 patient with MDS.
Parameters considered targeted were health status, time to grafting, overall survival (OS), disease-free survival (DFS), event free survival(EFS), indication and complications like death and its causes over a period of one year under hospital conditions. Additional quality of life was evaluated (subjectively) byEQ-5D questionnaire and visual analogue scale(VAS).
Financial accounting data extracted from administrative service took into account the costs of medication

Results: Overall survival at 15 months was 80%, death occurred in17.6% of cases. The most important complications in the early stage were: mucositis , infection and graft versus host reaction, and in the late stage bacterial infections and viral reactivation.

The results of the economic analysis: the average cost of HSCT(autologous +allogeneic)and expenses for the first year, in Timisoara was 31.908$, and in the world36000-88000$ for autologous HSCT and 96000-204000$ for allogeneic HSCT . ICER was 11.020,35 $ and ACER was 10.730,86 $, entirely justifying the opportunity of TCSH in Romania.

Conclusions

1.TCSH was indicated in extremely severe pathological situations, malignant disease, life-threatening (in 88.88% with unfavorable predictive factors) proved to be a therapeutic method to saving 82.4% of patients.

2. Despite in the extremely protective measures (average care, nutrition therapy, complex antibiotic prophylaxis with bacterial, fungal and viral agents ,prophylactic replacement with Immunoglobuline)

evolution was charged with numerous(62.74% of patients in the early stage and 29,41% in the late stage) complications, especially infections (64.7% of them) fortunately controlled in the majority of cases (87.9%). Deaths (17.6%) were determined in55.56% of cases by a chemoresistant malignant pathology, relapse and disease progression or second malignancy.

SECTION TRANSFUSION MEDICINE
EDUCATIONAL SESSION

E1. CAUSAL RELATIONSHIP BETWEEN BLOOD COMPONENTS SELF-SUFFICIENCY AND THE SECURITY INSURANCE OF TRANSFUSIONAL ACTIVITY IN CTS BRASOV.

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The transfusion security represents all phases of logistical and biological trials of transfusion of blood and human blood components. The transfusion security according to the WHO can only be met through five essential conditions (steps): providing a structure and national organization; the blood donor must be volunteer and unpaid; tehre must be a thorough testing and processing of all units collected; the rational clinicall use of blood; quality management through the implementation of quality systems that aim at securing the transfusion process.

Self sufficiency is the balance between demand and production. At the same time there should be a perfect balance between the transfusional security and the transfusional self suffiency locally.

I have studied the statistical data gathered in Blood Bank Brasov over the past five years regarding the number of outlets of blood taken from new donors in and the current ones. In paralell, I have studied the request/supply of human blood components.

Blood Bank Brasov respects (going through the five stages of safety) but there are times of the year when we cannot provide human blood components than in a variable proportion to the medical services providers.

The difficulties that we encountered in the path of a source of safe blood can be the following: lack of a plan or a national transfusional policy, lack of organized blood transfusion, lack of a recurrent blood donors or the presence of unsure donors, not making a through screening of the blood donated, the lack of founds or lack of testing kits and trained staff. Self sufficency is sometimes hard to be accomplished: having in mind that only 17-18% of the people are blood donors and that requests for blood production are signifiant higher. Deficiency of certain blood components is ranging between 80-90%.

E2. RISC MANAGEMENT IN DONOR SELECTION, IMPORTANT STEP TO INCREASE TRANSFUSION SAFETY.

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Blood Bank Ploiesti

Introduction: The objectives of any organization requires knowledge and multiple risk-taking. Risk management is a cyclical process with several distinct phases: risk identification, risk analysis and risk response.

Material and Methods: Identify all possible risks is the starting point for correct selection of donors. This is done by the management team using execution experience with staff who identify all risks in the context of formal meetings. It starts with drawing up a checklist that includes risk potential sources of selection that could be produced primarily by execution personnel that could generate such risks. Also, most often, the risks of internal environment must be checked execution personnel who interact with donors, which often is insufficient (chronic lack of staff in recent years) is unmotivated by the remuneration granted in general and relative to the same category of staff from other medical facilities, is totally unprepared for the communication and relationship with the public, which has big expectations that many consider offering more and gets very little in return. Objectives set by the management team too large, relative to the ability and opportunity to harvest transfusion centers can be a significant risk in the selection of donors. In this context, the objectives of any management team too much blood centers, being in accordance with the demands of hospitals, but in total contrast to the facilities and the number of staff, it handles a permanent risk in the selection of donors, which is hardly managed without significant allocations funds. Risk externally imposed by legislation and new legislation appeared to be managed.

Responding to risk management team needs to be prompt and to intervene in or even eliminate risk mitigation can be achieved through a range of tools such as: Because many risks in donor selection are outside staff reaction to risk Manager must start the training of medical personnel who work with potential donors. Judicious redesign teams work in collaboration to be perfect, redesigning processes and circuits for increasing the efficiency and fluidity donor route, with a decrease times waiting by increasing the efficient use of space and equipment. If risks are related to the execution times of activities, their scientific programming can mitigate risk within reasonable limits. Aims too high in the continuing growth of the collection, constantly increasing demands imposed by hospitals have sometimes adjusted.

Conclusion: All measures taken to identify the sources of risk, risk analysis and reaction to the conclusion that, after all, the performance of the risk management process in the selection of donors, regardless of environmental conditions, is given by the quality Manager transfusion and medical staff involvement.

E3. **KEY ELEMENTS IN TRANSFUSION SECURITY: REAGENTS QUALITY AND THE TECHNIQUES USED IN RED CELLS IMMUNOHEMATOLOGY.**

S. Sirian
Bucharest

Immunohematological califications for the blood donors and for the blood compatibility are done by the immunohematological tests.

Imunohematological qualification is ensured by quality of reagents, techniques, as well as the specific methodology applied in each situation.

The imunohematological reagents must be in accordance with the characteristics and international and national rules laid down, and all of the techniques used must be validated for a reagent and for a special equipment.

The reagents used for both haemagglutination techniques in liquid phase and solid phase are those for the detection of erytrocyte antigens (0AB, RH s.a.), red blood cells for the group 0AB and for research of irregular anti-erythrocyte antibodies 0AB and reagents for making antibodies anti-A and anti-B immune.

The quality of immunohematology reagents may be or may become inadequate, originally from the producer, or subsequently by improper transport or storage, or obsolescence, by overcoming the Terms of Use. Therefore, these reagents should be initially confirmed by the producer and certified by a national or international institute, and then the user will perform validation receipt and then will conduct daily internal quality control.

E4. **EXTERNAL QUALITY ASSESSMENT SCHEME IN IMMUNOHAEMATOLOGY ORGANIZED BY EDQM/CoE 2013: ROMANIAN EXPERIENCE.**

Dobrota Alina Mirella
Regional Blood Transfusion Centre of Constanta

Introduction: EDQM department of Council of Europe launched in 2010 a pilot project on External Quality Control Scheme targeting blood establishment laboratories in Europe. The project was developed in collaboration with European Committee of Expert son Blood Transfusion. The project plan includes external quality schemes for serology, molecular biology tests and immuno-haematology. Initially limited to a maximum of 60 participating laboratories per exercise, starting with 2012 the access has been extended to 100.The participation is free, participants having to pay only the transport fee.

Material and method: Participation to an external quality control scheme is a mandatory requirement and a mean to evaluate laboratories performance, namely the competence of technicians authorized to perform those tests. During 2010- 2012 Romania did not participate in this program for financial reasons and accountability constraints. In 2013, National Institute of Transfusion Haematology supported the participation of six blood establishments to the external quality control scheme in immuno-hematology. This scheme included testing of unknown samples for ABO, phenotype RH / KEL and extended phenotype Duffy, Kidd, MNSs, irregular antibody screening and identification. All these tests are included in the immuno-hematology

testing algorithm set up by NITH; all reagents are available upon request.

Results: Final results for 2013 exercise are introduced and discussed.

Conclusion: Participation of the 6 Romanian blood establishments to the external quality control scheme provided an opportunity both for objective evaluation of their competence in immuno-haematology and of the managerial capacity to manage this activity.

E5. **INFECTIOUS RISK MANAGEMENT IN BLOOD TRANSMITTED DISEASES LABORATORY.**

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*Bucharest BTC, **NITH

Risk Area. Registration Office. BTD testing. Laboratory 1.

Objectives. Correct registration of pilot test tubes in numerical order, according to donation barcodes. Correct validation of blood units. Donors correct information concerning testing results. Fulfillment of tests validation criteria. Compliance to the temporary/permanent donor deferral criteria. Correct interpretation of tests results.

Risk Description. Dissimilarity between donor name and barcode. Doubtful blood unit validation. Person substitution. Microplates contamination. Erroneous donor deferral data. Incorrect validation criteria.

Favourable conditions for risk occurrence. Incorrect labeling of pilot tubes after blood collection. Incorrect tests results registration. Tests results releasing without ID identification. Failure of laboratory equipment. Insufficient data. Incorrect calibration of equipment.

Risk Management Officer. Head do Laboratory + Deputy

Risk Management Strategy. Risk removal.

Internal Control Means. Data checking within informatics system. Double-checking the reactive samples records. Donor license and ID verification. Checking laboratory equipment, calibration and pipette. Double-checking data prior to registration. Internal control.

Observations.

E6. **CHALLENGES TO MANAGE RISKS RELATED TO HOSPITAL TRANSFUSION ACTIVITY.**

Dobrota Alina Mirella
Regional Blood Transfusion Centre of Constanta

Introduction: Transfusion therapy is still an indication with no alternatives in many clinical situations. Prescribers still choose to excessivelly use transfusion therapy, even when less risky alternatives do exist. Despite all measures to increase transfusion safety, a residual risk which may not be reduced to zero still stands, widely recognized by experts in the field, seemingly ignored by users and unknown by patients. Unjustified indications of transfusion therapy add additional risks to the patient, partially predictable. Both the attending physician and the patient must assume the inherent and predictable risks, according to their own responsibility related to the indication or informed consent for treatment.

Material and method: The presentation aims to analyze the potential risks, inherent or predictable, that may arise in the course of hospital transfusion activity. Thorough knowledge of every phase of the hospital transfusion activity, both organizationally and as a medical act, enables identification of the risks and potential measures to prevent / reduce them.

Transfusion and haemovigilence Committee has the essential role in promoting this attitude , mainly if supported by hospital management. Although the importance of transfusion therapy is recognized by many clinicians, a persistent resistance is still percieved against assuming the active role they should play according to national standards. As a consequence, responsibility to ensure quality and safe management of transfusion therapy is transfered to nurses.

Results: Given the lack of adequate information dissemination between HBB, Transfusion and haemovigilence Committee and clinical services and the passive attitude of the treating physicians, most of the preventable risks are ignored, denied or unidentified. Additional risks are generated by non-involvement and disclaimer.

Conclusion: Risks of transfusion therapy should be known by treating physician and communicated to the patient before prescribing this

treatment, as the basis for an assumed therapy. Knowledge of risk provides an opportunity to use transfusion therapy more efficient by streamlining.

E7. INCIDENTS POSSIBLE IN BLOOD BANKACTIVITY.

G. Hanganu, M. Catana, D. Gheorghe M. Coman
Blood Bank Ploiesti

Introduction: Romanian word: "He who does not work any wrong" is quite true nowadays. It is widely recognized that in everyday life, man is fallible. The desire for self-improvement of any organization makes any implemented quality management system, to be inserted into a general procedure for registration and resolution of errors / nonconformity, a general procedure for undertaking preventive and corrective actions.

Observation and analysis of systematic errors / nonconformities in an organization, awareness of all activities sensitive critical points in the process, awareness of errors that can occur during activities of daily medical attention, executive staff on errors that may occur in a work, determine significantly reduce the number of errors / nonconformities.

Material and Methods: Full transfusion center staff has a responsibility to report and record incidents occurring in daily activities. Prevention of unwanted incidents as preventing their recurrence in CTS work, recording and analyzing their periodical meetings is a way of improving operational work a transfusion center, with the ultimate goal of increasing the safety of transfusion. Have been recorded since 2012, all incidents occurring in the transfusion chain, which were observed by staff or executive staff. These were analyzed and remedied quickly, at the time of or reviewed periodically, depending on their impact on business, to avoid repeating them.

Minor incidents can occur without impact on transfusion safety but major incidents can occur that affect the quality of blood products and transfusion safety so. After notaries systematic incidents occurring in everyday life, and after examining the processes in the blood Ploiesti, observing all the critical points, we made a schedule unwanted incidents that if we study and acknowledge them as possible to occur in some ways, we have a good chance to avoid them, to proceed in such a manner as not to produce, and to assure the quality of blood products.

Results: We identified 97 possible incidents that may affect the quality of blood products center. We classified the business sectors and we discussed with staff to try to work in such a way as to avoid them.

Conclusions: Awareness that can go wrong, identification of critical points in the system do to work responsibly, taking all measures to avoid mistakes that can affect quality of care.

E8. THE INFORMATION LEVEL OF THE MEDICAL STAFF IN THE HEALTHCARE FACILITIES OF BRASOV ON THE HEMOVIGILANCE.

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The Blood Transfusions Center of Brasov

Introduction: Hemovigilance resides in the detection, gathering and analysis of all information on the unwanted and unexpected effects of blood transfusion.

In Romania, the law on the implementation of the hemovigilance system was adopted in 2006. As such, the Ministry of Public Health drafted the Norms on the organization of the hemovigilance system and traceability ensurance, the same as the regulation on the system of registration and reporting of incidence and side effects related of blood and human components collection and administration. Subsequently several legal amendments were made according to the European laws.

Objective: appreciation of the information level of the medical staff involved in the application of the hemovigilance procedures

Materials and methods: The research instrument that we used was the questionnaire. 35 medical stuff of different levels of education, the personnel employed in the blood transfusion units within the hospitals in the clinical departments using transfusion treatment and the within the Blood Transfusion Center of Brasov answered the questions of the questionnaire.

All the aforementioned classes of personnel are involved in the application of the hemovigilance procedures.

Results: the results of the questionnaire

processing are presented in detail in the paper.

Conclusions: although all the classes of medical staff analyzed according to the questionnaire have a certain level of knowledge, we noticed a slight differentiation depending on the specificity of the healthcare field the hemovigilance procedures are applied to. The level of information in the field of those involved in the hemovigilance network must be permanently improved. Together with pharmacovigilance, hemovigilance must be considered as part of the general vigilance in the health field.

E9. MAINTAINING QUALITY DOCUMENTS IN BRASOV BLOOD TRANSFUSION CENTER.

A.Munteanu
Blood Transfusion Center BRASOV

Introduction. Complying with the requirements of EU legislation, BTC Brasov, like each blood transfusion center in the country, had to realize and maintain a quality system based on the Guide on Good Practice of Directive 2003/94/EC and meet the requirements specified in Directive 2005/62/EC, transposed in our Law 282/2005.

The quality system is very important to ensure the quality and safety of blood components produced and supplied to hospitals and to ensure the safety and satisfaction of blood donors. To cover all activities that influence the quality of blood components produced by BTC Brasov, it was necessary to create rules, objectives and responsibilities clearly defined and to apply them through planning, quality control, quality assurance and continuous improvement.

Working method. This system has always tried to ensure that, if possible, the processes taking place in the BTC Brasov are specified in appropriate instructions, that they are carried out according to the principles of good practice and they respect current standards, domestic and international.

A representative of Quality Management has been appointed, in 2007 (RQM).

This, along with others in Brasov BTC drafted Standard Operating Procedures (SOP) that describe in detail the work of each department, General Procedures (GP) which establish rules of conduct and activities available for the whole institution, and all other internal documents of quality. All documents produced were recorded annually in the document control register.

The document control system involves also reviewing all documents, periodically, once a year, or by necessity when working procedures changes occur due either to change the law or to the acquisition of new equipment. Thus, in 2011, in the Laboratory of Biochemistry a change for all SOP has been required, due to purchase Vitros Biochemistry Analyzer and Hematology Analyzer MEK-6400.

In the Quality Control Laboratory, also due to purchase new equipment (Hematology Analyzer MEK-6400 and Bacteriology Analyzer BacT/ALERT 3D) all the control procedures were completely changed, both haematological and bacteriological control procedures.

The need to draft new operating procedures permanently appeared, due to trying to improve and diversify the blood testing methods, for example in the Laboratory of Immunohematology, where we introduced new techniques of donors extensive phenotyping.

RQM kept, in electronic format, all internal quality documents, quality control registers, year by year, records of reviews, year by year and all archiving documents.

Also archived in electronic format and on paper, all quality documents that are out of service, for a period of 15 years, according to the regulations.

Conclusions. All theese provide good traceability, according to the EU requirements. We tried that documentation is accessible to all (each containing a distribution list) and be drawn as clearly, while respecting data protection laws.
The BTC management always checks RQM activity, there is also a permanent information, consultation and cooperation, in both directions, as a mechanism for evaluation and continuous improvement. Where appropriate remedial measures are applied.

SECTION TRANSFUSION MEDICINE
ORAL PRESENTATIONS

C1. AVAILABILITY AND IRRESPONSIBILITY IN BLOOD DONATION.

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Bucharest Blood Transfusion Centre

"There is no substitute for blood, and somebody is in need for it every three seconds"
In accordance with the blood transfusion law, any person aged between 18 and 65 years, in good physical and mental condition is eligible for blood donation. Apparently, these criteria should give to a large part of population the opportunity to contribute to save the life of another human being. In fact, meeting the hospitals constant demands and ensuring blood sufficiency is a challenge for every country.

In Romania, the blood donors represent only 1,7% of population , in comparison with 10% in Denmark, 9% in United Kingdom, 8% in Nederland, 6% in Germany or 4% in Hungary. Thus, in our country we succeed to fulfill only 65% of the blood demands. Polls made during The Blood Donor Day revealed that 12% of population is donating blood for material purposes, 44% for friends or relatives, and 44% by their own conviction.

Regardless the motivations, any person willing to become a blood donor should comply not only with medical criteria, but also with the moral ones, meaning that one should observe a healthy of life in every way, which involve many qualities, such as morality, responsibility and desire to abide the blood donation procedures and regulations.

If the potential blood donor has an inappropriate moral conduct, neglects the blood donation rules, or has some other ulterior motives, that will induce a risk for both blood safety and donor's own health.

The expertise of some advanced countries shows us that our goals – safe blood transfusion and sustainable national transfusion system – could not be reach without the promotion of 100% volunteer and unremunerated blood donation, which has crucial role in this activity.

C2. CURRENT POLICY AND NEW STRATEGIES IN BLOOD DONATION PROMOTION.

F. Neagu, C. Ruxandra, D. Gosa, M. Popa, I. Cristea
Bucharest Blood Transfusion Centre

While some countries have solid transfusion system, based on 100% volunteer and unremunerated blood donation, some other countries, including Romania, are still depending, in different degrees, on blood donors' recruitment among patients' relatives/friends or among persons interested by material benefits. Even if the majority of population is aware that there not enough blood donors, the percentage of blood donors is only 1,7. The main reasons for refusing blood donation are the lack of spare time, fear of contracting a disease, the absence of adequate blood collecting facilities.
The Romanian legislation stipulated that "blood is a national resource; blood donation is a volunteer and non-remunerated act". This law also stipulates that the blood donor biological balance should be restored after blood donation. In this respect, the blood donor is provided with vouchers, in order to buy food and non-alcoholic drinks, fact that could induce the false impression of some remuneration. The challenge for Romania is to create the proper conditions for blood donation promotion and healthy and 100% non-remunerated blood donor recruitment, main elements for a stable and safe blood collection, as well as for a sustainable national transfusion system.

The World Health Organization and International Red Cross established a global agreement purposing the worldwide implementation of volunteer and non-remunerated blood donation, involving political and medical regulation at national level. In accordance with these regulation and in respect of the Romanian Law, the Bucharest Blood Transfusion Centre initiated new strategies in blood donation promotion an blood donors recruitment.

It is beyond any doubt that the transfusion system is an important part of any efficient medical system and should be based on an extended blood donation promotion and blood donors recruitment.

C3. **ASSESSMENT OF HIV, HCV, HBV VIREMIA IN SEROPOSITIVE BLOOD DONORS; IMPACT ON DONATED BLOOD NAT SCREENING STRATEGIES.**

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Background: Nucleic acid amplification techniques (NAT) has been gradually introduced worldwide, as a complement to serological testing aiming at reducing the residual risk of TTI (Transfusion Transmitted Infections), due to serological window or silent chronic infections. Triplex or duplex testing systems are comercially available and testing is performed either on ID (individual donation) or in minipools (MP) of 6 to 96 donations, according to national plolicies. Variable yields of NAT on serologically negative and serologically confirmed positives were reported for epidemiologically diverse areas, resulting in important differences in yield of window phase and occult infections. In Romania, blood donation screening for TTI relies on serological methods and previous occazional detection of viremic seronegative donations points to the need of introducing NAT. Local prevalences and levels of viremia among seropositive blood donors would contribute to establishing the pool size for NAT screenig. We report here the results of NAT testing on serologically confirmed donations.

Methods: Plasma samples from anti-HIV(201), anti-HCV(408) and HbsAg(366) confirmed donations were extracted with EZ1 DSP Virus Kit (Qiagen) and amplified with Arthus HIV, HCV and HBV kits (Qiagen) for viral load quantification.The LODs95% of the tests are 67, 34 and 3.8 IU/ml respectively. All samples were serologically confirmed by Inno-Lia Score(Innogenetics) for HIV and HCV, and by a confirmatory HbsAg assay, anti-HBc, HBeAg and anti-Hbe for HBV infection. For HCV positive samples with full developed serological profiles(316) and weak positive samples (92) with reactivities towards two different HCV antigens were tested.

Results: HIV-1: 198/201samples were had detectable RNA within a log c range of 1.7-7.4, except for one case which was repeatedly detectable under the LOD 95%. 91.9% of viral loads were over log 3.0 and 8.6% were over log 6.0, cumulating 41.2% of the recent infection cases. 3 seropositive samples were not detected by NAT, either due to the limits of the test or to originating from so called „elite controllers". HCV: Among the positive samples 71%(223/316) were detected, an yield comparable to those reported for the european countries, with 97.3% of viral loads ranging from log 3.0, and 31.4% over log 6.0. Only 4.9% were under log 3.0 and one repeatedly reactive under LOD 95%. For the weak positive samples only 8/92 were detected. HBV:90.2% of samples were detected with only 33.3% diplaying viral loads over log 3.0, and 11% repeatedly detectable under LOD 95%. 184/330(55.8%) of cases had viral loads between the LOD 95% and log 3.0, specific for the asymptomatic carriers, in contrast to 44/47 cases with viral loads over log 6.0 which were also HbeAg positive.

Conclusions: The local prevalences of blood transmitted viruses and the detection by NAT of HIV and HCV positive window-period donations during the last two years indicate that further reduction of the residual risk of TTI would occur only through introducing the NAT testing of all donations together with improving standards for donor selection. The adequate size of MPs has to be considered based on the distribution of viral loads in seropositive donors and the impact on the blood unit validation process. On the other hand,the existence of NAT negative HBV and HCV serologically positive blood donors has been reported worldwide, indicating that serological screening must be maintained evan with the most sensitive NAT performed on ID

C4. **ATYPICAL RESULTS IN SEROLOGICAL TESTS IN A CASE OF DE NOVO HBV INFECTION AFTER LIVER TRANSPLANTATION.**

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Background: The serological paterns that allow the diagnosis of HBV infection and the assesment of its clinical course are well established but, occasionally, atypical serological profiles are generated as the dynamics of the expression of viral proteins and the corresponding antibodies may vary during the infection natural course. Among the factors that can interrelate and may generate such uncommon serological profiles, infection with viral variants and/or host related factors(immune tolerance, cellular imune response and immunosupression) are frequenly reported. We present here the serological findings in a case of HBV infection after liver transplantation, in a HBV „naive" patient.

Methods: Four serum and plasma samples collected from the pacient prior to transplantation (1995-2012) were available and previously found negative for HBV and HCV. 11(I) and 16(II) months after transplantation serum and plasma samples were collected to determine the CMV status as suspicion of CMV infection/reactivation or rejection resulted from increasing ALT /AST levels, and subsequently immunosupressive therapy enhanced. Follow up samples were then collected at 18(III),19(IV) and 21(V) months after tranplantation. The pre-transplantation samples and the 1st(I) sample were initially tested for HBsAg EIA(EnzymeImmmunoAssay), anti-HBc, anti-HBs, anti-HCV, CMV-IgM and IgG antibodies and EBV VCA-IgM and IgG antibodies. On sample(I) CMV and EBV DNA were determined. The follow up samples were additionally tested for HBeAg, anti-HBe and HBV DNA.

Results: All the pre-transplantation samples tested negative for HBV and HCV markers and positive for CMV-IgG, EBV VCA-IgG and EBV EBNA-IgG. Sample (I) was negative in the curent EIA for HBsAg and anti-HBc and had the same serological profiles for CMV and EBV as the previous samples. CMV DNA was negative, but a weak EBV viremia was detected(132gEq/ml) as a possible result of EBV reactivation under immunosupression. Sample (II) was negative in the current EIA for HBsAg but positive for anti-HBc. Additional testing showed a high level of HBeAg, HBV DNA(log c=7.42IU/ml), HBsAg(1.0 IU/ml) in quantitative CLIA and a weak positive result in alternative HBsAg EIA. Retrospective testing of sample(I) showed lower HBeAg, HBsAg CLIA(0.31IU/ml), borderlier reactivity in alternative HBsAg EIA and high HBV DNA(log c=6.36IU/ml), characteristic of acute infection HBV. Initiation of antiviral therapy led significantly decreased the viral load over the next four months,down to logc=1.36IU/ml and to negative HBsAg and HBeAg.

Conclusions: We have confirmed a case of HBV acute infection with atypical reactivities in EIAs currently used for HBsAg screening. Low reactivities or lack of detection by such tests, with demonstrated sensitivities of less than 0.05IU/ml, despite the high levels of viremia are usualy indicative of viral variants, generated by mutations within the „a"determinat of HBsAg, which represents the very target of HBsAg screening tests. „Occult B infections" characterised by viremia together with anti-HBs and lack of detectable HBsAg, distinct from „negative window" cases are more frequently reported since the introduction of NAT screening of blood donations. Since for the reported case no other rick factors could be determined besides the graft and the blood products tranmission by transplat/tranfsusion cannot be ruled out and the donors should be investigated.

C5. **THE VALUE OF ANTI-HLA ANTIBODY DETECTION IN STEM CELL TRANSPLANTATION.**

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Introduction: One of the major factors that contribute to the successful of stem cell

transplantation is based on good HLA matching 10/10 compatibility between recipient and the donor. The number of patients, who received HLA mismatched stem cell transplantation significantly increased, including unrelated donors.
Recent studies suggested that the presence of donor specific HLA antibodies (DSA) in patients is associated with graft failure in HLA mismatched stem cell transplant.

Aim: The present study was to demonstrate if it is necessary to have our national strategy for determination of anti HLA antibody in patients who receive a mismatch stem cell transplantation. If the patients are antibody positive is good to avoid donors who possess the target Ag of HLA antibody,

Cazuistry: The authors presents four cases of patients with acute leukemia who are waiting a stem cell transplantation from unrelated donors. All cases had 1 class I mismatch between the recipients and the donor. The sera of the patients were analyzed for class I HLA antibody detection by Luminex bead technology, including mixed beads and single class I beads.

Conclusions: The results demonstrated the all the recipients sera had no donor specific antibody (DSA) against the mismatches of the donor. The authors discussed the possible impact of HLA specific antibody in patients sera.

The future studies try to establish the value of this antibody, as a routine test before transplantation.

C6. **THE IMPORTANCE OF CORRECT BLOOD TYPE.**

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The Blood Bank Ploiesti

Introduction: In over 100 years since the discovery of epochal transfusion practice, the physician Karl Landsteiner, correct blood type remains a topical issue.

Material and Methods: Case presentation: A 64 years women is hospitalized 20.09 in hospital SM for: malaise, asthenia and fatigue intense, marked pallor without bleeding syndrome. Laboratory examinations show: important inflammatory syndrome, severe pancytopenia, and hyperglycemia. On peripheral blood smears are seen with difficulty because of severe leukopenia few blasts. Biological values: Hb -5.1 g / dl, WBC - 1870/mmc platelets - 49.000/mmc, ESR -150 mm/1h, bilirubin -1 mg / dl, Fe - 309 mg / dl, urea - 35 mg / dl, creatinine - 0.6 mg / dl, ALT 42U / L, AST 31U / l, GGT-88U / l, total protein 6.6 mg / dl, blood smear shows: wrapping RBC, WBC: Bl 3%, S 50% E 2% Lf 43%, 2% Mo, EBL 4/100 elements, blood group AB +.

After consulting your doctor decides administration treatment with vitamins and red blood cells to treat anemia. It manages four CER.UA group AB +. Patient's condition does not improve, anemia is corrected, taking into observation the diagnosis of acute leukemia, the patient is transferred after 10 days of hospitalization Fundeni Hospital for further investigation.

On 23.10. patient is in hospital hospitalized J.N. the diagnosis of multiple myeloma, severe anemia. After clinical and biological evaluation of anemia observed due administration of packed red blood cells is decided. Pre-transfuzionale samples, group and Rh is done and it is found that the patient is B +. It manages two CER.UA B + despite the fact that the patient support group is AB + blood because it received the previous hospitalization, group AB + red blood cells. Analyzing blood demands we found that patient received nine waxed B + in JN Hospital, correction of anemia patients is a major challenge.

Results : The retrospective investigation we found that the first hospital admission in MS in UTS, carrying type blood was incorrect. Assistance from UTS conducted blood type Beth Vincent only method that showed group AB + because poliaglutination caused by myeloma UTS staff considering that only blood typing method Beth Vincent is sufficient. In subsequent discussions, we found that it was interpreted that the patient entered the hospital with Hb 5.1 g / dl and he was discharged with the same value in spite of 4 units of packed red blood cells received during the 10 days of admission. The fact that red blood cells were arranged in stacks, blood smear examination did not cause reevaluation blood group. Showed no symptoms inviting a noisy and incompatible transfusion has left serious consequences.

Conclusion: Making correct blood type remains a goal for many of the establishments in

hospitals. Preparing staff working in UTS is the first step in achieving transfusion safely. But the starting point in ensuring the safety of transfusion physicians we are training coordinators UTS.

C7. THE IMPORTANCE OF ERYTHROCYTE ANTIGENS A1 AND A2 IN TRANSFUSION CENTRE AND HOSPITAL.

G. Hanganu, D. Gheorghe, M. Catania, M. Coman
The Blood Bank Ploiesti

Introduction: Chemical erythrocyte ABH antigens has been a concern of immunology to the discovery of blood types, but was elucidated between 1960-1975. Though far discovered, the existence of two different antigenic structures with high applicability in transfusion practice, has still appropriate priority in the study of blood types. Material and Methods :Given these data, we tried to identify the existence of subgroups A1, A2, A1B and A2B in Ploiesti CTS donor population, and labeled as such blood products.

Over a period of three months, for a total of 1736 donors were identified in 1479 Group A Group A1 donors, and 297 donors with group A2 (A2 percentage being 17% of all donors A or otherwise computing A2 represents 20% of A1). 2 donors were identified antiA1 antibodies. The donor 320 of AB, A2B was 66 (20%). 2 A2B donors were identified antibodies A1. The products were labeled as such blood, plasma bags A2 and A2B anti-A1 antibody has been rebooted. Following the systematic determination of A1 and A2 subgroups we found that we fit the ratio between subgroup A1 and A2 in the data described in the literature up to 20%.

The usefulness of our approach would be full if and hospital establishments and would make identification of the two antigens and recipient patient.

Case Presentation: Patient 57 years with gastric bleeding esophageal varices and a history of gastrointestinal bleeding admitted, hemoglobin 6.5 g / dl, and requiring administration of packed red blood cells. Prescriber indicates transfusion 2 CER.UA. Pre-transfusion blood samples for determination of type is done both in the laboratory and in the hospital blood transfusion unit. In laboratory and UTS same difficulty occurs through blood typeping discrepancy between globular and the serum sample and send the sample to elucidate the CTS.

When CTS is performed by Beth Vincent and type blood by Simonin, using test red cells A, A1, A2, B; reagent agglutinates erythrocytes patient's anti A1. We conclude that it is a blood type A2 with anti-A1 and A2 send two compatible CER.UA who are transfused without problems. From the patient's transfusion history, we find that it has received in previous hospitalizations, erythrocyte mass 3 times in the last two years. We suspect that the number of units (80%) were immunized by the group A1 and the receiver.

Conclusion: Consider advisable systematic research in CTS sites A1 and A2 antigens, and UTS-A2 or A2B sites where recipients can be immunized by transfusion A1 or A1B. It is important to perform serum sample in both CTS and UTS RBC sites A, A1, A2, B, to increase safety transfusions.

C8. AN INHERITED RARE CDE HAPLOTYPE.

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Background: The Rh system is one of the most complex human blood group system. The Rh antigens are encoded by a complex RH locus wich has been located on the short arm of chromosome 1. Fisher-Race theory explains the transmission of the Rh characters in bloc, by a set of three closely linked genes, and Tippett's theory, validated by molecular genetic studies, shows that the RH loci of human genome consists of two closely linked homologous genes RHD and RHCE, which transmit the RhD and RhCCEe characters.

But for interpreting serological data, the Fisher-Race theory is still appropriate. The three pairs of alleles can comprise eight haplotype, which can form 36 different genotypes. From these genotypes only 18 different phenotypes can be recognized by serological tests with appropriate reagents.

Aim: Serological testing in a family for the evidence of the inherited rare CDE haplotype.
Subject, reagents, methods: A Caucasian male

immunological tested for the target donation for his wife. The same character in AB0, Kk, MN Ss, Pp systems between wife and husband. In Rh system was discordance between the two subjects: wife's phenotype was CcDee (32% in caucasians) and husband's phenotype was CCDEe (0,2% in caucasians).

Serological investigations in husband family releved CDE rare haplotype along 3 generations(grandfather, son and one of the nephwes), and consequently, the CCDEe phenotype.

Conclusions: The CDE haplotype is rare haplotype (second cathegory with under 1% frequency), resulting from "cross-over" between C and D factors of type 1 haplotypes. In our study it was transmitted along 3 generations resulting in three members CCDEe rare phenotype (0,20% frequency in caucasians). This situation for the subjects is very important in transfusion.

C9. COMPARATIVE STUDY ON IRREGULAR ANTIBODIES DETECTED IN MICROPLATES (DIAGAST) AND CASET (DIAMED / ORTHOBIOVUE) IN CTSMB.

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Lab.Imunohematologie C.T.S.M.Bucarest Roumanie

Objectives: The aim of this study was to monitor and to compare the different technique to detect irregular antibodies

Material/method: Equipments: fully automated system Qwalys (Diagast), Ortho BoiVue System, Dia Med System.
Antibodies screening on Qwalys:
Was used TCI with Erytrocytes Magnetised Technology (Diagast brevet) precoated microplate with human antiglobulin anti –Ig G and NanoLys (creates a density barrier between the serum and the AHG) and Hema Screen (magnetized red cells panel)
Antibodies screening on column
It was used Enzymatic test with hemagglutination micromethod in column (The Ortho BioVueSystem utilizes column agglutination technology comprised of glass beads contained in a column and Dia Med System utilizes agglutination in gel) as well as specific panels enzyme treated . Agglutination is represented by red blood cells retained on the suport .

It was used comparison from annual centralized data, archives data study and informatic sistem.

Results: 45 716 samples were processed during the year 2013 and 21,82 % cases were detected positive DAI Rh antibodies 45.04%, 24.78% other systems, 30.18% unidentified

Conclusions: To ensure the safety of transfusion it is recommended to utilised Coombs test and the Enzyme test in the detection of irregular antibodies.

C10. IMMUNOLOGICAL STATUS OF PATIENTS RECEIVING MULTIPLE TRANSFUSIONS IN MUNICIPAL HOSPITAL HATEG.

V. Halmagi*, C. Bichis**, D. Horvat**
* - DEVA BTC, ** - HUNEDOARA BTC

Introduction: Transfusion safety
Before you decide to support a patient's transfusion history should be investigated prior transfusion and getting file (immunological status of the patient).
Before a blood product transfusion is imperative to check:
-Concordance between patient identity on the product label and identify patient transfused
- Compatibility immunological product
- Date and time on the label and their compliance
- The integrity of the packaging (bag)
- Product appearance (color) and red blood cell concentrates aspects of coagulation and hemolysis
-No other product (drug or solution) should not be placed in the bag of blood product

Purpose, material and method: If the patient is known as politransfuzat shall be taken prior to transfusion test R.A.I. (And possibly Ac Anti-HLA) and we transfuse red blood cell concentrates izogrup. Test compatibility of donor red cells and recipient serum should be performed in all cases by the presence of irregular erythrocyte antibodies in patients who may develop hemolysis, and in all cases where a patient to be transfused.

In this paper I study the immunological status of many patients transfused, some politransfusions, Hateg Municipal Hospital.

Conclusions: Allogeneic transfusion is one of

the most common lifesaving procedures. It is, however, a dangerous therapy that is associated with severe complications, some fatal.

The most severe transfusion reactions are immunological fracvenses.

They are triggered by the incompatibility of the different blood group systems. Thus, they can occur against erythrocytes (hemolysis intravascular or extravascular), leukocytes, platelets, immunoglobulins (Ig) or other plasma antigens. Therefore, transfusion is under strict control biological and immunological compatibility.

C11. **CLINICAL CASES OF TRANSFUSION DEADLOCK RESOLVED AT THE DEVA HUNEDOARA CTSJ.**

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BTC Hunedoara-Deva

Introduction: The notion of deadlock transfusion relates to:
- the risk of reaching a blood transfusion refractory status making therapeutic act ineffective or
-occurrence of transfusion reactions may even have serious consequences for the patient.

It is a rare but not exceptional as frequently encountered to the pacient with multriple transfusions, multiparous pregnant women or patients with certain pathologies.

If the main cause is the order Immunohematology, the complexity of this phenomenon comes from the fact that it is very hard to find, then, a labile blood product compatible with the recipient of immunologically.

Purpose, matherial and method: Risk management deadlock transfusion

The interface between the prescriber, the doctor responsible for the UTS and the CTS (Blood Transfusion Centre) is fundamental to risk management impasse transfusional.The specific management of transfusion risk is restricted to patients requiring transfusion therapy in a time. You must follow the patient continuously from the correct indication of labile blood component.
The paper described three cases of deadlock resolved transfusion at our center.

Deadlock transfusion could be generated by several situations:
a) autoimmune hemolytic anemia
b) rare blood group
c) statements of comprehensive immunization
d) patients with immunoglobulin shortage
e) patients with malignancy

Conclussions: Transfusion AHAI can be problematic because the presence of autoantibodies in serum free or erythrocyte surface interferes with achieving pretransfusionale routine tests such as erythrocyte antibodies detection, extensive phenotype, compatibility testing direct and these must perform additional tests to transfuse the patient.

The main risk for transfusion in autoimmune hemolytic anemia, especially in the warm autoantibody is potential masking of interest transfusion alloantibodies by autoantibodies, the latter often recognizing the increased frequency red cell antigen.

C12. **TRASFUSION COMPATIBILITY IN MULTIPLE RED CELL ALLOIMUNIZATION**

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Background: Posttransfusional antierythrocytic alloimunisations depend on pacient's immunological status, antigen's immunogenicity and on the number and frequency of transfused unities. The presence of multiple alloantibodies is most frequent in subjects undergoing a chronic blood transfusion and less frequent in unique massive transfusion or in transfusion for a special, acute event. For these pacients is extremely complicated to find compatible red cells.

Aim: To perform the various immunohematological tests and methods for the selection of compatible red blood cells for the multiple alloimunised patients.

Materials, methods: Reagents for the hemaglutination in liquid phase and in column, at 40C, 220C, 370C, in saline with enzymes (papain, ficin, bromeline) and with antiglobulinic serum.

Case reports: There are some patients with multiple alloimunisations as: 1) anti c + Cw + s + Cob ; 2) anti Jk a + Lu a ; 3) anti S + Kp a, 4) anti E + Fy a + Le b; e. all. We present the techniques for the

establishment of specifities, clinical significance of the alloantibodies and the selection compatible blood.
Conclusions: The presence of multiple antibodies in a patient is a problem, because it is extremely complicated to find compatible red blood cells, in order to prevent some acute or retarded haemolytic transfusion reactions.

C13. **STEPS IN COMPATIBLE RED CELLS SELECTION IN HAEMOLYTIC AUTOIMMUN ANAEMIA.**

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Background:The Autoimmune Haemolytic Anaemia (AIHA) is an acute anaemia produced by the action of auto blood red cells antibodies. In acute hemolysis, only survival therapy is blood red cells transfusion. Because the large specifities of red cells autoantibodies (f. ex. a e, a I) it is very difficult the selection of compatible blood units.

Aim: Showing the steps, methods and techniques necessary for the selection of compatible red blood cells in an AIHA case.

Materials, methods: Reagents and techniques for in the liquid phase and in column haema-glutination, at +40C, +220C, 370C, in saline, with enzymes (bromeline, ficine) and with antiglobuline serum.

Case, report, results, conclusions: 84 years old pacient with acute anaemia (Hb 6,5%) All immunoserological tests showed poliaglutination, DAT positive. We performed all the steps recomanded in AIHA, on pacient's red cells and on his serum, for the correct phenotyping and for the establishment the type of AIHA. Finally the Pacient 0 blood group, ccdeekk RhK phenotype, DAT type C3d, cold aglutinius, titre 1/1024. For the red blood cells, the serum was prewarmed and diluted at ¼ in saline.

The appropriate izo group and RhK units were selected by IAT and saline technique, with special pretransfusion recomandations for the hospital physicians.

C14. **TRANSFUSION REGIMENS IN MAJOR THALASSAEMIA- NITH EXPERIENCE.**

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Background: Basic treatment in thalassemia major is the regular blood transfusions throughout life in parallel with iron chelation therapy. Current transfusion schemes for thalassemia major have main objective adequate suppression of bone marrow hyperactivity. There are: 1) super-transfusion regimen maintaining pretransfusion Hb over 11g/dl, 2) hipertransfusion regime with pretransfusion Hb values over 10g/dl and 3) moderate transfusion regimen with values between 9 and 10g/dl.

Aim: Analyses of blood transfusion regimens for major thalassaemia patients in NITH Subjects There are a number of 100 patients with β thalassemia major registreted at NITH level, aged 4 and 46, in chronic transfusion every 2-5 weeks. Our practice involves transfusion of leucodepleted red cell concentrates (pre-storage filtration) extended RhD-Kell phenotype, before each transfusion being performed cross-match tests and screening new irregular antibodies.

Most patients are in moderate transfusion regimen, maintaining pretransfusion Hb values over 9g/dl; it allowing normal growth and development of the body, conducting of regular physical activity and proper suppress bone marrow activity. There are also a number of patients (20-30%) who are rather in an undertransfusion regime with mean pretransfusion Hb about 8g/dl.

Conclusion: Optimal transfusion regimen is one that can correct severe anemia, which suppress ineffective erythropoiesis and minimizes iron accumulation from transfusion.

Sustained transfusion therapy maintaining pretransfusion Hb values over 9g/dl, alongside the needs of adjusted chelation therapy for each patient led to a significant increase in quality of life and its duration for thalassemia major.

C15. ROLE OF CENTRIFUGATION PARAMETERS ON PLATELETS CONCENTRATES QUALITY.

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Objective: the quality of platelet concentrates plays an important role in the effectiveness of transfusion therapy. As a result, this study aims at assessing the quality of platelet concentrates derived from platelets rich plasma. They were monitored the following quality parameters of blood components: volume, number of platelets, number of WBC; centrifugation parameters were: speed of centrifugation, time, temperature, acceleration and deceleration speeds. It was also evaluated the recovery of platelets in the final product.

Material and method: there have been evaluated 396 blood units collected during 6 months in CTS-Bucharest. There have been collected 450 ml blood in triple bags with 63 mL anticogulant CPDA-1. For processing, we used centrifuges Jouan K R4.22 and KRi samples have been tested on hematological Analyzer Nihon Cell-Dyn. Centrifuge parameters were permanently changed depending on the results obtained from the monitoring of hematologic parameters of platelet concentrates and intermediate products, in order to identify the optimal parameters of centrifugation.
Results: From all monitored quality parameters, the total number of platelets and the recovery degree were influenced in particular by the parameters of the first centrifugation, which must be soft enough for the vast majority of platelet to remain suspended in plasma. The second centrifugation was in all cases sufficiently energetic for an efficient sedimentation of platelets and played a minor role in obtaining an optimum concentration of platelets. The cellularity degree in plasma was under the calibration range of hematological analyzer, and was indicative only; the exact evaluation can be carried out only with a flowcytometer.

The total number of platelets in platelet concentrates units were processed statistically by calculating the averages and standard deviations.

Conclusions:
- Centrifuge parameters have a major importance in getting a platelet concentrate quality with a number of platelets enough to ensure the effectiveness of therapeutic product.
- For achieving required quality in platelet concentrates, centrifugation parameters must be continuously monitored
- The effects of changes in one or more of the centrifuge parameters are determined by the number of platelets in: whole blood, red blood cell concentrates, plasma-rich platelet, and the final platelet concentrate.

C16. PULMONARY HIPERTENSION ASSOCIATED WITH MAJOR THALASSAEMIA.

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Although the quality of life of patients with major thalassemia and its duration were dramatically improved in recent years, cardiac complications still represent an important cause of morbidity and mortality. Pulmonary hypertension appears to be a relatively common complication in patients with thalassemia major, echocardiography monitoring revealed elevated pulmonary systolic pressure. The causes of pulmonary hypertension are not always known, the most important being associated with thalassemia: chronic hypoxia, hemolysis, iron deposits in the liver, heart and lung, increased tendency of microthrombosis and splenectomy.

Annual heart evaluations performed for patients with thalassemia major revealed several cases with different degrees of pulmonary hypertension, two of them severe, symptomatic by decreased exercise tolerance, dyspnea to small efforts, mild leg edema. Both patients were older than 40 years, associated chronic hepatitis C, secondary osteoporosis, hypogonadism, splenectomy and cholecystectomy, and inconsistent transfusion history with long periods without blood transfusions.

For one of the cases was performed cardiac catheterization to confirm the diagnosis and initiate

specific vasodilator therapy with sildenafil, and oral anticoagulant therapy, which are yet well tolerated. The other case is pending confirmation of diagnosis and for both patients decided to intensify the transfusion regime and the chelation therapy supported.

C17. **ANAEMIC SYNDROME IN THYROID DISEASES.**

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Purpose of the study: we leave studied the incidence of the anaemic syndrome in thyroid diseases, and also established different etiologies of anaemias.

Patient and method: we leave studied 24 patients, that ewew investigated in the department of Haemstologcal Diagnosis of the National Transfusion Haematology Institute during one year (July 2013 – July 2014), after having been investigated in different Endocrinology clinics for various thyroid diseases. In these patients, we have followed: the levels of haemoglobin and packed volume, the mean corpuscular volume (MCV), the aspect of the peripheral blood smear, serum iron and vitamin B12 (cyanocobalamin), Coombs test.

Results: Patients. Aged between 29 and 82 were mostly females (only 2 male patients!). Half of the cases werw alredy diagnosed as chronic autoimmune thyroiditis, having … levels of anti-thyreoperoxidase; the other cases werw: hypothyroidism, hyperthyroidism, Basedow disease, toxic goitre, a.s.o. Thyroid diseases preceeded with one to thirty years the onset of anaemia. A part of the patients were already under L-thyroxine therapy when joining our department.

Anaemia was sleightin most cases (Hgb levels over 10 g/dl in19 patients), average in 3 cases (Hgb between 8-9 g/dl) and severe only in 2 cases anaemia was macrocytic (MCV between 95-125 fL), with a suggestive periplural blood film (macrocytosis, poikilocytosis, polichromatophilia, sometimes hypersegmentated neutrophils); 14 cases had low levels of seric cyanocobalamin / between 83-161 pg/mL); we mention a few cases with a suggestive smear but with normal levels of cyanocobalamin, in which we decided surveying these levels every 2 years.

As additional haematological .. we mention: iron deficiency (6 cases), myelodisplastic changes (2 cases), autoimmune haemolytic anaemia (2 cases); 2 cases associated leucopenia and 3 cases thrombocytopenia. Associated diseases: 2 cases of coeliac disease, 2 of hereditary spherocytosis and 1 case of psoriasis.

Conclusions:

1. The association of thyroid diseases and anaemia is much more frequent in females and affects all ages
2. thyroid disease usually precedes the onset of anaemia
3. Most frequently anaemia is macrocytic-megaloblastic and consists in cyanocobalamia deficiency
4. Patients with thyroid diseases need periodical surveying of cyanocobalamin levels.

C18. **THE USE OF DNA TECHNIQUES FOR DIAGNOSIS IN A CASE OF HIPERSIDEREMIC ANEMIA.**

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Is widely recognized that the diagnosis of anemia can be quite tricky and difficult in some cases. An accurate and reliable diagnositic is however crucial for treatment and monitoring of patients. In recent years, molecular biology and genetics of the various syndromes have been described in detail, revealing the wide range of mutations encountered in each type of inherited genetical disorders. That leaded to improve considerable the diagnosis of those syndromes.

Case report: male, 39 years old, diagnosed with Thalassemia minor in 1983 in our institute (HbF 11%) without any transfusion until now. After the diagnosis has not made any control until recently when he made a biliary colic which were admitted to gastroenterology service. Clinical and laboratory parameters as pallor, jaundice, hepato-splenomegaly, Hb 9 g / dL and ferritin 1700 ng / ml., raised the suspicion of hemochromatosis. The DNA tests for 11 of the most frequent mutation for hemochromatosis were negative.

Hematologic reevaluation raised suspicion of

Thalassemia intermedia. A number of 22 of the most common β thalassemia mutations specific to the Mediterranean have been tested by molecular biology techniques. Testing showed double heterozygosis: -101 [C> T] and Codon 8[-AA]

In INHT, the principal tools in thalassemia diagnosis were, for many years, classical Hb electrophoresis combined with peripheric blood examination on microscope. Since 2010, the new method - molecular biology was introduced. In our opinion, the classical methods remain the basis for routine diagnosis, but molecular biology allow to clarify ambiguous and difficult cases.

C19. **PLATELET COUNT VARIATION ON PLATELET CONCENTRATE DONORS WITH REPEATED DONATION BY APHERESYS.**

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B.B.T.C

Introduction: The platelet count of platelet concentrate by apheresys donors can vary with each donation session, thus setting their eligibility for the next donation.

Aim: The study of platelet count variation from one donation to the next on platelet concentrate donors with repeated, successive donations by apheresys.

Materials and method: The study was made on 163 platelet concentrate donors who donated at the Bucharest Blood Transfusion Center, for a period of one year, with 30 days interim between donations, on Haemonetics MCS +.

Results:It has been found that out of the 163 donors, 103 (63%) shown a decrease in platelet count, while 60 (37%) had an increase in platelet count on the following donation.

Platelet counts decreased with 1000/μL to 170000/μL and increased with 1000/μL to 100000/μL.

Donors with more than 5 successive donations registered a decrease in platelet count, over the initial value at first donation, in 67% of the cases and an increase in 33%, the variation ranging from 3000 /μL to 100 000 /μL.

Conclusions: Platelet concentrate by apheresys donors exibit a decrease in platelet count in 60% of the cases and an increase in 30%, at an interval of 30 days between donations, a pattern which remains constant even after more than 5 successive donations by apheresys.

NURSES SESSION

A2. HEMOPHILIA: PRACTICAL CONSIDERATIONS AND CURRENT MANAGEMENT OPTIONS.

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Hemophilia is a rare congenital bleeding disorder, resulting from a deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B) which manifests with prolonged or spontaneous, potentially life-threatening bleeding. Repeated bleeding episodes, most commonly in the joints and muscles, can also result in long-term, disabling complications.

The diagnosis of hemophilia is based on factor assays, bleeding patterns, and family history.

Hemophilia can be severe, moderate, or mild, depending on the degree of factor deficiency. A common long-term complication of hemophilia is permanent damage to the joints (hemarthropathy) caused by repeated bleeding episodes. Damage to the joint tissues can be seen after even short periods of exposure to small amounts of blood. Repeated bleeding into the muscle can also have long-term effects, with muscle and nerve damage potentially leading to contractures.

Treatment of hemophilia is comprehensive and focused on preserving both physical health and quality of life of individuals with the disorder. The primary goal of treatment is the prevention or cessation of bleeding episodes. Prompt treatment of acute bleeding episodes is essential to minimize long-term complications.

Available factor VIII and IX concentrates are either plasma-derived or produced via recombinant technology. Recombinant factor products are classified as first-, second-, or third-generation based on exposure to human or animal proteins. Recombinant factor VIII products are also classified as full-length or B-domain deleted proteins. However, the 2 main concerns with factor replacement are transmission of pathogenic viruses or prions and the development of inhibitors. Factors that may increase the risk of inhibitor development include severity of hemophilia, the type of genetic mutation, family history, and age at diagnosis and first treatment.

Patient education is also key in the treatment of hemophilia, not only to ensure that patients can recognize bleeding episodes quickly, but also to allow for a higher quality of life through an understanding of the disorder and the need for comprehensive care.

A3. EUROPEAN GUIDELINES AND PRINCIPLES OF HEMOPHILIA PATIENTS CARE.

As. Livia Neacsu
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The primary objective of haemophilia care consists of adequate replacement of deficient coagulation factor protein in order to prevent or resolve acute bleeding episodes. But, the management of haemophilia patient is complex requiring access to a range of services provided by a multidisciplinary team of specialists.

People with hemophilia are best managed in a comprehensive care setting, ensuring physical and psychosocial health and quality of life while decreasing morbidity and mortality.

The European Hemophilia Consortium launched the ten European Principles of Hemophilia Care endorsed by all EU member states in order to improve Hemophilia patient care in all European countries. According with these principles, in each country should be a central organization for hemophilia care and also a national hemophilia registry, comprehensive care and treatment centers for patient management and clinicians and patient representatives should be part of national hemophilia care decision making in partnership with authorities. Patients should have access to safe and effective concentrates at optimum treatment levels, prophylaxis treatment and home treatment, access to specialist services and emergency care and also to have access to treatment for inhibitors. Education and research is an important task for the future to secure high quality care (Colvin 2008).

Also, according with European recommendations, the minimum factor VIII consumption level in a country should be 3 I.U. per capita; in Romania the FVIII consumption level is 0.5 IU/capita (O'Mahony 2013).

In conclusion, patients with hemophilia must be treated in a comprehensive centers having access to a multidisciplinary team of specialists which can provide the best treatment plans in order to improve patients quality of life.

A4. **HEMOPHILIA MANAGEMENT– STILL A CHALLENGE IN CLINICAL PRACTICE?**

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Hemophilia requires a life-long commitment to the regimen of care. In persons and families affected by hemophilia, adherence is more difficult when a young child must be treated every other day or 3 times per week during prophylaxis.
The primary difficulty with adherence to a regimen of prophylaxis in young children is venous access which is an essential aspect of hemophilia care. According with WFH Guidelines, veins must be treated with care because they are the lifelines for a person with hemophilia.

Peripheral venous access is strongly encouraged in the Consensus Recommendations, if veins are adequate and if the patient is old enough. Most people using peripheral venous access use butterfly needles. With this method, a single needle is used for each treatment and then removed. This means that nothing remains under the skin — which results in reduced risk of infection and increased freedom.

Venous access devices should be avoided whenever possible but may be required in some children. Infection is the primary complication associated with central venous access devices (CVAD) and the most common reason for their removal. The blood clots are also a complication of CVAD. CVAD can be complex and require significant training. There is also risk of the device breaking or malfunctioning. For all of these reasons, the Consensus Recommendations suggest that CVADs be used only when peripheral access is not feasible and only for as long as medically necessary.
Drugs that affect platelet function, particularly acetylsalicylic acid and non-steroidal anti-inflammatory drugs should be avoided.
Physical activity should be encouraged to promote physical fitness and normal neuromuscular development, with attention paid to muscle strengthening.

In conclusion, peripheral venous access should be preferred for administration of replacement therapy in hemophilia patients and patient and family education is also an important in order to increase patient adherence to treatment.

A10. **MEDICAL STAFF AND BLOOD DONORS RELATIONSHIP: IMPACT ON BLOOD DONATION.**

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Even from the very beginning, the potential blood donor will have to interact directly with the medical assistant who will advise and provide him any necessary assistance during the blood donation performance. Thus, the medical staff will inform the potential blood donor about blood donation process, will perform individual evaluation of the potential blood donor, will perform blood collection and pre-donation tests, and provide emergency medical assistance, if any post-donation incidents occur.

This work is dedicated to the activity of medical assistants whose activity is directly related to the people, respectively to the blood donors. BTC medical staff is aware that medical profession should be perform with patience, generosity, compassion, and sincerity, its attention being focused on the person with “medical care needs” who is accepted “as it is”. In our specific activity, the expression “as it is” has a special meaning, the medical act being totally dedicated to the blood donor. The blood donor is a person aged between 18 and 65 years, totally open to blood donation and assuming completely this noble act. In consequence, the medical staff is nor confronted with the need and fears of a sick person searching for his health, but with a healthy person, sometimes too shy and nervous, sometimes too impetuous,

determinate to donate his blood in order to help the people in need.

The relationship between blood donors and medical staff is an every day challenge due to the individuals' diversity, people with different personalities, social status, culture level, religion or ethnicity. Therefore, the medical staff has the duty to develop a trustful and respectful relationship with the blood donor, serving the common purpose that is a safe and harmless blood donation, as well as a secure and conform blood unit. As a conclusion, we may say that blood donors represent a great variety of individuals, so the medical staff should comply accordingly.

Medical staff and blood donor interaction should be in accordance with the physical and mental status of each individual, in compliance with its understanding capacities that, in association with other helping elements, will lead to a successful blood collection. An inappropriate attitude of the medical might negatively influence the blood donor, generating suspicions and discomfort, affecting thus the blood collection activity. Medical assistant abilities are revealed by both technical and practical knowledge, as well as by the medical care provided for each individual, according to its specific personality and needs. Starting with potential donor registration, passing through all the procedures chain until a successful blood donation, the relationship between medical staff and blood donors prove to be a very important link within the transfusion activity.