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The national program for unrelated stem cells transplant – present and perspectives.

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Executive Summary

This document sets out the NRHSCVD Strategic Plan for 2013-2014. The plan is based on the consultation of the Scientific Council of NRHSCVD, staff consultation network of healthcare facilities that performing activities coordinated by NRHSCVD (HSC Donor Centers, HLA Laboratories, Collection centers and Transplant centers HSC). It is aimed at implementing the NRHSCVD Mission and Vision, in compliance with standards and policy of World Marrow Donor Association (WMDA) (http://worldmarrow.org/), which envisages:

1) To assure efficient provision of the hematopoietic progenitor cells for patients in need of a stem cell transplantation and to undertake the intervention in one of the Transplant Centres in the country.
2) To increase public and professional awareness of donation of hematopoietic stem cells as means to help patients;
1) To implement the international quality standards and procedures through education and studies;
3) To develop the governance structure for effective and accountable implementation of the mission and;
4) To maximize the available resources.

Present situation and the actual results after the first 6 months of implementation, difficulties identified and lessons learned were taken into account to determine future prospects for achieving the targets.

Key words: *standards and policy, unrelated donors, unrelated stem cell transplantation.*

GENERAL CONTEXT

Each year, thousands of people are diagnosed with severe, life-threatening conditions, like leukemia and other hematologic diseases. In some case, the only chance of saving these patients' lives is performing a hematopoietic stem cells transplant from a compatible donor. Some patients have a matching donor in their own family, but most patients rely on unrelated donors. The demand for unrelated hematopoietic stem cells donors has tripled in the last decade alone and might continue to increase during the next decade as well, as a consequence of smaller families. It is known that the interval from diagnosis to transplantation affects the patient's results in a negative way, providing unrelated donors being the main cause of these delays. Delays in finding unrelated donors are caused by: delays in referring the diagnosed patient to a transplant center as a result of waiting for results in the case of sibling typing; the lack of a matching donor (especially for ethnic minorities and/or in the case or rare HLA phenotypes); time and high expenses for checking the donor-patient compatibility if possible donors are HLA-typed at a lower or intermediate resolution; registered donor depreciation; donor ineligibility for health reasons and difficulties encountered with HSC transplants across international borders[1].

Because finding a compatible donor in the population group or the geographic area of the patient is more likely, most countries have established volunteer hematopoietic stem cells donor's registries. Registries worldwide share donors via an international database administered by Europdonor - Bone Marrow Donor Worldwide, which now **counter over 22 million of volunteer hematopoietic stem cells donors.** Each of these donors represents a chance at life for patients worldwide.

NRHSCVD is a public institution subordinated to the Ministry of Health. Its mission is to identify and ensure matched hematopoietic stem cells donors for all Romanian patients that need a transplant and don't have related compatible donors.

As of April of this year, the Romanian registry is recognized by the international community and it works according to the standards established by World Marrow Donor Association. It is in permanent contact with similar registries worldwide, registries that have common standards and work procedures. The World Marrow Donor Association represents 71 HSC donor registries, 140 cord blood banks, 350 collection centers and 1,259 transplant centers over 48 countries.

The Romanian registry is small: on September 1st 2013, in the NRHSCVD were 3,450 **HSC donors recruited.** The search database for potential donors matched with patients in need of transplants is incomparably larger due to the fact that it is interconnected with other international registries: the

[1] WMDA Strategic Plan for 2013 till 2017 (20130626-WBRD-Strategic Plan)

European Marrow Donor Information System and Europdonor - Bone Marrow Donor Worldwide.

As members of The European Group for Blood and Marrow Transplant, the Registry and the country's HSC Transplant Centers ensure European-level quality and security standards.

Furthermore, as a medical institution newly established in full swing of the global financial crisis, NRHSCVD's mission, of developing new healthcare services, is rendered even more difficult by the economic difficulties. As of April of 2013, when Government Decisions no. 124/2013 regarding the approval of national health programs for 2013 and 2014, was implemented, and the Ministry of Health order no.422/2013 regarding the technical norms of putting into practice national health programs for 2013 and 2014, the unrelated hematopoietic stem cells transplant program was approves. As a Management and Technical Assistance Unit of unrelated hematopoietic stem cells transplant program no.3.2, to ensure financial support, the Registry must raise to the challenge of identifying and selecting a matching donor for a patient, while maintaining efficiency and performance standards.

For NRHSCVD, the challenge lies with the **ethnic diversity of the available donors**, with improving HLA-typing resolution for Romanian donors and with carefully **monitoring the total costs of providing the HCS product for unrelated HSC allotransplant.**

Another challenge is **the continuous updating of quality standards and standard operational procedures** compliant with the international WMDA standards and **trains the staff** of Registry and the Registry network. The actual and exact knowledge regarding clinical benefits and economic consequences, facilitate the discussions of Registry with the transplant centers for selection of more suitable donors out of available options, such as the discussions with the Ministry of Health and other health authorities regarding **establishing the functional legal framework** for applying the international quality standards form Registry and all the health entities working with Registry.

1. Ensuring efficient supplying of HSC for patients in need of a transplant and carrying out transplant procedures in one of the country's Transplant Centers

One of the main activities of the Registry is to organize and maintain a complex database comprising both volunteer HSC donors and patients in need of a transplant. This database is secured according to all international standards and allows for the processing of HSC donation requests and HSC providing requests, both nationally and abroad.

The registry network includes 18 donor centers, 6 HLA laboratories, 3 collection/apheresis centers, and 3 transplant centers. With all the entities there are a written agreement and all of them have been implemented a minimum WMDA standards.

The Romanian software Prometheus allows the online connection with the EMDIS registries and a national virtual private networking between the head office, located in Bucharest, and keys locations of facilities that work with Romanian Registry. After the extension of the functionality of Prometheus IT system to the network of all the donor centers, laboratories, harvest centers and transplant centers, the virtual private networking allows the following functionalities:

- The 18 donor centers can register new donors. Blood samples of new donors will be sent to the HLA laboratory.
- The 3 partners transplant centers can register new patients. Blood samples of new patients will be sent to the HLA laboratory. The existing patient data and any modification are done by the registry staff on behalf of the request coming from the transplant center.
- The safe communication network is assured through VPN solution with the access (developed under the agreements with IRGHET - International Research Group on Unrelated Hematopoietic Stem Cell Transplantation, and with STS - Special Telecommunication Service).

During first nine months of the year 2013, the Registry performs searches in the local database for 17 foreign patients and 52 national patients with indication for unrelated hematopoietic stem cells transplant. One national HSC adult donor donated for an international patient (from Russia) and for two Romanian patients will carrying out transplant procedures in one of the country's Transplant Centers, with the donors provided by Romanian Registry. The transplant dates for those are fixed in October 2013 and the work-up of donors is ongoing.

The status for the national patients in need for an unrelated hematopoietic stem cells compatible donor is the following:

- **10 Romanian patients were transplanted** with stem cells from unrelated international donors provided by foreign registries: 1 Belgium (1), Poland (2) and Germany (7). The transplant procedures have been performed in all 3 transplant centers (3 in Timisoara, 5 in Bucharest and 2 in Targu Mures).
- Out of **32 active searches** performed for national patients, there are a number of 15 patients for which a compatible donor was identified and confirmed. For 7 patients the transplant plans have been set-up. There are 8 patients for which the Registry is coordinating the communication between the transplant centers of patients and the donor centers of the donors, for fixing

the most appropriate transplant plans and respectively work-up plans.

- **For 10 patients the search have been canceled** (they lost the indication for unrelated transplant, or without finding out an appropriate compatible donor they were transferred in other countries for alternatives therapy – hapllo-transplant or genic therapy)

The activities performed for the recorded patient's means a good coordination with HLA laboratories, donor centers, collection centers, transplant centers, and transport providers. This years the Registry coordinated related activities:

- 11 hand carry transports of HSC products (one was shipped by Romanian Registry and ten was transported from foreign Registries to national transplant centers).
- Over 50 international donors was extended HLA-typed
- 29 verification/confirmatory HLA-typing were performed by HLA laboratories in the country (1 - HLA laborator of District Emergency Hospital No.1 Timisoara, 7 - HLA laborator of Clinic Institute Fundeni Bucharest and 21 - HLA laborator of national Institute of Transfusion and Hematology Bucharest)

From the beginning of year 2013, 16 out of 18 donor centres started the recruitment of donors. The number of registered donors by donor centers is presented in the Table No.1.

The total number of recorded donor is 3,450, out of each less than half have been minimum HLA-ABDR typed (1,636 HSC donors), and are ready to be selected for a transplant (the detailed data are presented in the Table no.2). One third of the donors are registered by the centres located in Bucharest. 75% of registered HSC donors are also the blood donors. The age of recorded donors is equal distributed from 18 to 45 ages old (see the Figure No.1).

Perspectives:

- Improve the collaboration with international bodies and Registries: in the next year we plan to have on-line connection with all the EMDIS Registries, including the American (NMDP) and Brazilian where is special request to be fulfill (including the last two years of allotransplant history of all the transplant centers collaborated with Registry)
- To develop strategies to speed up the donor provision and to move to an efficient search
- Implementation a customised IT systems plan and improve performance and service: data of existing donors will be modified and validate and by the donor centers; data of existing patients will be modified and validate TC; the further integration of the software systems of the partner HLA laboratories and stem cell processing facilities.
- Implementation of ISBT 28 barcode for Romanian Registry
- Improving the performance of Registry by establishing resources to support the audit/accreditation activities
- Increase funding for core activities (performing the IDM and HLA typing, transport of blood sample and HSC products, work-up and monitoring of donors post-donation, monitoring of patients post-transplants)
- Increase the capacity of transplant centers
- Assuring sufficient specialize staff and the training according with up-date of standars and procedures.
- Improving the training and developing the education program for all the staff involved in the activities coordinated by the Registry (recruitment, testing, collection and transplant).
- To develop and implement a reporting system able to analyze the economics of hematopoietic stem cell donation of volunteer donors

2. Raising public and professional awareness regarding hematopoietic stem cells donation as a means of helping patients.

The Registry specific goals are to promote the interests of donors and to create public awareness for stem cell donation. In this respect, the Registry established a series of routines, procedures, safety measures, and insurance policies to protect the donors and to ensure proper conduct of the registry and its donor centers.

Start with 2009 when was issued the Governmental Decision for creating the Registry, the politicians recognize the global nature of issues. In June 2013 the Ministry of Health launched an information and communication campaigns “Donate a chance for life!”. During 10 days in Bucharest and 4 in Constanta district, over 500 persons were registered in the Registry as hematopoietic stem cells donors. In addition, few hundreds of people contact us (by mail or telephone) and express their willing to become donors.

For promotion of HSC donation, Registry develops a communication strategy. The main activities for implementing the strategy are:

- A set of information materials for donors was developed (brochure, leaflets, flyers)
- On the websites www.rndvcsh.ro and www.registru-celule-stem.ro are published useful information regarding donor centers, steps to became donor, etc.
- For the donors or the persons interested to became donors, a greenline is available - TelVerde 080088 STEM (7836).

Perspectives:
- To work with health authorities and specialist for develop and implement the communication strategy
- To have the necessary agreements and affiliations with national health authorities and health insurance authorities, concerning donor safety and insurance. These should include, without be limited series of routines, procedures, safety measures, and insurance policies to protect the donors and to ensure proper conduct of the registry and its donor centers, HLA laboratories, harvest centres and transplant centers (according with WMDA recommendations for donor workup and stem cell collection, it is therefore recommended that a national registry and harvest center are responsible, unless all legal and administrative aspects of donor workup, cell collection and donor insurance to be in place)
- To coordinate all the regulation and policies promoted with other bodies working in the field of cells, tissues and organs (including accreditation of National Agency of Transplant).

3. Implementation of the international quality standards and procedures through education and studies

All registries receive the WMDA qualification based on adherence to the benchmark standards[2]. For getting into the WMDA qualification process, the NRHSCVD should fulfil at least the following conditions:
- Compliance for all applicable standards, not just benchmark standards;
- Compliance with all of the WMDA standards labeled "must" or "shall" is required for approval of qualification.
- All the entities from the Registry network should comply with relevant WMDA standards (it is the responsibility of the Registry to ensure that).
- The Transplant Centers affiliated with the Registry and requesting a donor from another country meet standards designed to insure that donation of hematopoietic stem cells will only be requested for patients for whom transplantation is a medically acceptable procedure.
- The duties and responsibilities of each entity of Registry network must be documented in a written agreement
- Written policies and protocols for all procedures performed in the Registry (including standard operating procedures, guidelines and report forms).
- A mechanism like physician readily available to assist with routine medical decisions regarding donor selection and donation, and direct daily access to expert consultants in the areas pertinent to the operation of the registry to assist the registry in establishing policies and procedures.
- The staff of the Registry and network must be trained and knowledgeable about their duties. The Registry must conduct and document staff training and maintain training records and reference manuals.
- Sufficient communication links to facilitate searches
- Ensuring the confidentiality and protection of the identity of donors.
- Fully informed and legally valid written consent obtained from all adult volunteer donors at the time of workup.
- Signed consent obtained initially at the time of recruitment.
- Consent obtained if donor blood or other biological material or information is stored and/or used for the purpose of an ethically approved research project.
- Assuring the accuracy of tests (HLA, infectious disease markers, and other blood group markers). The histocompatibility testing of donors must include identification of HLA loci considered essential for transplant success.
- The Registry must maintain records of its activities and must maintain a database of volunteer donor information.
- All patient and donor communications and records must be stored to ensure confidentiality and to allow for traceability of the donors and steps of the donation process.

Perspective:
- HLA loci tested at intermediate resolution for HLA- A, -B, -C, -DR typed on volunteer donors at the time of recruitment, and a clear plan for change to antigen recognition site and single allele HLA loci tested.
- Plan to change your donor typing technology uses for donor registration from mostly PCR-SSO to PCR-SSP, within the next 5 years.
- Improve and standardize the typing technology use as a secondary method for donor testing (e.g. ambiguity resolution, primary technology failure, etc.)
- Increase the dedicated staff working with the Registry
- Develop the reference manual for staff training and assure the financial sustainability for cost of training (transport, accommodation, etc.)
- Increase the security level of database of Registry with electronic signature for all the

[2] WMDA Application and Review Packet for Qualification and Accreditation of Registries - 20120101-ACCR-Checklist

documents/information

4. The legal in the Context of the European Union, WHO, WMDA and other regulatory bodies

The development of governance structure for efficient and responsible implementation of NRHSCVD mission should be compliant with the European rules and with the regulations of other international bodies.

In this respect, in the first 18 months from the establishment of NRHSCVD, with the consultation of Scientific Comities, were initiated few regulations or have been proposed changes or completion of existing ones, regarding the following aspects:

- Issues the import/export authorization for stem cells products; define the traceability according with European Directives for covering the donated HSC product and the facilities in charge with donation, testing, collection, preservation, processing, transportation and transplant; accreditation of the health units from the Registry network (transplant centers, harvest centers, HLA laboratories) according with quality and safe requests of European Directives (law no95/2006 – Title VI)
- Approving the membership taxes payable by the NRHSCVD in order to be interconnected with international bodies in the field; defining the NRHSCVD responsibilities' in auditing and authorizing all the formal documents regarding WMDA qualification/accreditation; implementation of ISBT 128 as unique codification and labelling system according with the requests of European Directives (Governmental Decision No.760/2009)
- Establishing the structure of Scientific Council of NRHSCVD (Order of Ministry of Health no. 474/2011)
- Approval the eligibility criteria for a health facility for applying to organized a donor Center, HLA Laborator, Harvest Center or Transplant Center for unrelated stem cells transplant (Order of Ministry of Health no. 6/2013)
- Nomination of the health units which fulfil the conditions to be part of Registry network (Order of Ministry of Health no. 92/2013)
- Annual approval of expenditure with membership taxes (WMDA, BMDW, EBMT) and accreditation fees for HLA laboratories (Governmental Decision No.123/2013)
- Approval of unrelated stem cells transplantation program for years 2013-2014 (Governmental Decision no.124/2013 and order of Ministry of Health no.422/2013)
- Nomination of members of four regional commissions for evaluation of unrelated stem cells transplant indication

Perspective:

- The Registry must assume responsibility and establish procedures for all donor medical expenses including the pre-collection physical examination, the collection procedure and all post-collection medical expenses that are directly related to the donation. No donor should assume financial liability for any portion of the follow up testing and/or stem cell harvest/procurement process. The registry is responsible for all reasonable expenses incurred by the donor[3].
- Health insurance for the volunteer donors

Conclusions

Implementing the NRHSCVD Mission and Vision, in compliance with standards and policy of WMDA need a strategic plan, agreed by the health authorities and keys professionals in the field of stem cells transplant.

The strategic plan should be shared with the transplant physicians and general public, in the interest of patients in need for an unrelated stem cells transplant.

The NRHSCVD should implement recommended principles in its standards, working procedures, forms, etc. requested by WMDA in order to fulfil them and to apply for and eventually obtains WMDA qualification/accreditation.

References:

1. WMDA Strategic Plan for 2013 till 2017 (20130626-WBRD-Strategic Plan)
2. WMDA Application and Review Packet for Qualification and Accreditation of Registries – (20120101-ACCR-Checklist)

[3] Acording with 10.5 WMDA standars – Financial and legal liabilitis

Figure No.1: The group age of recruited donors

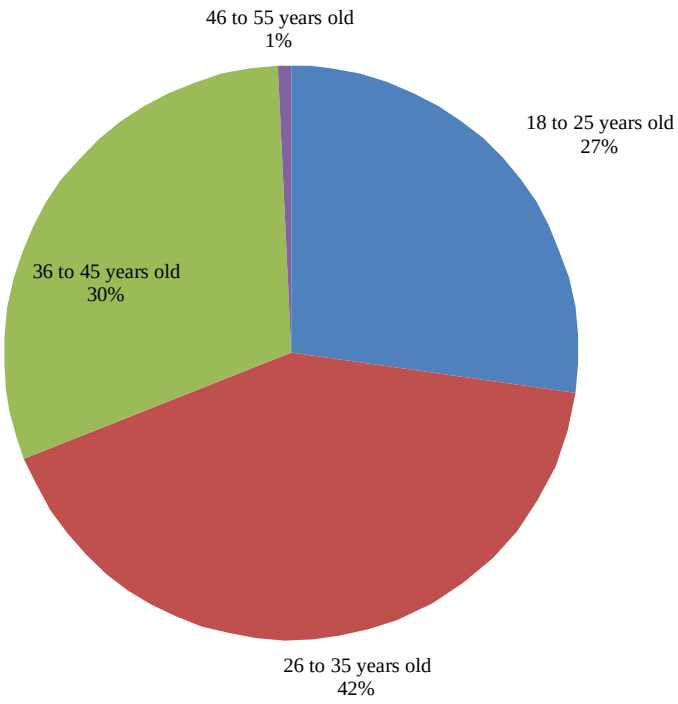

Table No.1: Total number of registered HSC donors and % of blood donors from total

DC ID	Name of Donor Center	Quarter 2			Quarter 3			Total 2013		
		Total count donors	Blood donors	% Blood donors	Total count donors	Blood donors	% Blood donors	Total Count Donors*	Blood donors	% Blood donors
RO1011	CTS Craiova	0	0	0%	0	0	0%	**0**	**0**	**0%**
RO1012	CTS Galati	92	63	68%	155	149	96%	**247**	**212**	**86%**
RO1017	CTS Slobozia	29	28	97%	148	147	99%	**177**	**175**	**99%**
RO1020	CTS Targu Mures	1	1	100%	19	14	74%	**20**	**15**	**75%**
RO1022	CTS Ploiesti	432	381	88%	26	23	88%	**458**	**404**	**88%**
RO1026	CTRS Timisoara	23	18	78%	85	85	100%	**108**	**103**	**95%**
RO1034	CTS Arad	0	0	0%	13	11	85%	**13**	**11**	**85%**
RO1035	CTS Bucuresti	504	448	89%	521	94	18%	**1,025**	**542**	**53%**
RO1036	CTS Olt	103	92	89%	266	261	98%	**369**	**353**	**96%**
RO1037	CTS Satu Mare	0	0	0%	0	0	0%	**0**	**0**	**0%**
RO1040	CRTS Cluj	2	1	50%	2	0	0%	**4**	**1**	**25%**
RO1041	CTS Oradea	84	74	88%	201	199	99%	**285**	**273**	**96%**
RO1044	CTS Braila	68	66	97%	39	37	95%	**107**	**103**	**96%**
RO1045	CTS Brasov	0	0	0%	97	88	91%	**97**	**88**	**91%**
RO1056	Institutul Clinic Fundeni Bucuresti	14	0	0%	1	1	100%	**15**	**1**	**7%**
RO1057	Spitalul Clinic de Urgenta Floreasca Bucuresti	19	14	74%	97	85	88%	**116**	**99**	**85%**
RO1058	Spitalul Universitar de Urgenta Bucuresti	6	0	0%	2	0	0%	**8**	**0**	**0%**
RO1018	Institutul Regional de Oncologie Iasi	5	1	20%	5	0	0%	**10**	**1**	**10%**
RO1001	NRHSCVD - TL	391	196	50%	0	0	0%	391	196	**50%**
	Total NRHSCVD	**1,773**	**1,383**	**78%**	**1677**	**1194**	**71%**	**3,450**	**2,577**	**75%**

**11 recruited donors was deleted due to medical or personal reasons*

Table No.2: HLA donor status report at 1 of September 2013

Donor Status*	Count total	% per DC	RO 1012	RO 1017	RO 1020	RO 1022	RO 1026	RO 1034	RO 1035	RO 1036	RO 1040	RO 1041	RO 1044	RO 1045	RO 1056	RO 1057	RO 1058	RO 1018	RO DC
Not typed (just IDM)	**1,717**	**49.93%**	115	177	18	19	114	11	488	342	4	207	39	57	3	106	4	10	3
A typed	**2**	**0.06%**	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
B typed	**2**	**0.06%**	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
C typed	**3**	**0.09%**	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
DR typed	**2**	**0.06%**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
AC typed	**4**	**0.12%**	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0
BDR typed	**9**	**0.26%**	0	0	0	2	0	0	3	0	0	0	0	1	0	1	0	0	2
CDR typed	**2**	**0.06%**	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
ADR typed	**2**	**0.06%**	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
BC typed	**3**	**0.09%**	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
ACDR typed	**12**	**0.35%**	2	0	0	1	0	0	5	0	0	2	0	1	0	0	0	0	1
ABCDQ typed	**1**	**0.03%**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AB typed	**7**	**0.20%**	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	3
ABC typed	**26**	**0.76%**	2	0	1	1	0	0	11	0	0	2	4	4	0	1	0	0	0
BCDR typed	**11**	**0.32%**	3	0	0	1	0	0	3	0	0	2	0	2	0	0	0	0	0
ABDR typed	**743**	**21.61%**	29	0	0	120	0	0	252	5	0	5	19	0	7	2	4	0	300
ABCDR typed	**739**	**21.49%**	74	0	1	300	0	2	184	22	0	59	44	30	1	9	0	0	13
ABDRDQ typed	**46**	**1.34%**	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	22
ABCDRDQ typed	**108**	**3.14%**	3	0	0	3	0	0	73	0	0	1	1	0	2	0	0	0	25
TOTAL	**3,439**	**100.00%**	**230**	**177**	**20**	**456**	**114**	**13**	**1,044**	**369**	**4**	**285**	**107**	**97**	**15**	**120**	**8**	**10**	**370**

**This report shows only active donors and temporarily unavailable for transplant (not typed HLA)*

The porphyrias – part II

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Abstract

CEP is an AR porphyria that results from deficient activity of URO-synthase and results in the accumulation of Uroporphyrin I and Coproporphyrin I isomers. The skin areas overexposed to sunlight are friable and blisters and vesicles are prone to rupture and infection. Skin thickening, focal hypo- and hyperpigmentation and hyperthricosis of the face and extremities are characteristic. The diagnosis is confirmed by the demonstration of significantly deficient URO-synthase activity or the identification of specific mutations of the UROS-gene.
The knowledge of these advances is relevant for hematologists because they administer the hematin infusions to treat the acute attacks in patients with acute hepatic porphiryas, perform the chronic phlebotomies to reduce iron overload, clear the dermatologic lessions in PCT, diagnose and treat the erithropoietic porphiryas, including chronic erythrocyte transfusions, B.M. or SCT transplants and experimental pharmacologic chaperone and stem cell gene therapies for CEP. These developments are relevant to update hematologists on the latest advances in these diverse disorders.

ERYTHROPOIETIC PORPHYRIAS

Classification

- Congenital Erythropoietic Porphyria (CEP);
- Erythropoietic Porphyria (EPP);
- X-Linked Porphyria (XLP)

Type	Deficient enzyme	Inheritance	Enzyme activity, % of normal	Increased porphyrin / porphyrin precursors		
				Erythrocytes	Urine	Stool
Erythropoietic porphyrias						
CEP	URO-synthase	AR	1-5%	Uroporphyrin I Coproporphyrin I	Uroporphyrin I Coproporphyrin I	Coproporphyrin
EPP	Ferrochelatase	AR	20 – 30 %	-Primarily free proporphyrin	-	Proporphyrin
XLP	ALA-synthase 2	XL	> 100%	Zn- and free proto-porphyrin	-	Proporphyrin

CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP)

- CEP is an AR disorder;
- The activity of URO-synthase is deficient and the result is an accumulation of uroporphyrin I and coproporphyrin I isomers;
- Uroporphyrinogen I is metabolised by uro-decarboxylase in coproporphyrinogen I, but the latter is not a substrate for copro-oxydase;
- CEP is associated with hemolytic anemia and severe cutanate photosensibility;
- The porphyrins in excess are also deposited in teeth and bones (1).

CLINICAL MANIFESTATIONS

- Severe cutaneous sensibility in early infancy;
- Hydrops fetalis, through a non-immune mechanism, is not uncommon;
- Skin overexposed to sunglight is red, friable and the blisters and vesicles are prone to rupture and infection;
- Healing is slow and the skin presents hypo and hyperpygmentatio areas, hypertrichosis of the face and extremities are chracteristic;
- Secondary infection and bone resorbtion may lead to

disfiguration of the face and hands;
- Retraction of the alar parts of the nose, of the eyelids, eyebrows, pavilions of the ear, resulting in a monstruos appearance of the patient;
- Hypertrichosis and hirsurtism results in a lion or simian-like appearance of the patient;
- The teeth are reddish brown and in UV light emanate a reddish-crimson fluorescence due to the erythrodontia which appears mainly because of the protoporphyrin crystals accumulated in the dentine;
- Hematological myeloid malignancies and especially secondary MDS without any connections to the apparition of UROS or GATA 1 mutatios (2) ;
HEMATOLOGIC MANIFESTATIONS
- Moderate-hemolytic anemia due to the excess of porphyrins in erythrocytes;
- Clinical: splenomegaly;
- On exposure to U.V., the erythrocytes, normoblasts and reticulocytes from the perypheral blood and bone marrow present fluorescences;
RENAL MANIFESTATIONS
- Urine is reddish-brown, alkaptonuria, urine that taints the lingerie;
- Uro and coproporphyrin >500 mg/24 hours;
LABORATORY DIAGNOSIS
-Uroporphyrin and coproporphyrin (type I isomers)accumulate in the bone-marrow, circulating erythtrocytes, plasma,urine and faeces;
- The diagnosis is confirmed by demonstrating the deficient URO-syntase activity or by identifying specific mutations of the UROS-gene;
- The disease can be detected antenatally by measuring porphyrins in the amniotic fluid and URO-syntase activity in cultured amniotic cells or chorioral villi;
- More than 35 UROS-mutations have been identified, including four in its erythroid specific promoter and genotype/phenotype corrrelations in patients with severe manifestations;
TREATMENT
- Chronic transfusions of red cell mass can be started antenatally;
- Protection from sunlight is essential, superinfections or traumatisms should be avoided;
- Bone marrow and cord blood transplant have proven effective in several transfusion-dependent patients;
- Modern treatment for the UROS-mutation (C73R) with chaperone and/or protease inhibitor was reported;
ERYTHROPOIETIC PORPHYRIA (EPP) and X-LINKED PORPHYRIA (XLP);
- EPP is an AR disorder, resulting from mutations in the FECH-gene, with heavy reduction of the activity of the enzyme;
- It is the most common porphyria in adults and children;
-XLP, a clinically distinguishable form of EPP only by demonstrating the presence of mutations in the last exon of ALAS 2-gene (3).
CLINICAL MANIFESTATIONS
- Cutaneous photosensibility usually beginning in early childhood, redness, itchting that lasts for hours, diffuse oedema, vesicles and blisters persisting for hours or days, manifestations occuring within minutes of sunlight exposure;
- Electric light, heat, traumatisms can trigger the symptoms;
- Purpura, oedema, agitation, shivers or even collapse;
CLINICAL MANIFESTATIONS
- Permanent sequelae which can persist on the exposed regions (nose, cheekbones, forehead, the back of the hands and fingers);
- Hyperkeratosis, scars, vesicles and blisters;
- Other clinical symptoms: biliary colic (pigmentary lithiasis in women), intrahepatic cholestasis, (lethal) liver failure;
BIOLOGICAL DIAGNOSIS
- The primary source of excess of protophorphirin in EPP and XLP in the B. M. reticulocytes and erythrocytes will exhibit red fluorescence when examined by fluorescence emission microscopy at 620 nm with excitation at 405 nm;
- Demonstrating increased protoporphyrin - in EPP, erythrocyte protoporphyrin is mostly bound to Hb and not complexed with Zn (as in XLP);
- Normal urinary levels of Urinary levels of porphyrins and porphyrin precursors;
- Mutational analysis confirms mutations of the FECH and ALAS 2-genes;
- IVS 3-48T>C (25% of transcripts normal FECH) in cys, followed by a low expression of the allel in trans, but with a normal activity of FECH;
PATHOGENESIS
- Protoporphyrin accumulates primarly in B.M. reticulocytes during Hb synthesis and then appears in plasma, is transported to the liver and excreted in bile and faeces. Protoporphyrin can be deposited in the skin and blood vessels and can be activated by light, causing cutaneous photosensivity.
- Protoporphyrin is insoluble at neutral pH, due to an excess, form crystalline structures in liver cells and can decrease hepatic bile flow in bile fistula rats, causing progressive liver disease in EPP due to a constant accumulation of protoporphyrins in the liver, erythrocytes, plasma and causing photosensitivity;
- Hepatic complications appear especially in AR forms of EPP because of 2-FECH mutations and in XLP.
TREATMENT
- Solar light protection;
- Beta-carotene (oral), 120-180 mg/dl, causing carotenodermia that improves tolerance to sunlight;
- Treatment with alpha-MSH-analogue which darkens the skin and can increase tolerance to sunlight exposure;

- Plasmapheresis;
- Treatment with intravenous HEMIN;
- Liver transplant and administration of intravenous HEMINE and sessions of plasmapheresis;
- Treatment of hepatic complications: Cholestyramine, activated Charcoal (may interrupt the enterohepatic circulation of protophorphyrins and promote their fecal excretion);
- Treatment with MSH (melanocyto-stimulating-hormone) because the darkening of the skin may be followed by a painless tolerance to sunlight exposure;

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Second ESCMID Conference on Invasive Fungal Infections

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The second ESCMID Conference on Invasive Fungal Infections (IFI) was held between 16 and 18 January 2013 at Universita Cattolica Sacro Cuore in Rome.

The lecturers were experts from England, France, Switzerland, Germany, Turkey and Italy.

The conference was attended by specialists in infectious diseases, clinical microbiology, as well as specialists in other fields dealing with immunocompromised patients : haematologists, oncologists, intensive care practitioners.

On the first day, current issues related to fungal infections epidemiology were discussed, Aspergillus species distribution and yeasts forming the object of separate discussions. Aspergillus is the most commonly diagnosed pathogen, followed by Candida spp. Other fungal agents are rare. The mortality rate due to aspergillosis declined from 60 -70% 10 years ago to 40% today. There is no change in the 30 - 40% mortality due to Candida spp. fungal infections.

Discussions were held about IFI incidence and progression evaluation in patients with malignant blood diseases. Please find below some of the data that were outlined:

- rate of Aspergillosis, depending on the underlying disease: auto-BMT: 7%; allo-BMT: 25%; malignant blood diseases: 28% ; transplant for solid tumors: 9% ; AIDS: 8%; the remaining percentage: other diseases: auto-immune, pulmonary.
- time of IFI onset in patients with AML : 57% during the first induction therapy; 11% during the second course of induction therapy; 6% during consolidation; remaining percentage: during the hematopoietic stem cell transplantation.
- Aspergillus spp. fungal infection site: lungs 69%; sinuses 15%; CNS 7%; blood 1%; other sites: the remaining percentage.

With regard to the IFI diagnosis, the importance of bringing together clinical, laboratory and imagistic aspects was underlined. Here are some ideas:

- the CT scan indicated upon the first suspicion of fungal infection (of sinuses, chest or abdomen) allows for the diagnosis and implicitly the early treatment (antifungal and surgical, as applicable), resulting in the improvement of overall survival in fungal infections.
- Microbiology testing of pathology specimens collected by bronchoalveolar lavage, fine-needle aspiration biopsy or surgical biopsy reveal key information to help define the fungal infection.
- non-invasive diagnostic tests are criteria supporting positive diagnosis of fungal infections. Such tests are:

1. C-reactive protein (CRP), procalcitonin, IL6 (for fungi and bacteria);
2. panfungal PCR and 1.3 beta D glucan (for fungi);
3. PCR, galactomannan (GM), capsular antigens (gender-specific tests);
4. PCR (species specific tests).

The second part of the day was dedicated to fungal infections prophylaxis and treatment.

The efficiency of antifungal prophylaxis in patients with acute myeloblastic leukemia was analyzed.

Some ideas are listed below:

- Posaconazole is the anti-fungal agent most frequently used for the prophylaxis of fungal infections in patients with severe post-chemotherapy neutropenia. Fluconazole ranks second.
- Although Itraconaxole is reputedly more toxic and expensive than other antifungal agents, it is preferred in some hematopoietic stem cell transplant centers in Germany.
- The groups receiving prophylactic treatment with posaconazole and itraconazole were compared. The number of IFI cases in the itraconazole group was larger, but there was no difference in terms of the IFI-related mortality rate. Therefore, there are no overall survival differences between the group of patients treated with prophylactic posaconazole and the itraconazole group.

The empirical and the pre-emptive therapeutic approaches of the antifungal treatment were discussed, including their related pros and cons.

Here are some examples:

- supporters of empirical treatment: a fungal infection is suspected in patients with malignant blood diseases treated with cytostatic drugs and persistent fever under broad-spectrum antibiotic therapy and empirical antifungal therapy is started after 3 - 5 days of fever persistence despite broad-spectrum antibiotic treatment. Certain studies show the significantly low incidence of suspected/proven IFI in patients receiving empirical anti-fungal therapy (7.4%) versus patients undergoing pre-emptive treatment (23.7%) ($p<0.001$). According to these studies, the death rate due to IFI was

lower in the case of empirically treated patients versus pre-emptive treatment.

· advocates of pre-emptive treatment draw a comparison between the outcomes of the two therapeutic approaches in patients with hematopoietic stem cell transplantation. Pre-emptive treatment is based on PCR tests. An empirically treated group is compared to a group receing pre-emptive treatment undergoing bi-weekly PCR screening throughout the hospitalization, followed by weekly PCR screening until day 100. The following findings were made: 1. early mortality rate reduction (day 30) in the group undergoing pre-emptive treatment and 2. there were no differences between the two groups in terms of mortality, on day 100 and on day 180.

· the following commonsensical conclusion was reached: the pre-emptive approach is logical, feasible and "safe". However, since not all sites have the capacity to perform CT and GM and PCR screening as often as necessary, empirical treatment is the standard practice in smaller sites. Large sites should embrace a pre-emptive approach.

The last part of the conference was reserved to:

1. Antifungal resistance: mechanisms of fungal resistance to antifungal agents, new rapid detection strategies of fungal resistance, azole resistance impact on the clinical progression in invasive aspergillosis.
2. New developements in fungal infection diagnosis procedures: spectrometry detection of fungi, update of molecular diagnosis tests for mold and yeast infections, role of in vitro susceptibility testing in routine clinical practice, new perspectives in antigen detection for the diagnosis and treatment of invasive fungal infections.

The XXI-Th. National Conference of Clinical and Transfusional Haematology Abstracts

CLINICAL HAEMATOLOGY SECTION – EDUCATIONAL SESSION

E1. DISCORDANT, COMPOSITE AND TRANSFORMED LYMPHOMAS.

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Discordant lymphomas

Discordant lymphomas (DL) are rare entities characterised by the simultaneous presence of two distinct types of lymphomas in different anatomic sites.
This peculiar presentation has to be differentiated from the so called "composite lymphoma" wich is occurrence of two or more morphologically and immuno-phenotyically distinct lymphoma clones in a single anatomic site, that is within a single organ or tissue.

Discordant morphology has been documented in 14 to 38% of patients undergoing multiple biopsies. Most frecvently, however, discordance is documented as a bone marrow (BM) infiltrate predominantly composed of small cells, compatible with infiltration by low-grade NHL in patients with large-cell lymphoma in an extramedullary site.

The reasons for BM discordance are unclear, but proposed mechanisms include tumor evolution, transformation of a low grade NHL microenvironement or the presence of two unrelated neoplasms.

There may be an increased risk for late relapse in patients who have apparently achieved CR after therapy. Although both tumor components are clonally related in majority of cases and the DLBCL might be regarded as transformation of a clinically innaparent low-grade NHL, there is evidence of a distinct clonal B-cell proliferation in a significant minority of cases.
Rare cases of DL consisting of splenic mantle cell lymphoma and marginal zone lymphoma involving the bone marrow and peripheral blood, and DL consisting of MALT lymphoma of parotid gland and follicular lymphoma of the small intestine was described.

Another examples includes MALT lymphoma of intestine and follicular lymphoma of the bone marrow. Therapy of DL is directed against the more aggressive component.

Composite lymphoma

Composite lymphoma (CL) is a rare occurrence of 2 or more morphologically and immunophenotypically distinct lymphoma clones in a single anatomic site.
Many combinations of CL have been reported including multiple B cell lymphomas, B cell and T cell lymphomas, non Hodgkin's Lymphoma (NHL) and Hodgkin Lymphoma (HL) and complex B cell, T cell and HL cases.

The morphologic criteria must be confirmed by one or more tests, including immunohistochemical analysis, flow cytometria, immunophenotyping, conventional cytogenetic tests, FISH and/or molecular biology studies. Results are more accurate using the laser capture microdisection method.

It must be carefully diagnosed because the multiple disease entities may have entirely different natural histories, prognosis and treatment modalities.
Careful study of CL may clarify the possible pathogenetic mechanisms of the interrelationship of clonal evolution in lymphoma.

Several theories were proposed including clonal selection with additional mutational accumulation, genomic instability with genetic predisposition, a common precursor cell and the aid of viral factor, mostly EBV.

The incidence has been reported to vary between 1% and 4.7%.

Composite lymphomas have the similar prognosis that of more aggressive component.
Therapy in the rare situations of so called "composite" lymphomas has traditionally been directed against the more aggressive subtype.

In case of association HL with low-grade B cell lymphoma the treatment of choice is chemotherapy (for HL) in association with rituximab (for low-grade NHL).
In fact the treatment of composite lymphomas must be individulizated according the components of CL.

Transformed lymphomas

Evolution of an indolent non Hodgkin lymphoma (NHL) to an aggressive histology is know as histologic transformation (HT). HT is a frequent occurrence for all subtypes of indolent B cell lymphoproliferative

disorders.

This complication presents with a rapid change in the clinical behavior of disease with evidence of a highly proliferative malignancy having a propensity to involve extranodal sites.

The frequency of HT is dependent on the definition of HT, length of follow up and whether biopsies or autopsies were performed to document HT.
The risk of HT is approximately 3% per year for patients with indolent lymphoma.

The clinician should suspect HT in case of: rapid growth or discordant growth between various disease sites, sudden rise in LDH, unusual extranodal involvement new and persistent onset of B symptoms, new hypercalcemia, high SUV (standard uptake value) at PET imaging. Biopsies should be directed to the site of greatest FDG activity.

Acquisition and accumulation of additional genetic abnormalities appear to be responsible for HT. The overwhelming majority of cases of transformed lymphomas are clonally related to initial indolent lymphomas.

No single cytogenetic abnormality appears to be associated with HT. Specific genetic lesions have been identified. These include alterations in genes regulating proliferation, control of cell cycle and apoptosis: C-MYC and C-mYC regulated genes CDKN2a, CDKN2b, TP53, BCL2.

Patients with HT generally have a poor prognosis, with a median OS of aproxymately one year.

The good prognostic factors included: prior complete response of indolent lymphoma, CR following HT, normal LDH activity, absence of marrow involvement and of systemic B symptoms, limited stage disease, treatment with CHOP-like regimen.

The most commonly employed treatment regimens for patients with HT include conventional therapy (CHOP or its variants) radioimmunotherapy and high-dose therapy followed by hematologic stem cell transplantation.

E2. GOALS OF THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults. Although significant advances have been made in the treatment of CLL in the last decade, it remains incurable. Although there is no cure for CLL, the disease is treatable and current standard chemotherapy regimens have been shown to prolong survival.

Historically, the goal of treatment for CLL patients has been palliation of symptoms, and treatment was usually continued until disease-related symptoms were resolved.

While clinical staging systems have been used to stratify patients into risk categories, they lack the ability to predict disease progression or response to therapy.
In clinical trials, the tumor load that remains after therapy in patients with chronic lymphocytic leukemia can be quantified by modern minimal residual disease (MRD) technology with a 1000- fold higher sensitivity compared with clinical staging.

MRD levels independently predict OS and PFS in CLL. Low-level MRD does not equal complete disease eradication, but it is an important prognostic factor in a non-curative treatment setting. Therefore, MRD quantification might serve as a surrogate marker to assess treatment efficacy in randomized trials before clinical end points can be evaluated.

Rituximab mediates CDC, ADCC, and direct cell apoptosis in the treatment of B-cell malignancies. Currently clinical data have placed rituximab as a standard addition to front-line and subsequent lines of therapy for CLL improving progression-free survival (PFS) and overall survival (OS) and minimal residual disease (MRD).

The future of treatment in CLL it is represented by GA101 , a glycoengineered anti-CD20 antibody, which means specific sugar molecules in GA101 were modified to change its interaction with the body's immune cells and to bind to CD20 with the aim of inducing direct cell death.

The primary endpoint of the study with GA101 was PFS with secondary endpoints including overall response rate (ORR), overall survival (OS), disease-free survival (DFS), minimal residual disease (MRD) and safety profile.

Except for allogeneic stem cell transplantation, the use of MRD to tailor treatment in individual patients outside clinical trials is currently discouraged.

E3. PROGNOSTIC FACTORS WITH THERAPEUTIC IMPLICATION IN DIFFUSE LARGE B-CELL NON-HODGKIN LYMPHOMA`S

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The International Prognostic Index (IPI), originally established to predict outcome of patients with aggressive lymphoma treated in the pre-rituximab era,

has been confirmed to be a valid prognosticator for patients receiving rituximab, with the differences between the four risk groups (low, low-intermediate, high-intermediate and high) being smaller, yet significant compared to the pre-rituximab era.

Apart from the IPI, there are other subsets of diffuse large B-cell lymphoma (DLBCL) that are characterized by criteria not included in the IPI or are too rare to be recognized in multivariable analyses. This applies to: very old patients (>80 years), histological subgroups like DLBCL with immunoblastic or plasmablastic morphology, Epstein-Barr (BV)-positive B-cell diffuse large B- cell lymphoma of the elderly, the germinal center versus the non-germinal center subgroups,
DLBCL with MYC breakpoints (including double-and triple hit DLBCL), expression of MYC together with BCL2 protein, patients presenting with skeletal involvement, central nervous system (CNS) involvement during the course of disease, represent a subpopulation with an almost always fatal course.

A major improvement in treatment outcome has been achieved by adding rituximab to CHOP-like regimens. The revised IPI or "R-IPI" has three risk groups as suggested by Sehn *et* a/. treated with R-CHOP. The differences became smaller between the four risk groups under R-CHOP, demonstratrated that the IPI is still valid in the R-CHOP era and has a significant prognostic power.

ln the Mega-CHOEP trial, young patients with aalPI of 2 had a 3-year survival of 90%, and aalPI of 3 73% after 8 X R-CHOEP-14. In young patients, only the high-risk group with a 3-year survival of less than 75% represents a clinically relevant risk group. Related to toxicity of CHOP- 14 and CHOEP-14, combinations with targeted therapies like bortezomib, lenalidomide or ibrutinib are currently being evaluated in this population of young patients with high-risk DLBCL.

In elderly (age 61-80 years) DLBCL patients, with a 3-year overall survival of 88% for low-risk, 78% for low-intermediate, 67% for high-intermediate and 58% for the high-risk group, all but the low- risk group have a high risk of failure and must be improved. The increased toxicity in elderly patients as hematotoxicity, and strategies pursued include dose-dense of rituximab, adding other CD20 monoclonal antibodies or antibodies directed against targets other than CD20, addition of lenalidomide to R-CHOP, or lenalidomide or enzastaurin for maintenance therapy.

In a study of morphological and immuno-histochemical biomarkers in elderly patients treated both with and without rituximab within the RICOVER-60, immunoblastic morphology emerged as a robust, significantly adverse prognostic factor. In multivariate analysis adjusted for the factors of the IPI, the immunoblastic subtype was an independent predictor for EFS.

EBV-positive B-cell diffuse large B-cell lymphoma of the elderly is an EBV-positive clonal B-cell lymphoid proliferation that occurs in patients over 50 years of age and predominantly in elderly patients without any known immunodeficiency or prior lymphoma. These patients are diagnosed at older age, present with elevated LDH, poor performance status, B symptoms, and frequent skin and lung involvement. B symptoms and age over 70 years, but not IPI, appear to be reliable prognostic factors.

Age is one of the strongest prognostic factors in the IPI, not related to increasing comorbidities of elderly patients, but also because adverse biological features like the ABC-type and MYC breaks are enriched in the elderly population. While the IPI discriminates between patients aged 60 years or under and those over 60 years, a modification of the IPI, the IPI for elderly patients or E-IPI, was suggested using 70 instead of 60 years as a cut-off point to delineate older age as a risk factor. There are fewer prospective data available for octogenarians or nonagenarians, even though this population of DLBCL patients is increasing fast.

Male gender is a negative prognostic factor in (elderly) patients treated with rituximab, because female patients have a higher benefit from the addition of rituximab to CHOP chemotherapy than male patients. This is due to the slower rituximab clearance in elderly females that results in higher serum levels, longer serum half-life elimination time. No pharmacokinetic data are available for young DLBCL patients.

Bulky disease was an independent risk factor in the MInT study in young patients with an aalPI of 0 or 1 and bulky disease, despite the fact that nearly all patients with bulky disease had received radiotherapy to the respective area. For elderly patients with bulky disease, the results of the RICOVER-noRX study also suggest a benefit of additional radiotherapy, at least in patients achieving a PR or less. While skeletal involvement (localized or diffuse) was not a risk factor in the pre-rituximab era, it evolved as such when rituximab was given. Involvement of the central nervous system (CNS) is a serious and mostly fatal complication of DLBCL and remains to be so in the rituximab era. Several retrospective studies suggest that iv. HD-MTX can reduce the incidence of CNS involvement in patients at increased risk. The situation is less clear in younger patients for whom a group at significant risk for CNS involvement (elevated LDH, advanced stage) develops CNS disease in only 6.5% of the cases.

Chromosomal instability and changes confer a worse prognosis, and the expression of certain microRNAs and proteins has been reported to be

associated with a favorable (BCL6, CD10, HIF-la, HLA-DR, IRF4/MUM1, LM02; CD30) or an adverse (BCL2, CD5, indolamine 2,3-dioxygenase, high Ki-61, mutated p53, VEGFR2, Skp2) outcome.

In contrast to «single molecules, the analysis of the entire exome by GEP studies identified three biologically and prognostically relevant subtypes of DLBCL: the activated B cell (ABC)-like DLBCL, the germinal center (GC)-like, the mediastinal large B-cell lymphoma based on cell-of-origin (COO) gene signatures, with the activated B-cell (ABC) type being associated with an inferior outcome compared to the germinal center (GC) type.

ABC- and GC-like DLBCL differ with respect to the cell of origin, pathogenetic mechanisms and prognosis: the GC/non-GC was shown to be a prognostic factor independent of the IPI in patients treated with CHOP only, the GEP added to the predictive power of the IPI, the IPI added to the predictive power of the GEP in patients treated with CHOP-R.

In many B-cell lymphomas, chromosomal translocations are biological and diagnostic hallmarks of the disease. A subset of these lymphomas has structural aberrations affecting the myc locus that is associated with a poor prognosis independent of clinical risk factors. MYC- break positive DLBCL cases may also co-express high levels of BCL2, and up to half of these cases have a concurrent translocation involving BCL-2. These double-hit (DH) lymphomas are defined by a chromosomal breakpoint affecting the MYC/8q24 locus in combination with another recurrent breakpoint, *e.g.* a t(14; 18)(q32;q21) involving BCL2.

Regimens effective in Burkitt's lymphomas that incorporate HD-MTX such as the (CODOX-M/IVAC regimen), will improve the outcome of DH lymphomas.

In contrast to MYC translocations, observed in 5% of the cases and had a median OS of less than one year, MYC protein expression was associated with an inferior PSF and OS only when BCL2 protein was co-expressed. The patients with DLCBL co-expressing MYC and BCL2-proteins by IHC have a poor prognosis.

E4.**ADULT T-CELL LEUKEMIA/LYMPHOMA, A CHALLENGE OF DIAGNOSIS AND TREATMENT**

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Adult T-cell leukemia/lymphoma is an aggressive neoplasia, characterized by proliferation of activated peripheral T-cell lymphocytes, promoted by HTLV-I (human T-cell lymphotropic virus I) retrovirus infection.

The endemic areas for HTLV-I infection, which also have the largest experience with ATLL, are Japan, Carribean, Central and South America, Tropical Africa, and also Romania. The incidence of infection in Europe in low. The studies show that there are around 20 million HTLV-I positive adults and children worldwide, out of which 1-4% will develop ATLL, in around 30-50 years after the infection. The primary event is the viral trigger, but the lymphoproliferation becomes subsequently independent from the viral stimulation.

ATLL has four major clinical presentations: acute-leukemic, lymphomatous, chronic and smouldering. The first two forms are the most frequent and the most aggressive, with poor prognosis, a medium survival of months, severe immunosuppression and intrinsic chemoresistance – poor response to classical CHOP regimen, the conventional therapy still widely used in Europe; this response in frequently followed by relapse.

Although there are no wide trials, the studies of the Japan Clinical Oncology Group (JCOG) proposed combined chemotherapy regimens with higher efficiency such as LSG15 - VCAP-AMP- VECP. This protocol had a complete response (CR) significantly higher than CHOP-14, but a similar rate of overall response (OR), with a higher toxicity for LSG15. The 3-years survival is superior for LSG15 (24 vs 13% for CHOP-14). Still, because vindesine and ranimustine and not available usually (including in Romania), some authors propose Hyper-CVAD regimen, although with a lower efficiency.

Recent studies introduce humanized monoclonal antibody anti-CCR4 mogamulizumab, added to LSG15 regimen, with a higher rate of CR (~50%) and OR (~85%), especially efficient for acute and lymphomatous forms, with a longer median survival (~8.6 months), without an increase of toxicity. On the other hand, mogamulizumab is not widely available outside Japan.

The treatment of acute and lymphomatous forms must include prophylactic intrathecal chemotherapy, because there is a 10-25% incidence of CNS involvement.

Other treatment options may include antiviral therapy with interferon and zidovudine, especially for leukemic forms, but also for chronic and smouldering ones, for which long term survival is notably improved. Nevertheless, since the HTLV-I retrovirus is usually latent in ATLL patients, the efficiency of the antiviral therapy probably has a different mechanism.

There are also some small studies which discuss a (limited) efficacy in ATLL for other therapies, such as: alemtuzumab, arsenic trioxide with or without interferon, all-trans-retinoic acid, pralatrexate (anti-

folate), bortezomib, forodesine (purine nucleoside phosphorylase inhibitor), histone deacetylase inhibitor, or lenalidomide.

In spite of all these, for acute and lymphomatous forms, allogeneic stem transplantation remains the only option with a better rate of long term response.

E5. **B-CELL RECEPTOR SIGNALLING. PATHOGENETIC AND THERAPEUTIC IMPLICATIONS IN B-CELL LYMPHOPROLIFERATIONS.**

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B-cell lymphoproliferations account for approximately 90% of all lymphoid malignancies in our geographical area. Over the past decade, a decrease in mortality has been noted, mainly due to the incorporation in chemotherapy regimens of the anti-CD20 monoclonal antibody rituximab. However, rituximab represents only a semi-targeted therapy, targeting both malignant and non-malignant CD20 positive cells; besides, CD20 is not part of any significant malignancy-related signaling pathway.

B cells produce immunoglobulins (Ig) that specifically bind antigen and that are initially expressed on cell surface (sIg). sIg cooperate with the Ig-alpha (CD79a) and Ig-beta (CD79b) chains to form the B-cell receptor (BCR) complex. Antigen binding to BCR leads to downstream events, leading to the phosphorylation of several nuclear effectors that activate transcription, cause growth and proliferation of the B cell and memory cell formation. BCR signaling also leads to pathologic B-cell clone amplification and contributes to disease initiation and progression in several B-cell malignancies as well as in some B-cell dependent autoimmune diseases. The main lymphoid malignancies that are BCR-signaling dependent are chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL).

Lately, BCR signaling has been investigated as an attractive target for small molecules, due to the fact that several downstream BCR pathway components are tyrosine kinases, especially the Lyn, Syk and Btk kinases. Several molecules targeting these kinases are currently in clinical trials – dasatinib (anti-Lyn, Btk), fostamatinib (anti-Syk), ibrutinib (anti-Btk), GS-1101 (anti-p110s). The anti-Btk (Bruton tyrosine kinase) inhibitor, ibrutinib, is currently the most promising clinically-tested BCR pathway inhibitor. Several phase 1 and 2 clinical studies have shown very promising results, especially in the setting of relapsed/refractory CLL and MCL and phase 3 trials are currently under way.

In conclusion, targeting the BCR pathway with small-molecule tyrosine kinase inhibitors may be incorporated in B-cell malignancy armamentarium as an "intelligent" approach in the near future.

E6. **MANAGEMENT OF ACUTE PROMYELOCYTIC LEUKEMIA IN 2013.**

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Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by the morphology of leukemic blasts (abnormal promyelocytes), a life-threatening coagulopathy combining disseminated intravascular coagulation, fibrinolysis and fibrinogenolysis, specific reciprocal translocation t(15;17) fusing the promyelocytic leukemia gene (PML) to the retinoic acid receptor alfa (RAR), specific sensitivity to the differentiating effect of all-trans retinoic acid (ATRA) and the proapoptotic effect of arsenic trioxid (ATO). The introduction of ATRA and, more recently, ATO into the therapy of APL has revolutionized the management and outcome of this disease. The treatment strategies using this agents in combination with chemotherapy (CT) have provided excellent therapeutic results: high CR rates (90-94%) and high 5 year DFS rates (> 74%). Once a diagnosis of APL is suspected upon morphologic criteria the disease should be managed as a medical emergency that requires the following rapid and simultaneous actions: a) start treatment with ATRA without waiting for genetic confirmation of diagnosis; ATRA is known to rapidly ameliorate the coagulopathy; b) initiate supportive measures to conteract the coagulopathy and decrease the risk of fatal hemorrhage. Coagulopathy should be treated rapidly with fresh frozen plasma, fibrinogen or cryoprecipitate and platelet transfusions to maintain fibrinogen concentration above 100-150 mg/dL and platelet count above 30-50 x 10^9/L, which should be monitored at least once a day. This measures should be more agresive in patients with higher hemorrhagic risk

(older patients, patients with hyperleukocytosis, patients with increased level of creatinine); c) confirm diagnosis in bone marrow at the genetic level. Demonstration of the t(15;17) or its counterpart, the PML/RAR hybride gene by conventional karyotyping, FISH or RT-PCR is mandatory because the efficacy of differentiation treatment based on retinoids is strictly dependent on the presence of the PML/RAR fusion in leukemia cells. RT-PCR is the "gold standard" approach for confirming a diagnostic of APL. RT-PCR allows definition of the type of PML-RAR isoform and the target for monitoring MRD. Morphologic diagnosis in bone marrow, although highly predictive of the specific genetic lesion in hypergranular tipical cases is considered insufficient. A morphological suspicion of PML-RAR positive APL can be remforced by the study of the characteristic immunophenotypic features of blast cells by multiparameter flow cytometry. Once the diagnosis has been confirmed at the genetic level, targeted induction therapy should be promptly started with ATRA combined with anthracycline based-chemotherapy. 20% patients with APL treated with ATRA (and ATO) can experience the APL differentiation syndrome and treatment with dexamethasone should be promptly started at very earliest sign or symptom. Distinct from AML, early morphologic evaluation of bone marrow has no value in APL. Morphologic features in bone marrow during differentiation therapy can be misleading and some-times erroneously interpreted as resistence. This features showing delayed maturation or persistance of atypical promyelocytes are occasionally detectable several weeks after the start of treatment (up to 50 days). An accurate assessment of RT-PCR status of the end of consolidation is crucial because patients who show residual PML/ RAR transcripts at this time point are candidate for further intensification, whereas those who test PCR negative would proceed to receive maintenance. The small fraction of patients who test PCR-pozitiv for the PML/RAR hybride gene at the end of consolidation (molecular persistance) have a dismal prognosis and should receive additional therapy aimed at obtaining molecular remission including ATO, novel agent auto or allogeneic hematopoietic stem cell transplantation (HSCT). For patients with hyperleucocytosis is reasonable to recomand a stringent monitoring, at least every 2 months in the early postconsolidation period and there after every 3 months for two years. The APL status has evolved from highly fatal to highly curable.

IE7. **MOLECULAR PROGNOSTIC FACTORS IN ACUTE LEUKEMIA**

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In hematological malignancies, acute leukemia has a special place due to adverse prognosis and clinical course. Introduction of new molecular biology methods has identified a number of prognostic factors that allow stratification of patients into risk groups. Using these prognostic factors into clinical practice has made the treatment of acute leukemias currently be considered an example of personalized medicine.

Materials and methods: We have analyzed 290 cases of acute leukemia (adults and children) admitted to our institute in the period 2008-2012.

Samples were analyzed using multiplex RT-PCR that allows identification nine of the most common fusion genes in acute leukemias: MLL-AF4, BCR-ABL1, AML1-ETO, PML-RARA, CBFb-MYH11, SIL-TAL1, E2A -PBX1, TEL-AML1 and MLL-AF9. AML samples were analyzed for insertion in tandem FLT3 gene (FLT3-ITD mutation) and C-teminal region insertions in the NPM1 gene. For AML samples were also analyzed for the expression of WT-1 gene. Quantification of transcript and minimal residual disease were determined using Nested RT-qPCR and for Fusion genes identified.

Results: Using nine fusion genes as a prognostic marker allowed stratification into risk groups 36% of patients (105 of the 290 cases analyzed). For patients with AML, in 26% of cases tested were identified insertions in the C-terminal region of the gene NPM-1 and in 18.2% of cases were found FLT3-ITD mutation. Minimal residual disease follow-up was done using RT-qPCR in 36% of cases and this allowed early identification of cases with relapse or treatment resistant. In acute myeloid leukemia, the level of WT-1 expression is a prognostic marker and, also, a marker for monitoring minimal residual disease in 22% of cases.

Conclusions: The molecular biology methods used in this work have allowed the classification of patients in risk groups and the use of adapted risk therapeutic modalities - standard chemotherapy followed or not by allogeneic bone marrow transplant. On the other hand, monitoring of minimal residual disease allows the

patient's risk reassessment after treatment (or after certain phases of treatment) and early identification of cases with relapse or treatment resistance.

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E8. REFRACTORY / RELAPSED MULTIPLE MYELOMA - STANDARD AND CLINICAL TRIAL THERAPY

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Multiple myeloma (MM) is by definition a chronic disease that usually requires more than one regimen. So the second line treatment is necessary in most cases of multiple myeloma.
In practice, we meet two groups of patients with MM who require second-line therapy:
- MM refractory - nonresponsive disease, or progression within 60 days after the last treatment
- MM relapsed - the disease progresses to more than 60 days after first line therapy and require treatment
First-line treatment is standardized in therapeutic protocols, and usually include strengthening auto / allo stem cell transplant (SCT) to eligible patients. But complete answer does not exceed 20%.
International treatment guidelines recommendations (ESMO) are usually vague and general practitioner needs explicit customized recommendations.
In this context, second-line therapy in myeloma refractory / relapsed should be assessed and customized according to the time of relapse, the associated comorbidities, including post-induction complications and eligibility for stem cell transplantation.

Recommended therapeutic guidelines mention the possibility of using the initial therapy in case of relapse in distance, especially new molecules (thalidomide, bortezomib, lenalidomide) in combination.

An example of the second-line treatment protocols is shown in the HOVON guidelines which recommended retreatment if relapse occurred more than 1 year and at least VGPR type response (very good partial response). Otherwise, the combination of bortezomib in 11 cycles or lenalidomide until progression.

A more detailed guide is in the NCCN guidelines, which direct second-line therapy in stages, depending on eligibility for transplantation, the response to previous therapy, comorbidities.

Selection regimens must be a rational process based on patient characteristics.
Patient selection is based on:

- patient characteristics, cytogenetics, FISH, flow (NCCN)
- Renal function
- performance score
- preexisting peripheral neuropathy
- cytopenia, anemia, and gastrointestinal toxicity may limit treatment

Supportive treatment is also an important aspect in the treatment of patients with multiple myeloma.
The combinations available are different for refractory / relapsed MM:

- Bortezomib
- Bortezomib / dexamethasone
- Bortezomib / liposomal doxorubicin
- Bortezomib / thalidomide / dexamethasone
- Cyclophosphamide / bortezomib dexamethasone
- Dexamethasone / cyclophosphamide / etoposide / cisplatin (DCEP)
- Dexamethasone / thalidomide / cisplatin / doxorubicin / cyclophosphamide / etoposide (DT PACE) ± bortezomib (VTDPACE)
- High-dose cyclophosphamide
- thalidomide / dexamethasone
- Melphalan / Prednisone
- Melphalan / Prednisone / Thalidomide
- Bortezomib / Melphalan / Prednisone / Thalidomide
- Vincristine / Adriablastine / Dexamethasone (VAD)
- Auto / allo SCT

Besides the combination with established drugs, clinical trials are an important option that allows access to certain therapies. The actual use of lenalidomide is possible in most clinical trials with the introduction of new drugs: carfilzomib, elotuzumab, pomalidomide, bendamustine, vorinostat, Tanespimycin, etc..
I n conclusion, lenalidomide and bortezomib regimens are effective in relapsed / refractory myeloma, and using weekly bortezomib decreases the risk of discontinuation. Also, the combination of thalidomide in refractory myeloma combinations should be supplemented with the increasing number of drugs in combination. Clinical trials are recommended as the first option for eligible patients. Stem cell transplantation should be reviewed in all patients with relapsed multiple myeloma.

E9. THE PORPHYRIAS.

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Traditionally, the porphyrias have been classified as either hepatic or erytropoiethic, depending on the primary site of the overproduction and accumulation of porphyrin and porphyrin precursors, although some porphyrias have overlapping features. For simplicity, we have classified the eight major porphyrias into three groups: (1) the four acute hepatic porphyrias, (2) the single hepatic cutaneous porphyria (PCT) and (3) the three erythropoietic cutaneous porphyrias.

The acute hepatic porphyrias includes AIP,HCP,VP (AIP is the most common). The major manifestations of these disorders are acute neurological attacks, abdominal pain, cramps, constipation, abdominal distension, increased bowel sounds, nausea, vomitting, tachicardia, hypertension, menthal symptoms, chest pain, headache, muscle weakness, tremors,disuria, bladder distension. Once a biochemical diagnosis is established , mutation analysis of the genes for AIP (HMBS), HCP (CPOX),VP (PPOX) and then ADP (ALAD) should be undertaken.

CEP is an AR porphyria that results from deficient activity of URO-synthase and results in the accumulation of Uroporphyrin I and Coproporphyrin I isomers. The skin areas overexposed to sunlight are friable and blisters and vesicles are prone to rupture and infection. Skin thickening, focal hypo- and hyperpigmentation and hyperthricosis of the face and extremities are characteristic. The diagnosis is confirmed by the demonstration of significantly deficient URO-synthase activity or the identification of specific mutations of the UROS-gene.

The knowledge of these advances is relevant for hematologists because they administer the hematin infusions to treat the acute attacks in patients with acute hepatic porphiryas, perform the chronic phlebotomies to reduce iron overload, clear the dermatologic lessions in PCT, diagnose and treat the erithropoietic porphiryas, including chronic erythrocyte transfusions, B.M. or SCT transplants and experimental pharmacologic chaperone and stem cell gene therapies for CEP. These developments are relevant to update hematologists on the latest advances in these diverse disorders.

E10. THE CLASSIC BCR/ABL–NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (MPN, MPD): ESSENTIAL THROMBOCYTHEMIA. ACTUALITIES IN DIAGNOSIS, PATHOGENESIS AND TREATMENT.

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The myeloproliferative disorders or neoplasms (MPN) are heterogeneous diseases that occur at the level of a multipotent hematopoietic stem cell. They are characterized by increased blood cell production and virtually normal cell maturation. Essential thrombocythemia (ET) is currently classified, with the polycythemia vera (PV), and primary myelofibrosis (PM) as a classic myeloproliferative neoplasms (MPN) BCR/ABL negative. ET is characterized by persistent thrombocytosis, excessive proliferation of megakaryocites in the bone marrow, normal erythrocitic mass and the absence of prominent bone marrow fibrosis.

The molecular pathogenesis of the MPN has been poorly understood until 2005, when an unique acquired clonal mutation in *JAK2* was reported by five different research teams in around 50% of patients with ET, PM and the vast majority of PV.A unique valine to phenylalanine substitution at position 617 (V617F) in the pseudokinase autoinhibitory *JAK2* domain causes the constitutive activation of the *JAK2*-STAT signaling pathway and leads to autonomous cell growth in a cytokine - independent manner. *JAK2V617F* has been adopted in the new WHO diagnostic criteria for ET, PV and PM.

Up to one-half of patients with ET may be totally asymptomatic at presentation. The remaining patients may report “vasomotor“ symptoms or manifest thrombotic and hemorrhagic complications, which are the main causes of morbidity and mortality in ET. Most patients with ET enjoy a normal life expectancy, without associated disease-related complications. The delayed development of either acute myeloid leukemia or post- ET myelofibrosis is unusual, 2 and 4 percent respectively.

The optimal therapeutic strategy intended to prevent vascular events is depended on the presence or absence

of thrombotic risk factor, requiring prognostic stratification in risk groups depending on age, thrombotic history.

BONE MARROW TRANSPLANTATION SESSION

T1. LATE COMPLICATIONS AFTER ALLOTRANSPLANTS WITH HEMATOPOIETIC STEM CELLS OBSERVED IN THE FUNDENI CENTER OF BONE MARROW TRANSPLANTATION – BUCHAREST.

Dan Coliţă, Zsofia Varady, Alina Tănase, Oana Crăciun, Alexandra Mărculescu, Didona Vasilache, Camelia Dobrea, Daniel Coriu
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Between 2003 and 2013 in our Center were done 457 auto- and allotransplants with peripheral hematopoietic stem cells (380 and 77 respectively). We report here the observations regarding the adult patients (18 – 60 years) who received allografts. These were collected from HLA-identical donors (60 brothers/ sisters and 2 unrelated). The majority of cases (41) were acute leukemias (myeloblastic > lymphoblastic > biphenotypic). The other cases were severe aplastic anemias (7), lymphomas Hodgkin and non-Hodgkin (5), chronic myelocytic leukemias (4), myelofibrosis with myeloid metaplasia (2), lymphocytic chronic leukemias (2), refractory anemia (1). The late complications after transplantation were considered those who supervene, after 100 days from the transplantation. The conditioning treatments were myeloablatives [BuCy – 25, TBI in association – 12] and, also, nonmyeloablatives – Fludarabine – based. All 7 cases of SAA received ATG-based schedules. The grafting was recorded in 10-14 days. At the boundary of 100 day after the transplantation all patients present 97 – 100% donor chimerism a performance IK of 70 – 100%, normal temperature and received immunosupression (Cyclosporine, Tacrolimus, Mycophenolate Mofetil). The complications recovered after the day + 101 were the chronic form of graft – versus – host disease) (cGvHD), menstrual disfunctions, hypothyroidia, malabsorbtion/malnutrition, cataract, xerostomia, xerophtalmia, postcorticotherapy diabetes melitus, hirsutism and dislipidemia. In 7 cases were recorded the reactivation of CMV infection. The most frequent and severe was cGvHD. It supervened in 22 cases independently of the conditioning schedule and drove to exitus in 2 cases. In 7 cases cGvHD arrive de novo. In the other 15 cases at was preceded by the acute form installed immediatly posttransplant. At the end of the study 26 pts. were alive (41%). Other causes of exitus were the relapse of the initial disease (7), the graft rejection (2), the sepsis ± MSOF (9), IFI (1), hepatic failure (2), lung failure (3). All patients in life were in complete remission of the primary malignant disease. The overall survival is 17,2 months.

T2. MATCHED UNRELATED BONE MARROW TRANSPLANTATION IN A CASE OF SEVERE APLASTIC ANEMIA WITH MAJOR BLOOD GROUP INCOMPATIBILITY.

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We present the case of a 19 year old male patient with severe aplastic anemia with no response to immunosuppressive treatment and no HLA matched family donor.

We obtained through the Romanian Registry of Voluntary Donors from the Polish Registry fully matched HLA compatible donor with major blood group incompatibility the donor being B+ and the recipient O+. We obtained bone marrow with a number of 681.04×10^8 nucleated cells and 3.63×10^6/kg CD34+ stem cells in a quantity of 1875 ml. The bone marrow had to be processed with the Cobe Spectra Aphaeresis Machine for the erythrocytes and plasma depletion. The conditioning consisted in Fludarabin,Cyclophosfamide, ATG and the immunosupression with tacrolimus and low dose methotrexate according to the M.D. Anderson protocol. We present the difficulties, complications and the results of this case.

T3. CMV MONITORING AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION. THE EXPERIENCE OF BONE MORROW TRANSPLANT DEPARTAMENT I.C. FUNDENI JANUARY 2011 – JUNE 2013.

Alina Tănase, Anca Coliţă, Ileana Constantinescu, Ana Moise, Laura Ştefan, Oana Crăciun, Zsofia Varady , Dan Coliţă
Bone Marrow Transplantation Center, Fundeni Clinical Institute, Bucharest, Romania

Introduction:

Despite recent progress regarding the diagnosis and

the development of prophylactic, preemptive and curative treatment, cytomegalovirus (CMV) infection is still a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT).
Patients and methods:

We monitored with qPCR DNA-CMV, from January 2011 to June 2013, 54 patients after allogeneic HSCT.
Standard prophylactic treatment was Acilcovir 1000mg per day per os during transplant procedure and 180 days after allogeneic HSCT.
Primary CMV infection – was defined as one positive PCR assays in a previously seronegative patient.
Recurrent CMV infection – was defined as one positive PCR assays detected in a seropositiv patient.

High risk or standard risk patients category – was defined based on CMV serology before allogeneic HSCT in receptor and donor. CMV seropositive patients with CMV seropositive or seronegative donor are considered high risk.
We assessed – patient and donor CMV status before allogeneic HSCT, fever, cytopenias and severity of cytopenias, treatment and days of treatment.
Preemtiv treatment was Valganciclovir 2 x 5 mg/kg/day.
qPCR DNA-CMV results were recorded as number of viral copies per milliliter.
Results:

Eleven of 54 (20%) patients developed at least one positive PCR assay
All the data below regards the 11 patients with at least one positive PCR assays.
Nine of 11 (82%) patients with at least one positive PCR belonged to high risk category.
Two of 11 (18%) patients developed primary CMV infection. The remaining 9 (82%) recurrent CMV infection.

Six of 11 (54,5%) patients developed CMV positive PCR in first 180 days after allogeneic HSCT.
Fever was present in 4 of 11 (36%) patients . Eight of 11 (73%) patients presented thrombocytopenia oh which 25% severe thrombocytopenia. One of 11 (9%) patients presented neutropenia with no case under 500 neutrophils/mm^3. Five of 11 (45%) patients presented anemia.

One of 11 (9%) patients presented pancytopenia.
All patients with positive PCR received preemptive treatment (Valgancyclovir 2 x 5mg/kg per day).
Median time of treatment was 27 days.
Three of 11 (27%) patients acute or chronic GVHD occurred together with CMV positive PCR.
Conclusions:

CMV monitoring after allogeneic HSCT is an important standard in the following up of the patient, allowing early treatment of CMV reactivation or primary infection.

T4. DONOR LYMPHOCYTE INFUSION - THERAPEUTICAL METHOD IN ALLOGENEIC STEM CELL TRANSPLANTATION. FUNDENI EXPERIENCE

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Allogeneic stem cell transplantation is a therapeutic method with curative intent in hematological malignancies, but also in non-malignant illnesses. From the '90 the graft versus leukemia effect is proved as the NK and T lymphocytes action. They could react with major or minor histocompatibility antigens and also with tumor specific antigens. The donor lymphocytes infusion (DLI) could be used as prophylactic or curative cell therapy reinforcing the antileukemic effect of the graft for those who relapsing after transplant.
In our center 79 allogeneic stem cell transplants (64 adults and 15 children) were performed between 2001 and september 2013. Three of them were with unrelated donor stem cells, in collaboration with our Romanian National Stem Cell Registry.

The diagnosis of the 64 adult patients were: 27 AML; 12 ALL; 4 biphenotipic AL; 7 severe aplastic anemias; 5 lymphomas; 4 CML; 2 MMM; 2 CLL and 1 MDS. From these 64 adult cases we had 11 relapses and 2 rejections. We used DLI in 5 cases (in the case of the 2 rejections, 2 AML and 1 ALL).
From these 5 cases were we used DLI, one case were successful. It was a 28 years old female patient with AML2 in CR1, transplanted from her sibling donor with Bu/Cy conditioning. She had an early relapse at 7 months and she received chemo 2+5 with a new complete remission and 2 doses of DLI at 30 days interval. She developed a limited chronic GVHD responsive to Metilprednisolon. Now she is in remission with complete chimerism at 24 months after the second dose of DLI.

T5. APLASTIC ANEMIA IN CHILDREN AND ADOLESCENTS –RESULTS OF STEM CELL TRANSPLANTATION, FUNDENI TRANSPLANT CENTER EXPERIENCE

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Background. Aplastic anemia (AA) is a rare disease in children; it's most commonly idiopathic and less often due to a hereditary disorder. However, hereditary bone marrow failure syndromes should be considered in both children and adults before the institution of any therapeutic treatment plan. Hematopoietic stem cell transplantation (HSCT) is the only treatment that definitively restores normal hematopoiesis. HSCT is appropriate: (1) as initial definitive treatment when a HLA-identical sibling donor is available in a patient with Severe aplastic anemia (SAA) or Very severe aplastic anemia (VSAA), (2) after failure of 1 or (traditionally) 2 courses of immunosuppressive therapy (IST) in a patient with SAA or VSAA who has an acceptably matched alternative donor, (3) in patients with constitutional Aplastic anemia who are transfusion dependent and who have a HLA-identical donor related or unrelated.
Aim. We analyzed the transplant indication, complications and results in a cohort of 9 children with AA transplanted or followed in Fundeni Clinical Institute.
Patients & Methods. Nine patients with AA - 5 males, 4 females, age range 4-13 years - were transplanted and monitories in Fundeni Clinical Institute, Pediatric Bone Marrow Transplant Department between 2005-2013. The donor was in 6/9 cases a HLA identical sibling and in 3/9 cases a 10/10 compatible unrelated donor. In 3 cases the transplant procedure has been performed abroad – 2 cases - Israel, 1 case – Italy. The stem cell source was: bone marrow (BM)- 1/9, cord blood (CB)- 1/9, peripheral stem cells (PSC) – 7/9. The conditioning regimen included ciclophosphamide, rabbitt ATG, +/- Fludarabine. GVHD prophylaxis consisted in standard CsA and short MTX with Leucovorin rescue. Chimerism analysis has been performed using STR technique.
Results. Follow-up range was 120-1660 days. Five patients are alive, in good clinical condition. The chimerism analysis shows in 4/5 cases complete donor chimersim, in 1/5 cases stable mixed chimersim. We registered 4/9 deaths: graft failure – 1 case, graft rejection – 1 case, hemophagocitic syndrome-1 case, infection – 1 case. Only 3 patients developed GVHD and received immunosuppression.
Discussions/Conclusion. The distinction between constitutional and acquired aplastic anemia is critical for the choice of the best conditioning regimen: reduced intensity regimen including Fludarabine for constitutional and standard CFA-ATG for acquired aplastic anemia. The number of blood transfusions before transplant seems to be the most important prognostic factor for engraftment.

T6. DHAP VS. IGEV AS MOBILIZATION TREATMENT IN PATIENTS WITH LYMPHOMAS- FUNDENI CLINICAL INSTITUTE EXPERIENCE

Alexandra Mărculescu, Carmen Eleonora Călugaroiu, Alina Daniela Tănase, Zsofia Varady, Oana Gabriela Crăciun, Daniel Coriu, Anca Coliţă, Luminiţa Dumitrache, Constantin Arion
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This work represents a 3 years retrospective study (January 2010 - December 2012) that wants to compare different mobilization regimens used in patients with different forms of malignant lymphomas.
Material and Methods:
There have been accomplished 154 hematopoietic stem cells harvest for 146 patients with different forms of non-Hodgkin's lymphomas (LMNH) or different forms of Hodgkin's Disease (BH) with ages between 6 and 61 years in Clinical Institute Fundeni, in the period mentioned above.
In most cases a single apheresis procedure was enough, but 2 procedures were needed in 4 patients and 3 apheresis procedures were needed in a single patient.
DHAP regimen was used in 73 patients (40 females with ages between 12 and 56 years and 33 males with ages between 17 and 61 years); R-DHAP regimen was used in 4 females from this cohort.
IGEV regimen was used in 42 patients (21 females with ages between 21-53 years and 21 males with ages between 11-55 years); R-IGEV was used in 1 female and 1 male from this cohort.
HD-Etoposide regimen was used in 10 patients (5 females with ages between 19-58 years and 5 males with ages between 25-48 years).
Others regimens (R-ICE, HD-CFA, Ifosfamide-Vinorelbin-Dexametasone, Ifosfamide-Idarubicine-Etoposide, CHOP) were used as a mobilization treatment for a few patients in which neither DHAP nor IGEV regimen could be used for objective reasons.
Only G-CSF +/- Plerixafor alone (without

chemotherapy) was used in 5 patients (2 females with ages 28 and 42 years and 3 males with ages 14-25 years).
Results and Conclusions:
In (R)-IGEV cohort: 2,38% (1 patient) needed 5 days G-CSF; 11,90% (5 patients) needed 6 days G-CSF; 52,39% (22 patients) needed 7 days G-CSF; 16,67% (7 patients) needed 8 days G-CSF; 9,52%(4 patients) needed 9 days G-CSF; 4,76% (2 patients) needed 10 days G-CSF; 2,38% (1 patient) needed 12 days G-CSF. The smallest graft = 1,65 x 10^6 CD34+cells/ body weight recipient and the largest graft = 56,63 x 10^6 CD34+cells/b.w recipient in this cohort.
In (R)-DHAP cohort: 1,37% (1 patient) needed 5 days G-CSF; 8,22% (6 patients) needed 6 days G-CSF; 27,4% (20 patients) needed 7 days G-CSF; 32,88% (24 patients) needed 8 days G-CSF; 12,33% (9 patients) needed 9 days G-CSF; 12,33% (9 patients) needed 10 days G-CSF; 1,37% (1 patient) needed 11 days G-CSF; 4,1% (3 patients) needed 14 days G-CSF. The smallest graft = 1,51 x 10^6 CD34+ cells/b.w recipient and the largest graft = 31,28 x 10^6 / b.w. recipient in this cohort.
In conclusion, there is no significant differences between DHAP and IGEV regimen as mobilization treatment, although it seems that IGEV has a small advantage over DHAP.

T7. THE NATIONAL PROGRAM FOR UNRELATED STEM CELLS TRANSPLANT – PRESENT AND PERSPECTIVES.

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Romanian National Registry of Hematopoietic Stem Cell Voluntary Donors (NRHSCVD)

This document sets out the NRHSCVD Strategic Plan for 2013-2014. The plan is based on the consultation of the Scientific Council of NRHSCVD, staff consultation network of healthcare facilities that performing activities coordinated by NRHSCVD (HSC Donor Centers, HLA Laboratories, Collection centers and Transplant centers HSC).
It is aimed at implementing the NRHSCVD Mission and Vision, in compliance with standards and policy of World Marrow Donor Association (WMDA) (http://worldmarrow.org/), which envisages:
1) To assure efficient provision of the hematopoietic progenitor cells for patients in need of a stem cell transplantation and to undertake the intervention in one of the Transplant Centres in the country.
2) To increase public and professional awareness of donation of hematopoietic stem cells as means to help patients;
3) To implement the international quality standards and procedures through education and studies;
3) To develop the governance structure for effective and accountable implementation of the mission and;
4) To maximize the available resources.
Present situation and the actual results after the first 6 months of implementation, difficulties identified and lessons learned were taken into account to determine future prospects for achieving the targets.

T8. THE ROLE OF ANTITHROMBIN-III THERAPY IN REDUCTION OF HEMATOPOIETIC STEM CELL TRANSPLANTATION RELATED TOXICITY.

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Introduction: Antithrombin III (ATIII) is a potent inhibitor of the coagulation cascade. It is a nonvitamin K-dependent protease that inhibits coagulation by lysing thrombin and factor Xa. Antithrombin III's activity is markedly potentiated by heparin; potentiation of its activity is the main mechanism by which both heparin and low molecular weight heparin result in anticoagulation.

Acquired antithrombin III deficiency is a deficiency of antithrombin primarily due to consumption. It is observed in situations in which activation of the coagulation system is inappropriate. Common conditions that result in acquired antithrombin III deficiency include disseminated intravascular coagulation (DIC) , microangiopathic hemolytic anemias due to endothelial damage and veno-occlusive disease in patients undergoing allogeneic bone marrow transplantation.

A hypercoagulable state has been shown to follow high-dose chemotherapy for bone marrow transplantation (BMT). Deficiency of the natural anticoagulants, antithrombin III (ATIII), protein C and protein S correlates with organ dysfunction following bone marrow transplantation (BMT).

Material and methods: We treated 8 patients pre-emptive or with severe post-BMT organ dysfunction

with ATIII concentrate (Baxter AT III). Indications for treatment included ATIII anticoagulant level less than 80% and life-threatening single or multiorgan dysfunction. All patients were loaded with 50 units/kg ATIII/ day for 3-5 days. Clinical improvement was seen within 1-7 days of start of therapy in all patients.The study group was composed of 4 childrens (age range 3 years 7 months-16 years) and 4 adults (age 35-56) who underwent allogeneic peripheral stem cell transplantation from February 2012 to March 2013: 6 patients from related matched donors and 2 from unrelated matched donors.The underlying disease was: myelo-monocytic AML 2 cases, B-ALL 3 cases, aplastic anemia 1 case, JMML 1 case, beta thalasemia major 1 case. Total and conjugated bilirubin, aspartame, alanine and γ-glutamyl transferase, prothrombin time, activated partial thromboplastin, protein C and S and fibrinogen were determined at baseline and three times per week thereafter until at least day +30 after HSCT. ATIII activity (normal range 70-120%) was measured at least three times per week beginning prior to conditioning (baseline) and ending at day +40.

Results: From the study group 6 patients received pre-emptive replacement with ATIII, 1 patient in condition of multiorgan failure and 1 in confirmed VOD.Four from the pre-emptive group are in remission after day+100, one relapsed and died from progressive AML at 6 months after BMT, one died at day +45 by hemofagocytic syndrome.In cases of graft failure and multiorgan dysfunction and VOD death occured at day +64 respectively +38.

Patients receiving pre-emptive or therapeutic AT-III replacement therapy had no detectable toxicity or adverse effects.

Conclusions: Significant improvements in organ dysfunction following ATIII treatment in this small study supports a causal relationship between ATIII deficiency and post-BMT chemotherapy-induced organ dysfunction. In conclusion, the encouraging results of this study suggest that this antithrombin III treatment should be further considered in patients with allogeneic bone marrow transplantation with or without defibrotide.

T9. **IMPAIRMENT OF LIPID METABOLISM AFTER BMT.**

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Background: Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. Dyslipidemia may be manifested by elevation of the total cholesterol, the "bad" low-density lipoprotein (LDL) cholesterol and the triglyceride concentrations, and a decrease in the "good" high-density lipoprotein (HDL) cholesterol concentration in the blood. Dyslipidemia is a major vascular risk factor and cardiovascular disease is the main cause of mortality in the whole world.

Dyslipidemia comes under consideration in many situations including diabetes , a common cause of lipidemia , excessive alcohol consumption, cholestatic liver diseases, nephrotic syndrome, chronic renal failure, hypothyroidism, cigarette smoking, obesity, drugs . Most common classes of drugs that are used to treat hypertension and are known to cause dyslipidemia are diuretics , beta blockers and methyldopa, and chemotherapy for cancer. Dyslipidemia is one of late complications after BMT. Stem cell transplantation is a treatment for hematologic malignancies and some nonmalignant diseases, with significant improvement in survival. Also, a list of late complications is increasing with longer follow up times.

Aims: This study aims to analyze lipid profile in patients who followed BMT and to established their impact on long term survival.

Materials and methods: We analyzed 221 patients who underwent BMT between 2008 and 2013 in Bone Marrow Transplantation Department Timisoara . International recommendations for monitoring long-term survivors of HSCT suggested a series of tests from time to time. The patients were stratified by age, sex, race, type of BMT, source of stem cells, hematological disease, body mass index, another comorbidities like obesity, hypertension, diabetes. Laboratory parameters

were : total lipid level, LDL level, cholesterol level, triglyceride level, LDL-C and HDL-C .For changes in lipid metabolism the patients were treated with hipolipidic diet and oral agents : statins and fibrates.

Results: From 221 patients we had 72 with dyslipidemia: 71 with overproduction and 1 with deficiency.

The patient with hypolipidemia is a young boy, 12 years old at the time of transplantation for Hodgkin lymphoma , with vegetarian diet and levels of lipid below the lower limit of normal (Total lipid =3.7 g/l; Cholesterol = 2.6 mmol/L;Triglyceride =0.57 mmol/L)

The rest of the patients were with high levels of lipid and their dates are: 38 female patients and 33 male patients; repartition after age : 18-26 years : 8 patients, over 26 years :63 patients.62 cases with autologous BMT and 9 with allogeneic (3 with chronic hepatic GVHD) . Diagnosis was: acute lymphoblastic leukemia 4, acute myeloid leukemia 5, Hodgkin lymphoma 17, non-Hodgkin lymphoma 14, multiple myeloma 30, others 1.Patients with comorbidities: 29 with obesity, 20 with arterial hypertension, 12 with diabetes.Type of dyslipidemia: 4 patients with hypertriglyceridemia 4 (Mean value 2,63 mmol/ L), hypercholesterolemia 20 (Mean value 5,52 mmol/l), mixed dyslipidemia 47 patients. Treatment for dyslipidemia: diet 71 patients, statins 12, fibrates 4, statins+fibrates 1.

Summary/Conclusions: In our study, dyslipidemia occurs in 32,58% of patients with BMT, over 26 years old, approximately equal between men and women. The most common disorder was multiple myeloma, followed by associated with diabetes (16,90%) and hypertension (28,17%) and almost half with obesity (40,84%). All the patients were treated by diet and lifestyle changes and 23,94 % follows medications; lipid monitoring is performed every three months .

In conclusion, common cause of dyslipidemia, such as obesity, primary genetic lipid disorders, significant alcohol intake, uncontrolled diabetes, complications of the primary disease, treatment and transplantation can worsen dyslipidemia. Also, endocrine changes such as hypogonadism, hypothyroidism, deficiency in growth hormone after BMT may predispose to insulin resistance and metabolic syndrome. Chronic GVHD of the liver with severe cholestatic liver disease has been associated with severe hypercholesterolemia in adult and pediatric allogeneic patients. Drugs used to treat GVHD (Glucocorticoids, Cyclosporine, Tacrolimus, etc.) can cause dyslipidemia by altering the mechanism of lipid synthesis. Patients who develop dyslipidemia in an early or intermediate posttransplantation period may still have increased risk of cardiovascular events over the long term and they should be consider to add medication if lipid levels remain above goal for a patient's risk category.

There are two goals for lipid-lowering therapy: to reduce risk of future cardiovascular events and to prevent risk of pancreatitis in patients with severe hypertriglyceridemia. The American Heart Association guidelines recommend therapeutic lifestyle change (diet and physical activity) . Pharmacologic agents for dyslipidemia are statins and fibrates. A proper selection for treatment and dose is important because the drug-drug interactions (Ex. Cyclosporine-Statins) and the adverse events.

CLINICAL HAEMATOLOGY SECTION
ORAL PRESENTATION SESSION

C1.PRELIMINARY RESULTS OF THE CLL11 (BO21004) TRIAL

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Background:

Chronic lymphocytic leukemia (CLL) is characterized by the expansion of the malignant clone leading to compromised bone marrow and immune function. One important goal of therapy is to reduce the tumor burden and to restore normal haematopoetic function. In preclinical experiments, the novel type II, glycoengineered anti-CD20 mAb obinutuzumab (GA101) demonstrated increased B-cell depleting activity in blood, lymph nodes and spleen of cynomolgus monkeys compared to rituximab (R) (Mössner et al., Blood 2010). We participated in a large randomized, prospective phase 3 trial (CLL11, BO21004) in untreated CLL patients with coexisting medical conditions to compare outcome and safety of GA101+Clb (GClb) or rituximab+Clb (RClb) with Clb alone. During the safety run-in phase we observed a very rapid removal of lymphocytes from peripheral blood of six patients after first administration of GClb (Goede et al, Leukemia, 2013). We present the changes in B-cells, hemoglobin (Hb), platelets and neutrophils from baseline to end of treatment as observed in the stage 1 analysis of the CLL11 trial.

Methods:

Treatment-naïve CLL patients with a Cumulative Illness Rating Scale (CIRS) total score >6 and/or an estimated creatinine clearance (CrCl) <70 mL/min were eligible. Patients received Clb alone (0.5 mg/kg po d1, d15 q28 days, 6 cycles), GClb (100 mg iv d1, 900 mg d2,

1000 mg d8 and d15 of cycle 1, 1000 mg d1 cycles 2-6), or RClb (375 mg/m2 iv d1 cycle 1, 500 mg/m2 d1 cycles 2-6). The B-cells, Hb, platelet and absolute neutrophil count (ANC) counts were measured at baseline, during treatment and at the end of treatment. Immunoglobulin levels were measured in all patients at baseline and at the end of treatment.

Results:

The median number of treatment cycles received was 6 in all treatment arms and patients received similar total cumulative doses of Chlorambucil. Both RClb and GClb induced a rapid and more profound reduction in lymphocytes than Clb alone. GClb achieved lymphocyte reduction faster and to a deeper level than RClb (Table 1).

In the Clb treatment arm, the median hemoglobin level remained relatively stable during treatment, while a clear trend towards an improvement was observed in the anti-CD20 containing arms.

Platelet count slightly decreased during Clb treatment and improved with GClb and RClb. Neutrophil count dropped below baseline in all three treatment arms. There were no changes in the median IgA, IgG and IgM levels for all three treatment arms during chemoimmunotherapy.

Conclusions:

The addition of CD20 monoclonal antibodies to Clb leads to a faster and deeper decrease of lymphocytes from peripheral blood than Clb alone. GClb induces an almost immediate and complete lymphocyte depletion whilst RClb acts more gradually. Despite the profound B-cell depletion with GClb and RClb, immunoglobulin levels remain unchanged until the end of treatment. Overall the data suggests that GA101 may have superior B-cell depleting activity compared to rituximab which could result in enhanced recovery of bone marrow function and increased efficacy as previously reported (Goede et al JCO 2013 abstract 4005 ASCO).

Table 1: Absolute lymphocyte count (median, range)

	BL	C1D15	C2D1	C4D1	C6
Clb	71.07 (4.64-437.86)	47.30 (2.88-296.13)	23.17 (0.88-221.55)	11.30 (0.52-195.80)	7.51 (0.60-158.50)
G-Clb	54.36 (0.41-764.00)	1.05 (0.04-33.92)	1.02 (0.05-126.00)	0.90 (0.10-20.20)	0.83 (0.14-6.63)
Clb	71.07 (4.64-437.86)	47.30 (2.88-296.13)	23.17 0.88-221.55)	11.30 (0.52-195.80)	7.62 (0.60-158.50)
R-Clb	52.69 (0.00 – 558.72)	10.14 (0.00 – 426.00)	3.42 (0.00-120.90)	1.61 (0.00-62.27)	1.26 (0.00 – 83.50)

C2. **PROGNOSTIC FACTORS ASSOCIATED TO THE BIOLOGY OF THE TUMOR PROCESS IN CLASSIC HODGKIN LYMPHOMA**

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Classical Hodgkin's lymphoma (CHL) represents 11% of all lymphomas but, with the highest incidence among young adults with ages from 20 to 34 years. Using current treatment strategies, over 70% patients can be cured. Still, 15-20% patients in early stages (Stage I, II), as well as 35-40% of those in advanced stages (III and IV) present relapses or resistance to first line therapy.

Progress made in the past 15 years allowed a better understanding of the biology of CHL and prognostic implications regarding disease evolution and therapeutic response.

Hodgkin's Lymphoma represents a peculiar type of cancer by the fact that malignant cells (Reed-Sternberg cells - RSC) represent only 2% of the tumour mass, the rest being represented by a rich inflammatory infiltrate consisting of a great variety of non-malignant cells (B and T lymphocytes, plasma cells, eosinophils, histocytes, fibroblasts, granulocytes) which form a RSC-driven micro-environment that ensures tumour cell survival.

RSC derive from B lymphocytes of the lymphatic germinal centre which have suffered a clonal rearrangement of genes coding for immunoglobulin heavy chains (Ig) and which are deficient in Ig surface expression and the expression of genes involved in differentiation. Despite these phenomena, RSC does not undergo apoptosis due to the disturbance of several signalling pathways as a consequence of mutations, fusions and altered expression of genes involved in these mechanisms. Changes in the expression of these genes may influence disease progression and therapeutic response.

RSC synthesizes and releases a variety of cytokines and chemokines which are responsible for recruiting the cells forming the tumour micro-environment.

The prognostic importance of cell composition in the non-malignant infiltrate around RSC has been observed ever since the description of histological subtypes. Gene expression and phenotypic changes in the cells that form the tumor microenvironment may influence the disease outcome.

Several studies have shown, as well, the predictive value of the serum levels of chemokine and cytokine molecules and their soluble receptors' presence in serum.

Research in biological characteristics of both RSC and cells and soluble factors of the tumour micro-environment represent the latest line of research in this pathology, in the attempt to describe biomarkers with a prognostic role.

C3. **BENDAMUSTINE BASED THERAPY FOR INDOLENT LYMPHOPROLIFERATIVE DISEASES - FUNDENI DEPARTMENT OF HEMATOLOGY EXPERIENCE.**

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Over the past decade the addition of rituximab to conventional chemotherapy has revolutionized the treatment of B-cell malignancies. Although patients with indolent lymphoma usually respond very well to first-line treatment, most of them eventually experience relapse, and the disease remains largely incurable.

Treatment options are limited once patients relapse, therefore, it is necessary to explore alternative therapies for advanced B-cell NHL and CLL.

Bendamustine, a unique cytotoxic agent with alkylating and antimetabolite properties, has been used for decades in Germany for NHL, CLL and multiple myeloma. In 2008, bendamustine was approved by the US FDA for the treatment of CLL and rituximab-refractory indolent B-cell NHL. In the European Community, bendamustine has been approved for rituximab-refractory indolent B-cell NHL and only for those patients with CLL in whom first-line treatment with fludarabine combinations is not appropriate.

In Romania we have little experience with bendamustine.

In our department nine patients diagnosed with CLL or indolent NHL (small B cell, follicular, marginal zone and mantle zone lymphoma), in the refractory/ relapsed setting were treated with bendamustine in combination with rituximab. We made a retrospective analysis of these cases in order to make a comparison with data available from the most important clinical trials and to provide practical advices about the management of bendamustine therapy in indolent NHL and CLL.

Six patients have completed the planned 6 cycles treatment. Bendamustine in association with rituximab demonstrated efficacy in six patients, one had complete remission and the rest of five achieved partial response. The median duration of response was 6 month. Two patients were not evaluable. One patient had progressive disease. There were 2 treatment related deaths: one patient presented neutropenia grade 4 and died from infection and one had thrombocytopenia grade 3 and died from posttraumatic intracranial haemorrhage.

Our results confirm the role of bendamustine in CLL and indolent NHL treatment. The toxicity profile was acceptable. Bendamustine plus rituximab therapy was more efficient in patients with relapsed disease than in those refractory to previous lines of treatment. The best results were obtained in patients treated with this regimen earlier in the disease course.

C4. THE ROLE OF THROMBOPOIETIN RECEPTOR AGONISTS (TPO-R) IN THE TREATMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA OF ADULT- THE EXPERIENCE OF FUNDENI CLINIC OF HEMATOLOGY

Iulia Ursuleac, Adriana Coliţă, Mariana Vasilică, Răzvan Stoia, Bogdan Ionescu, Sorina Bădeliţă, Daniel Coriu, Radu Niculescu
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Introduction: TPO-R agonists represent an available therapeutical option for those patients with chronic immune thrombocytopenia (ITP) refractory or with contraindication for splenectomy. The two agents are: Eltrombopag (Revolade) with oral, daily administration and Romiplostim (N'Plate), with subcutaneously, weekly administration. Clinical trials showed their efficacy in order to maintain a safe number of platelets ~50000/mmc, prevent fatal bleeding and offer to the patients a good quality of life with few adverse reactions.

Matherial and method: a clinical epidemiological retrospective study of 33 patients with ITP, treated with TPO-R agonists in Fundeni Clinic of Hematology between 2011-2013.

Results: therapeutical indications were – third line therapy (failure after splenectomy and corticosteroids): 6 patients; bridging through splenectomy -11 patients; second line therapy- 13 patients (4 patients with hepatitis C and B active infection, who undergone concomitant antiviral therapy, maintaining normal level of platelets).The age was variable, between 21-68 years old. 27 patients received Eltrombopag and 9 patients Romiplostim. 2 patients received both Eltrombopag and Romiplostim. 29 patients responded with platelets above 50000/mmc, 9 of them had a sustained response, maintained after stopped medication: 4 after Romiplostim therapy and 5 after Revolade treatment. 2 patients were lost from evidence. During treatment were reported as adverse reactions headache, artralgias and one female patient had a transient episode of hepatocytolysis and cholestasis, but without clinical evidence.

Discussion: the TPO-R agonists are a good option for the treatment of ITP refractory patients, for splenectomy bridging or for those with antiHCV/HVB therapy. The terapy is safe, with minimum adverse events. Because of the financial reasons the period of the treatment is limited.

C5. IMPACT OF MOLECULAR BIOLOGY ON PROGNOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA

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The t(15;17), characteristc of acute promyelocytic leukemia (APL), results in the PML/RARα fusion transcript, which can have three major isoforms as a result of the occurrence of different breakpoints within the PML gene: intron 6, bcr-1 (L, long form); exon 6, bcr-2 (V, variable form) and intron 3, bcr-3 (S, short form). The influence of breakpoint locus on the clinical features and response to treatment are still controversial. In this paper we aimed to determine whether the three isoforms PML/RARα transcript is associated with differences in pre-treatment clinical parameters and are predictive of response of treatment.

The **material** is represented by 41 cases of acute promyelocytic leukemia analysis in Molecular Biology Laboratory of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute in I 2008-VIII 2013. The material comes from three sources: 20 cases from Center of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute, 6 cases from Fundeni Pediatric Clinic, 6 cases from Coltea Clinical Hospital. In 29 cases, the determination of PML/RARα fusion gene (L, V, S isoforms) was performed at diagnosis. 16 of 29 newly diagnosis cases were monitored in dynamic. 12 cases diagnosed prior 2008 which were monitored for minimal residual disease (BMR) and are in hematologic and molecular remission.

Method for determining of PML\/RARα gene fusion isoforms was Nested PCR.

Results: Of the 29 cases of APL from three clinics of Hematology, analyzed at diagnosis, 14 (48%) patients had been diagnosed with bcr-1 (L-form), 6 (20%) patients with bcr-2 (V-form) and 9 (31%) patients with bcr-3 (S-form). For the 20 cases admitted in the Center of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute were able to analyse the

clinical and Hematological pre-treatment data. Of these 20 cases: 11 (55%) cases were bcr-1 (L-form), 3 (15%) cases were bcr-2 (V-form) and 6 (30%) cases were bcr-3 (S-form). There were no differences regarding age, gender, basic status of performance, clinical signs of coagulopathy. But differences were notted between L and S isoforms on the following hematologic features at presentation:

a)3/6 (50%) of the cases with S isoform (bcr-3) had the WBC > 10,000\/µl to 2/11 (18%) of the cases with L isoform (bcr-1).

b)3/6 (50%) of the cases with S isoform (bcr-3) had the phenotype M3 v. All patients with L isoform (bcr-1) presented with classical hypergranular form of LAM3 . 1 patient with bcr-2 isoform presented with the microgranular form and increased number of leukocytes (56,000\/µl).

c)FLT3-ITD mutation was detected in 2/6 (44%) cases with S- isoform (bcr-3) and was not discovered in any of the 11 cases with L- isoform (bcr-1).

d)CD56, CD2, CD 34 antigens were not investigated systematically. CD 56 was found in a case of APL with S- isoform (bcr-3), increased number of leukocytes (64,000/µl) and M3 v.

e)Regarding to relapse risk Sanz score, 3/6 (50%) of patients with S isoform (bcr-3) were included in the high risk group compared to only 2/11 (18%) of cases with isoform L (bcr-1).

All 12 patients diagnosed and treated before the year 2008 which were followed in the dynamic of BMR using nested PCR remained in molecular remission. 20 patients treated in the center of Hematology and bone marrow transplantation during the period 2008-2013 received treatment with ATRA + chemotherapy (Fenaux and Sanz PETHEMA protocols). 2 patients with LAP-bcr1-isoform, from intermediary risk group died at induction due to differentiation of syndrome according to ATRA that couldn't be overcome by treatment with dexamethasone. Of 18 patients, 15 are in molecular remission, 1 patient is in hematologic remission, but PCR + and 2 patients relapsed: at 4 months (a 50 years old patient with M3-v, S-form, bcr-3, FLT3 +, CD56 +, high risk, L > 10,000\/µl) and at 48 months (a 43 years old patient with M3, L-form, bcr-1, intermediate risk).

Conclusions: the cases of PML\/RARα S form (bcr-3) were associated with negative prognostic indicators (increased number of leukocytes, M3 v variant; FLT3, CD 56) but the therapeutic strategy adapted for high risk factors was able to obtain complete hematologic and molecular remissions as long as L forms PML/RARα.

In the cases of APL with resistant disease or molecular relapse it is recommended emergency treatment in order to obtain a new complete hematologic and molecular remission that will need to be consolidated according by their status of PCR +/- with blood stem cell transplantation (autologous / allogenic) or ATO or innovative therapies.

C6. **DETECTION OF MALIGNANT CELLSIN CEREBROSPINAL FLUID, PLEURAL- AND PERITONEAL EFFUSION BY FLOWCYTOMETRY**

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Immunophenotyping by multiparametric flowcytometry (MFC) is indispensable in diagnosis and follow-up of hematological malignancies. MFC allow to evaluate multiple antigens simultaneously making it possible to identify and characterize the malignant cell population in cerebrospinal fluid, pleural and peritoneal effusion.

We present 3 cases in which immunophenotyping by flowcytometry was helpful in detection of malignant cells from different body fluids other than peripheral blood or bone marrow.

In case 1 – a 34 year old female patient with treated acute lymphoblastic leukemia (ALL) – the immunophenotyping from cerebrospinal fluid identified the relapse in central nervous system (CNS) by the presence of the lymphoblasts positive for CD19, CD10, CD22 and Cd34.

In case 2 – a 46 year old male patient with plasmocytoma localized on vertebral column - immuno-phenotyping from pleural effusion revealed malignant plasma cells negative for CD45, CD19, CD20 and positive for CD38, CD138, Cd56.

In case 3- a 3 year old boy – the primary diagnosis of Burkitt-lymphoma was made on MFI of ascitic fluid. Monoclonal Kappa+ B-cell population was identified with the following antigenic profile: Cd19+, CD20+, CD10+, CD22+, CD79b+, CD38+, HLA-DR+, CD5-, CD23-, FMC7-, CD200-, CD27-, LAIR1-, CD103-, CD11c-, CD34-.

Immunophenotyping by multiparametric flowcytometry could improve the efficiency of detection of CNS involvement and it is an important and rapid method to identify and characterize the malignant cells in serous effusion such as pleural and peritoneal effusion.

C7. NEXT GENERATION SEQUENCING FOR THE STUDY OF ACUTE MYELOID LEUKEMIA. A PILOT STUDY FOR THE TARGETED SEQUENCING OF LEUKEMIA-ASSOCIATED GENES USING THE GS JUNIOR PLATFORM

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Introduction.

Acute myeloid leukemia is a hematological disease associated with cytogenetic abnormalities at the hematopoietic stem cell level. Leukemogenesis involves several "hits" targeting genes that that regulate cell growth and differentiation, leading to maturation arrest and aberrant proliferation. The genes that are most requently mutated are NPM1, RAR, CBF, as well as FLT3, c-kit and MLL. Recent studies have shown that some other genes may take an active part in the development of the AML phenotype, such as CBL, KRAS and TET2. The purpose of the present pilot study was to observe the mutation status of these genes with the help of next generation sequencing technology in a number of three AML patients at the time of diagnosis.

Materials and Methods.

The aim of this pilot study was the Targeted Sequencing of AML-associated genes using the GS JUNIOR platform from Roche, and the genes studies were TET2, CBL and KRAS. For this, the peripheral blood collected from the patients at the time of diagnosis was separated using standard density gradient centrifugation, and DNA was extracted from the white blood cells. The amplicons used for sequencing were obtained using the GS GType TET2/CBL/KRAS Primer Set which consists of oligonucleotide PCR primers for the amplification of exons 3-11 of TET2, exons 8-9 of CBL, and exons 2-3 of the KRAS genes. This process also included the attachment of molecular identification labels (MID) to each patient sample. The amplicon libraries that were obtained were quantified, quality checked and amplified by means of emulsion PCR, then the samples from all three patients were sequenced in a single run on the GS Junior machine.

Results and Discussions.

Upon completion of the sequencing run and data analysis, we have observed a total number of eight mutations, of which seven were in the TET2 gene, one in the CBL gene, and none in KRAS. Three of the mutations in TET2 were deletions at intron levels, the other four were substitutions, while the mutation observed in the CBL gene – homozygous in all three patients – was an exonic deletion which is most likely to cause a frame shift. Mutations of these three genes alter cellular biology at multiple levels and require not only the activation of receptor proximal signaling events but also an increase in cellular glucose metabolism. Pathways that are activated by CBL gain-of-function mutations can be efficiently targeted by small molecule drugs. Since the results of this pilot study may bring knowledge with the potential of influencing therapeutic approaches for AML, further research will be performed in this direction.

C8. THE RELATIONSHIP BETWEEN FACTOR V LEIDEN, PROTHROMBIN G20210A AND *MTHFR* MUTATIONS AND THE FIRST MAJOR THROMBOTIC EPISODE IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA.

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Background/aim

Arterial and venous thrombosis are the most frequent complications in patients with polycythemia vera and essential thrombocythemia. We sought to demonstrate a possible contribution of the Factor V Leiden, Prothrombin G20210A, and *MTHFR* 677 C>T and 1298 A>C mutations to the thrombotic risk in patients with polycythemia vera and essential thrombocythemia, along with other biological features of these patients.

Material and methods

We included 86 patients with polycythemia vera, of which 34 (39.5%) had major thrombosis, and 95 patients with essential thrombocythemia, of which 22

(23.1%) had major thrombosis. Factor V Leiden, Prothrombin G20210A, and *MTHFR* 677 C>T and 1298 A>C mutations were genotyped by PCR-RFLP and AS-PCR techniques.

Results

In the whole cohort of patients, only the Factor V Leiden mutation was significantly associated with both arterial and venous thrombosis, in univariate and multivariate analysis (OR = 4.3; 95% CI = 1.5 – 12.5; p=0.008 and OR = 4.3; 95% CI = 1.2 – 15.9; p = 0.02, respectively). Other factors significantly associated with thrombosis in both univariate and multivariate analysis were: male sex (OR = 2.8; 95% CI = 1.4 – 5.4; p = 0.002 and OR = 3.5; 95% CI = 1.6 – 7.6; p = 0.002, respectively) and the *JAK2* V617F mutation (OR = 5.5; 95% CI = 2.1 – 15; p =0.0001 and OR = 6.9; 95% CI = 2.2 – 21.2; p = 0.001, respectively).

Conclusions

In conclusion, among the four mutations analyzed (Factor V Leiden, Prothrombin G20210A, and *MTHFR* 677 C>T and 1298 A>C), only Factor V Leiden is a major contributor to thrombosis in polycythemia vera and essential thrombocythemia.

C9. **REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS AND THROMBOCYTOSIS WITHOUT JAK2 V617F MUTATION- REPORT OF THREE CASES**

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Abstract: In the WHO classification, there is a provisional entity called - Myelodysplastic / Myeloproliferative Neoplasm, Unclassifiable (MDS/MPN,U). Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) was included in this category. Recently published studies report a small percentage of patients with RARS-T. Sixty percentages of these have JAK2 V617F mutation, which can suggest the coexistence of two pathological conditions (MDS and MPN). In this paper, we analyzed three patients diagnosed with RARS-T in Hematology Department, Fundeni Clinical Institute, during the period 2005-2011. The patients were investigated with cytogenetic exam and molecular biology. In these three cases were identified morphological features of multilineage dysplasia (two lineage dysplasia in two cases and three lineage dysplasia in one case). In two cases thrombocytosis was under 1000 x 10 ^{3}/μl and clinical evolution was similar to the myelodysplastic syndrome (transfusion dependent anemia with response to administration of erythropoietin). In the third case, the platelets were over 1000x10 ^{3}/μl and with respons to the treatment with Hydrea, which improved anemia. JAK2 V617F mutation was not identified in any case.
RARS-T remains a provisional entity and requires a complex investigation of patients for the correct diagnosis of these patients. Therapeutic options should be personalized to each case in part because there is not yet a standardized treatment of these patients.

C10. **BLOODSTREAM INFECTIONS IN PATIENTS WITH MALIGNANT DISEASES**

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Key words: malignant diseases, multidrug resistant strains, bloodstream infections, extended spectrum β-lactamases, Gram negative bacilli, febrile neutropenia

We studied the resistance phenotypes of the bacteria isolated from patients having positive BSIs, during a three years period (325 strains). The study group: 219 patients had malignant diseases and 106 patients (the control group) had other conditions. We determined the minimal inhibitory concentrations (MICs) with the Vitek 2 compact system and reported them as percent susceptibility to the main /class representative antibiotics used to treat/ prevent BSIs. The resistance mechanisms detected with the same system were confirmed by molecular biology. The pulse field electrophoresis (PFGE) determined the phylogroups of the Gram negative bacilli.

Results: BSIs OR= 1,24, (CI 95% 0,65-1,91), z= 1,64, P=0,1). Gram-negative bacilli n=181, 55,86%; *Enterobacteriaceae* n=124, 48,33%; *P. aeruginosa* n=35, 10,80%. The Gram-positive cocci n=131, 40,43%. The staphylococci n=97, 29,94%, *S. aureus* (n=54, 16,05%), coagulase negative spp (CNS) (n=44, 13,89%). The streptococci: enterococci (n=18, 5,56%), from polimicrobial samples. The fungal BSIs n=6, 1,8%. In the study group the Gram negative bacilli were two to eight times more frequent; *S. aureus* was the dominant pathogen, in both groups; *E. faecium* was predominant in the study group (n=5, 71,4%).

The cumulative susceptibility to 3rd generation cephalosporins (CG III), malignant diseases: *E. coli* n=56, 58,93%; *K. pneumoniae* n=16, 44,44%, *P. aeruginosa* n=35, ceftazidim = 16,67%. ESBL production:: *E. coli* n=20, 35,71%, *K. pneumoniae*: n=7, 43,75%. The carbapenems had the highest susceptibility values. *S. aureus* was methicillin-resistant 74,19% .

Molecular determinations: 65 strains. Phylogroups of *E. coli*: group A, intestinal (n=20, 48,65%), group B2

extraintestinal (n=5, 13,51%). ESBLs belong to the CTX-M group, 50% isolated from hematologic malignancies (n=31). CTX-M group 1 (n=16, 43,24%), **(**CTX-M-15), mostly in hematologic malignancies. Resistance to fluoroquinolones 3rd generation is the product of the mutant gene aac(6')-Ib la *E. coli :* n=11, 29,73%.

Conclusions: The main pathogens in BSIs in patients having malignant diseases are the Gram negative bacilli. They are MDR (mostly by ESBL of CTX-M 15 type), susceptible only to carbapems (the Enterobacteriaceae) and to colistin P. aeruginosa, as a result of frequent antibiotic treatments. The Enterobacteriaceae belong to the phylogroups A and B1. The BSIS in patients with malignant diseases are a result of the digestive mucosites.

CLINICAL HAEMATOLOGY SECTION - POSTERS SESSION

P1. CASE PRESENTATION: PROGNOSTIC FACTORS, CLASSIFICATION, AND RISK MODELS FOR OVERALL SURVIVAL PREDICTION IN MDS PATIENTS.

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The myelodysplastic (MDS) are a very heterogeneous group of myeloid disorders characterized by peripheral blood cytopenias and increased risk of transformation to acute myelogenous leukemia (AML).

Prognosis of MDS patients can be predicted by different risk models. Some are disease-related (FAB, WHO, IPSS, WPSS, MDACC) and some are patient-related (MDS-CI):

– FAB and WHO classifications do not account for cytogenetics
– IPSS is the best model for newly diagnosed patients, but can underestimate transfusion dependency and cytogenetics
– WPSS can be used at any time during the disease course, but can underestimate cytogenetics
– MDACC is dynamic and can be applied to patients with prior therapy, secondary MDS, CMML
– MDS-CI assesses the additional risk of comorbidities

We present the case of a male patient of 65 years old, diagnosed in Nov 2009 with MDS–RCMD with RS (WHO 2008); IPSS Int-1; WPSS Int; MDACC Low risk; MDS-CI Low risk.

The patient was transfusion – dependent, with 3-4 units /month.

After 12 months (Nov 2010): he was symptomatic with macrocytic anaemia (Hb 7.9 g/dL) and ferritin levels: 1121. No LIC or cardiac T2 available; No test for HFE gene mutation; No clinical signs of haemochromatosis. The treatment was Erythropoiesis-stimulating agent and Red cell transfusion + iron chelation.

September 2012: The bone marrow aspirate shows erythroblastopenia, multilineage dysplasia and 25% ring sideroblasts; Chest CT scan shows no modifications. He starts Cyclosporine 5 mg/kg – 8 mg/kg with no hematological response.

January – May 2013: Supportive care with transfusions: 6 units/month and iron chelation

Progressive heart failure appear - The patients is now MDS-CI Int - High risk. ECOG 3.

May 2013: The bone marrow aspirate shows erythroblastopenia, multilineage dysplasia and 20% ring sideroblasts; WPSS score: Intermediate

The Immunosuppression and transfusion – dependence influence the prognosis. In June 2013 the patient diagnosis is stafilococcal endocarditis and he dies, with no sign of leukemic progression.

It has become apparent that the natural history of patients with lower risk disease is very heterogeneous. Presence of comorbidity had a significant independent impact on survival and a prognostic score could be developed that assesses the additional risk of comorbidities.

IPSS is now replaced by a new revised score (IPSS-R) and by the incorporation of new molecular markers recently described.

P2. DIFFERENTIAL DIAGNOSTIC PROBLEMS INVASIVE FUNGAL INFECTION IN A PATIENT PRESENTING ACUTE LYMPHOBLASTIC LEUKEMIA WITH HIGH DOSAGE CHEMOTHERAPY.

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Patients with acute leukemia, especially in relapse, and polychemotherapy have a high risk ,during re-induction chemotherapy, to develop severe invasive

fungal infection, with a high mortality rate.
We present the case of a 43 years old patient with recurrent acute lymphoblastic leukemia , for which she received high dose chemotherapy associated with nucleoside analogues. During post-therapy aplasia the patient developed a febril sindrome without a clinical infectious source, with normal pulmonary sounds and negative blood culture. The chest radiography and CT were sugestive for a fungal infection. In this context the patient recived a broad spectre antibiotherapy associated with antifungal therapy.

The evolution was however unfavorable, requiring a monitorization of the patient in ICU.

It was carried out a alveolar fibrobronhoendoscopie and it was performed cultures and smears from the alveolar lavage fluid, with a negative result. We also tested CMV (PCR), Ag Legionella-negative, Galactomannan- negativ serum, BAC-negative for Aspergilus. Neutropenia is recovered, but the patient becomes hemodynamic unstable, requiring vasopressor therapy in progressively higher doses and invasive cardiac monitoring (PICCO). Neurological manifestations also appeared this is why we suspected an infiltration of central nervous system, however with normal CT brain and cerebrospinal fluid morphological examination.

The progression of the pulmonary lesions detected on a new chest CT required a thoracotomy with a pulmonary biopsy and pleural drainage, however the anatomical pathologic exam and the cultures didn't evidentiat any infectious lesions.

High dose antibiotherapy was continued based on the clinical suppositions, but the evolution of the patient was unfavorable, and the death occured.

Fungal infections continue to represent a frequent and serious complication in patients with hematological diseases, especially among those with an acute pathology. It is important an early detection of the infectious agent becouse it is crucial to administrate a specific treatment.

P3. EVOLUTION IMMUNOSUPPRESSIVE THERAPY WITH MONOCLONAL ANTIBODIES IN A PATIENT WITH SEVERE HEMOPHILIA A, FACTOR VIII INHIBITOR.

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Hemophilia A is an inherited blood disorder, because of mutation HEMA gene on the X chromosome, characterized by a deficiency of blood clotting protein, Factor VIII (FVIII), accompanied by hemorrhagic manifestations typical. Patients with severe hemophilia produce less than 1% of normal clotting factor affected the administration of FVIII are addicted to treat or prevent episodes of bleeding.They can inherite allo-anticorpiin after the administration of FVIII, a situation that requires special care because of the high risk of mortality increaseang bleeding complications develop.
This paper presents the case of a patient diagnosed at severe haemophilia A-form, with massive bleeding that despite administration of FVIII rFVII maintaince the bleeding active hemostatic serious (hemohidrotorax 2/3 lower left, retroperitoneal hematoma, retrogastric the fall in hemoglobin to 2.6 g%). It raises suspicion of the presence of factor VIII inhibitors, confirmed the investigation specialist (80 u Bethesda) needing treatment immunosuppressive monoclonal antibody and Ciclofosfamidasi desensitization treatment with high doses of FVIII.
The management of inhibitors of the hemofilici patients is an ongoing challenge that requires induction of immune tolerance by using a technique the most successful desensitization observed in patients with low titer inhibitors (<5 u Bethesda), which are treated immediately after detecting a allo-antibodies, and include the use of immunosuppressive therapy and also with repeated infusions of FVIII high titer with high results in studies to date.

P4. THE IMPORTANCE OF MORPHOLOGICAL EXAM OF PERIPHERAL BLOOD IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA.

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Introduction: In patients with Essential Thrombocytosis/Primary Thrombocythemia/ET, associated with sustained megakaryocyte proliferation and increased number of circulating platelets with various morphological, biochemical, and metabolic platelet defects- a blood smear is essential to validate the automated cell counts (CBC).

Methods: We present a patient: female, age 62, with ET for 20 years, with several thrombotic episodes and splenectomy in antecedents, treated mainly with hydroxyurea and aspirin. We performed automated

CBC for the platelets number (PLT) and cytologic exams of blood smears. CBC were performed on a Coulter AC.T Diff analyser; we examined the blood films MGG stained on Nikon Eclipse E 200 microscope.

Blood smears were obtained both from peripheral and capillary blood. We evaluated results over ten weeks period, once a week, after a reversible thrombotic episode.

Results: PLT, on automated CBC: 281.9 +- 33.25×109/L; estimated on blood films: 559.5 +- 66.02×109/L.

The peripheral films show large platelet aggregates, varying degrees of platelet anisocytosis with larger atypical forms and frequent megakaryocyte fragments. These facts generated significant differences between counts.

Discussion / Conclusion: In ET, morphological and functional defects of platelets interfere with automated counts.Microscopic exam of blood smear is mandatory in order to ensure the optimal management of these patients.

P5. INVASIVE FUNGAL INFECTION ASSOCIATED WITH PULMONARY TUBERCULOSIS IN A PATIENT WITH HAIRY CELL LEUKEMIA.

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Hairy cell leukemia is a chronic lymphoproliferative syndrome with increased infectious risk secondary to humoral immunity and cellular damage by cytopenias that are amplified by treatment with nucleoside analogues. Fungal and atypical mycobacterial infections associated with severe neutropenia shows a common complication that occurs in patients with hairy cell leukemia under treatment with nucleoside analogues.

This paper presents the case of a 64 years old patient diagnosed with hairy cell leukemia in December 2012 under therapy with Litak (2-CdA, cladribine) 0.1 mg / kg / day for 7days. At time of diagnosis the patient had marked physical fatigue; in haematologic plan - grade III neutropenia, normal clinical examination with normal CT scan.

The evolution is marked by persistent severe neutropenia with febrile syndrome occurring in parallel with the installation of a severe progressive cholestatic syndrome with hyperbilirubinemia, hepatic cytolysis syndrome and inflammatory syndrome, which required a clinical and imagistic reevaluation. The case presented many problems of differential diagnosis: autoimmune pathology, complex infectious pathology or tumor pathology. CT examinations revealed pulmonary nodular lesions with central necrosis. In the bronchoalveolar lavage Aspergillus filaments were revealed. Antifungal treatment is administered with the persistence of a febrile syndrome, which is why another coexistent infection is suspected and later confirmed by the presence of Koch bacillus in bronchial alveolar lavage.

The conclusion: the importance of the diagnosis and identification of the pathogen agent whith invasive methods including lung biopsy samples from immunosuppressed patients who have a febrile syndrome with no response to treatment.

P6. MEGALOBLASTIC ANEMIA ASSOCIATED WITH DEMIELYNATING MEDULLARY DISEASE IN A VEGETARIAN PATIENT.

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A 44 years old woman was admitted in our clinic with severe fatigue, anasarca, dyspnea, walking disorder (ataxia), numbness and pallor. She had a vegetarian diet history of 7 years (with no meat, dairy products or eggs), she had a 6 months history of menstrual cycle anomalies, hyposmia and hypogeusesthesia, a 2 months history of calf edema that worsened in the past 2 weeks.

The clinical exam showed altered general state, severe asthenia, dyspnea, she was oriented and pale, had anasarca, ataxia and paraesthesiae. The pulmonary exam showed absent murmur and pleural friction in both bases. She had accelerated intestinal transit, pollakiuria; the breasts had infiltrated teguments that looked like an orange skin.

The blood count presented with macrocytic anemia (Hb 6.5 g/dl, Ht 19.2%, MCV 103.2 fl, MCH 34.9 pg), leucopenia with left shift to metamyelocyte (WBC 2580/μL, Mt1 Band 3 N51 B1 L38 M6) and thrombocytopenia (PLT 61000/ μL). The blood film had polychromatic and oxyphile macrocytes, fragmented red cells, teardrop RBC's, ovalocytes and basophilic stippling, a picture suggesting megaloblastic anemia. The bone marrow showed megaloblastosis.

She had hypoalbuminemia (2.6 mg/dl), hepatocytolysis (65 U/L), BI 2.26 mg/dl, LDH 2668 mg/dl, negative DAT, cold haemagglutinin - 1/32, without cholestasis, muscle wasting (low Cr – 0.4 mg/dl), hypocalcaemia (7.7 mg/dl), low B12 (13

pmol/l). HBV, HCV and HIV viral markers were negative. CA 125 was raised (169.4 U/ml).

The neurological consult diagnosed subacute combined degeneration of the spinal cord and paraparaesis. The spinal cord MRI showed a wide demyelination of the cervical and thoracal spinal cord (affecting the posterior spinal cord). The mamography and gynecological exam were normal. The pulmonary radiography showed bilateral effusion.

The superior digestiv endosopy showed a palle and discretely friable stomach mucosa. The abdominal-pelvine echography showed hepatomegaly and ascites. The toraco-abdomino-pelvin CT showed an heterogenous uterus and multiple tracks of collateral periuterine circulation.

She was diagnosed with severe protein-calorie malnutrition and megaloblasic anemia due to nutritional B12 deficiency, associated with combined degeneration of the spinal cord and the demyelination of the cervical and thoracal posterior spinal cord.

The patient was given substitutive treatment, and the evolution was with hematological (Hb 11.3 g/dl after 2 months, normal erythrocyte indices, leukocytes and platelets) and biological improvement. Se had a slight improvement of the ataxia, with paraparaesis, and discrete calf edema after 2 months. The menstrual cycles normalized and the smell and taste senses improved. She kept her strict vegetarian diet.

P7. SUSTAINED REGRESSION OF THE DISEASE UNDER THERAPY WITH LEVOFLOXACIN IN TWO CASES OF MYELODYSPLASTIC SYNDROME.

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We are presenting two cases of patients with myelodysplastic syndrome- RAEB and trilinear damage on therapy with levofloxacin that had sustained regression of disease:

The first is the male patient CG, born in 1943 and diagnosed with myelodysplastic syndrome-AR in 2008, with favorable evolution until May 2012, when the disease progression appears. Haematological evaluation indicates pancytopenia and medulograma reveals a rate of 8% blasts. Patient accepted only supportive therapy until November 2012, when returns with worsening suffering by non tuberculous L4/L5 infection surgically solved and treated with levofloxacin. Marrow puncture performed on this occasion indicates the percentage of myeloblasts increased to 15% and cytogenetic analysis did not indicate significant changes; clinico-biological evolution is favorable and medulograma later in December 2012 indicate partial correction of cytopenias and the percentage of blasts decreased from 15% to 4%. The patient mantained levofloxacin therapy and evaluations of May and July 2013 indicate minimal changes of myelodysplastic nature and percentage of blasts of 6%.

The second case is the patient IM, female, born in 1948 and diagnosed in September 2012 with myelodysplastic syndrome associated with myxedema and severe autoimmune hemolytic anemia. The marrow puncture at diagnosis indicates myelodysplastic syndrome and the percentage of blasts is 5% . After correction of myxedema and controling the hemolysis, the patient is revalued hematologically (in November 2012) and there is progression of disease with increasing percentage of blasts to 10%. The patient presents as chronic associated pathology pyelonephritis and for its recurrent exacerbation we initiated antibiotic therapy with levofloxacin. Evolution was favorable clinically and hematologically, so the hematologic evaluation in March 2013 indicated regression of disease with partial correction of cytopenias and the percentage of blasts in her bone marrow decreased from 10% to 4%;

Observations are arguments for the already stated role of quinolones, imposing their effectiveness in expanding research on serious diseases such as myelodysplastic syndrome.

P8. EVALUATION OF ACID-BASE BALANCE IN MEDULLARY JUICE FROM PATIENTS WITH MALIGNANT AND NON-MALIGNANT HEMATOLOGIC DISEASES.

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This paper presents the case of 39 patients with hematologic malignancies and non-malignant on which we performed a bone marrow puncture and evaluated its acid-base balance. Diagnoses were most varied: acute myelogenous leukemia, chronic myeloid leukemia, multiple myeloma,Waldenstrom's disease, myelodysplastic syndromes, lymphomas, megaloblastic anemia, immune thrombocytopenic purpura, collagen disease, liver cirrhosis secondary hypersplenism, anemia paraneoplastic mastocytosis. The preliminary results indicate significant differences in Ph of marrow juice; it was pathologically altered in 74% of subjects with hematologic malignancies and

37.5% of subjects with benign hematologic diseases. It was also noted the modification of Na + ions concentration, pathologically altered in 74% of subjects with hematologic malignancies and 25% of subjects with hematologic non maligne diseases. Simultaneously there were significant increases in the concentration of K + ions to 59% of subjects with hematologic malignanacies and 50% of subjects with non-hematologic malignancies. We also noted severely low blood glucose values in marrow juice (< 27mg%) in 11% of subjects with hematologic malignancies. The data represent a starting point for research of the pathophysiological significance of this changes.

P9. ETIOPATHOGENY OF THE FEBRILE SYNDROME ASSOCIATED WITH HAEMATOLOGICAL MALIGNANCIES.

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Understanding the etiopathogeny of the febrile syndrome associated with haematological malignancies is involving maximal difficulties and costs.

There are two situations: prolonged febrile syndrome and the febrile syndrome associated with diagnosed hematologic malignancies. In prolonged febrile syndrome pathogenesis the hematologic malignancies may be involved in a proportion of up to 10%, which means that we have to consider their diagnosis procedures in this cases .In the second situation it is necessary to specify if the fever is favored expression of infectious complications, of immuno-suppression or both : hematologic expression and infection.

In this paper we propose a hierarchy of information brought in clinical and laboratory diagnosis. First there are cultures: bacterial or fungus (blood cultures, urine cultures, coprocultures, crop secretions, cannula, serous effusions, bronchial aspirate). Equally important are evaluating serological Borrelia infections, HIV, HBV, CMV, legionella, fungus and the cytological / HP for Leishmania, Toxoplasma, mycobacteria. We must not forget inflammation evaluations of specific proteins: fibrinogen, CRP, immunoglobulins, ferritin, TNF and very important procalcitonin values. The results represent a starting point for an interdisciplinary discussion.

10. THE RELATIONSHIP BETWEEN ACCEPTANCE, ANXIETY AND HEALTH STATUS IN HEMATOLOGICAL PATIENTS.

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Psychological accepting is presented in the scientific literature from different points of view : from the rational- emotional and behavioral therapy (REBT) and from the Acceptance and Commitment Therapy (ACT) as having an impact on health and genetic status, and also a therapeutic role in reducing emotional and mental disorders.

Given the impact of psychological factors on somatic health in hematological patients, this study wishes to highlight the relationship between acceptance and other medical indicators in hematological diseases and healthy individuals. For this purpose we used a lot of 60 hematological patients and a lot of 60 individuals without hematological pathology. The results are interpreted both intra-group and inter-group, respectively, and the future research directions are also discussed.

P11. ASPECTS REGARDING THE EVOLUTION OF A GROUP OF PATIENTS WITH MYELODYSPLASTIC SYNDROME IN THE HEMATOLOGY CLINIC OF ARAD

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Myelodysplastic syndrome is a relatively new pathological entity, and, because of its preleukemic character it has been carefully studied in the recent years. This paper aims to present the evolution of a group of 16 patients followed in the past year in the Hematology Clinic of Arad. Patients' data were collected retrospectively, paying attention to the diagnostic criteria and the type of disease, to the onset of the disease, treatment protocols and disease evolution. There were a couple of patients with MDS RAEB 1 and 2 which, under tretinoin treatment, had a favorable evolution in time without progression toward acute leukemia. The study also emphasises the transfusion dependance in this group, the onset of secondary hemochromatosis and the iron chelation therapy and its

effects on those patients. Being a group of disorders that are usually difficult to diagnose, myelodysplasia is often underdiagnosed; the therapy with tretinoin, initiated at the proper moment after the diagnosis, has, in our clinic experience, a positive impact on the disease, prolonging the survival of these patients.

P12. **AUTOIMMUNE HEMOLYTIC ANEMIA – CASE REPORT.**

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Autoimmune hemolytic anemia is a disorder that affects the quality of life of a patient by its somewhat unpredictable occurrence of the acute episodes and the constant dilemma concerning its idiopathic or secondary occurrence.

In this paper the authors wish to present the case of a woman with autoimmune hemolytic anemia, diagnosed in 2003, followed in the Hematology Clinic of Arad. In the first years, the acute episodes of disease appeared every few months ; since 2010, the acute episodes became more frequent, even at three weeks. The patient was complex investigated for a possible disorder that could lead to a secondary hemolysis ; the clinicians weren't able to detect such a cause. In parallel with increasing the frequency of the acute episodes, the patient presented a marked increase in the size of her spleen, which rised the suspicion of a splenic lymphoma. The patient refused any intervention that could establish the diagnosis of a lymphoma. The patient's condition gradually deteriorated until March 2013. She was periodically followed and the patient came to the Hematology Clinic of Arad in March 2013 with a clinical status much improved overall, biological - mild anemia and the splenomegaly greatly reduced in size compared to previous hospitalization (1 month before). In the meantime, the patient didn't take any medication but folic acid therapy. This patient has raised the interest of the team of clinicians from the Hematology Clinic of Arad, this paper also wishes to debate the possible causes that could lead to patient improvement.

P13. **PROGNOSTIC VALUE OF THE INTERNATIONAL STAGING SYSTEM IN MULTIPLE MYELOMA. COMPARISON WITH THE SALMON DURIE STAGING SYSTEM.**

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Background: Multiple myeloma (MM) is a malignant monoclonal gammopathy characterized by bone pain, lytic bone lesions, hipercalcemia, anemia, impaired renal function, bone marrow plasmacytosis. Since 1975 the Salmon Durie staging (SDS) system has been used for the evaluation of prognosis of these patients, a staging system that takes into account the clinical features listed above. The subjectivity of the interpretation of bone lesions, an important part of the staging system, and the poor positive predictive value of the Salmon Durie system has led to the design of a new staging system, the International Staging System (ISS) in 2005. ISS takes into consideration the serum levels of beta 2 microglobulin and albumin. Several comparisons of the two systems have demonstrated the superiority of ISS.

Aim: To compare the prognostic value of the ISS and Salmon Durie staging system for patients with multiple myeloma treated in the hematology department in Cluj-Napoca

Material and method: The study is a prospective analytic study of the prognostic value and survival rates of 47 MM patients treated in 2012-2013 in the hematology department in Cluj-Napoca, that were grouped according to both systems. 30 patients (63%) were female and the median age was 60 years (range 82–42). Evaluation of response included immunoglobulin levels, serum and urine immunofixation, radiologic evaluation of the plasmacytoma, bone marrow aspiration/biopsy –plasmacytosis, and were done at the end of 8 cycles of chemotherapy.

Results: According to the Salmon Durie staging system 9% of our patients were stage I, 28% stage II, and 63% stage III. Using the ISS, 28% were stage I, 30% stage II and 41% stage III. Until now 17 patients had undergone one or two evaluations, 10 of which had a complete response and have stopped treatment. Among the complete responders, 8 patients were in stage III SDS and 2 patients were in stage II SDS, while according to ISS, 5 patients were in stage III, 3 patients were in stage II and 2 patient was in stage I. The non-responder group was formed of 7 patients who had stable disease (5 in stage III SDS and 2 in stage II SDS; according to ISS, 4 patients were stage III 3 patients

were stage II and 1 patient was stage I) and continue treatment with 2-nd line therapy. One patient, who initially had a complete response (stage IIIB SDS and stage III ISS) had an early relapse (6 months after autologous stem cell transplantation). Forty-six patients are currently alive and continuing observation regularly at 6 months interval; one patient (stage IIIB SDS and stage III ISS) died 18 months after diagnosis.

Conclusions: Between the responders group and the non responders group there were no differences according to Salmon Durie system, stage III patients forming the majority in both groups (80% and 71%), while, according to the ISS there were differences between the two groups: in the responders group the majority were stage I (50%), while in the non responders group stage II formed the majority of patients (44%). Therefore, the Salmon Durie staging system has a poorer prognostic value than the ISS, because it tends to confer a poor prognosis to the majority of patients.

P14. PROGNOSTIC FACTORS, THERAPEUTIC STRATEGIES AND CHRONIC LYMPHOCYTIC LEUKEMIAEVOLUTION.

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Chronic lymphocytic leukemia is one of the most common and heterogeneous hematologic malignancies. Finding a curative solution and prolonging survival is the objective of numerous cases and clinical trials . Diagnosis is based on the interlocking of classic elements and newly identified prognostic factors but time to first treatment is still an open issue. CD38 , ZAP 70 IgHV mutation status of genes and cytogenetic changes are proven to negatively influence the evolution of chronic lymphocytic leukemia . Whether through aggressive rapid evolution or by the difficulty of obtaining a complete remission or risk of early relapse. Adapted to these prognostic factors, combinated therapeutic regimens have proved to be effective in achieving a durable complete remission, new agents, with encouraging partial results, being studied. Alkylating agents were a basic treatment of chronic lymphocytic leukemia for decades also combinations with standard chemotherapy (CHOP) and regimens with fludarabine (FC). Randomized trials have demonstrated the efficacy of immunotherapy in patients with adverse prognostic factors .

The present observational retrospective / prospective study are evaluated 145 patients with chronic lymphocytic leukemia admitted in Coltea Clinical Hospital Bucharest between January 2005 - December 2012. Were analyzed risk factors (clinical, laboratory, biological) and patient survival was evaluated according to them, the disease stage and the therapeutic regimens used. A different pattern even in patients with the same stage at diagnosis is put on different regimens but also on factors related to the patient.

Requiring initial screening, for comparative purposes, a current and growing importance has minimal residual disease, its absence at the end of treatment represents a strong positive prognostic factor.

P15. DIFFUSE NON-HODGKIN'S LYMPHOMA WITH LARGE B CELLS AND LEUKEMIC PICTURE.

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It is the report of a case of 70 years old, without notable antecedents, wich presents from 10 days fever, icterus and profound asthenia. At admission was retained damage of the general status, icterus, bruises at the phlebopunctions, splenomegaly, Hb: 11 g/dL, Ht 32%, Retic: 0,8%, L: 15.430/L, Tr: 50.000/L. The formula revealed polymorphic mononuclear cells (25%) with blastic appearance (big diameter, round or lobulated nucleus, fine chromatine, prominent nucleoli, scanty basophilic cytoplasma) and in plus My: 1%, Mt: 1%, N:4%, S:54%, Ly:5%, Mo:10%.

Total bilirubin level 16 mg/dL; indirect bilirubin level 11 mg/dL; ALT: 76 n/L; AST: 212 n/L; TP: 4,6 g/dL, Alb: 2,10 g/dL, Glucose: 65 mg/dL; Urea: 65 mg/dL; Creatininemia: 0,8 mg/dL; LDH: 1359 nmol/L; 2m: 8,86 mg/L; Fbg: 421 mg/dL; INR: 1,18; APTT: 33 sec.; IgA: 421 mg/dL; IgG: 840 mg/dL; Coombs test: neg.

Bone marrow aspirate: rich cellularity, 30-40% mononuclear cells, high diameter irregular cutting nuclei, proeminent nucleoli, basophilic cytoplasm. Cellular mitoses are present.

Flow cytometry of the bone marrow aspirate revealed an infiltration with monoclonal B cells, Cd45+, with moderate internal complexity (cca 24% of total) which expresses CD38, CD19het., cCD79a+++, CD22het., CD5-/+ het., CD43+(low), CD79B+, CD11c+/-, FMC7+/-, CD20, +, CD34-, Tdt-, CD23-, CD10-. Conclusion: aspect compatible with the diagnosis of NHML with large B cells. Bone marrow biopsy: hypercellularity (75/25), malignant interstitial infiltration (35-40%) with large polymorphic cells;

nuclei with lobi (frequent), fine chromatine, 2-4 nucleoli; G/E=3/1, normal maturation, scatterese maturing megakaryocytes.
At the immunohistochemistry, CD20 diffuse expressed in the tumor; Cyclin D1=neg.; CD3 neg. in the tumor, CD30 neg., CD34 neg.

Conclusion: diffuse NH Lymphoma with large B cells (Cd20+) in the bone marrow.
Abdominal computer tomography: splenomegaly (15/8cm); polyadenopathies perigastric, coeliac zone, retroperitoneal, inter aorto-cave.
Pulmonary rx.: normal.

Treatment: antibiotics, antimycotic, hepato-supportive, followed by Dexamethasone + Cyclophosphamide + Vincristine (as a prephase) and later Hyper-CVAD (2 cycles) with complete remission, but with a relapse in 3 months affecting the brain, meninges, the cervico-thoracal teritories. The treatment with HD-MTX plus i.t. MTX, VCR + Procarbazine (R-MVP) was not efficient.

This case underline the importance of taking together the date of clinic, morphology, immuno-phenotyping, histopathology, immunohistochemistry for the correct establishment of the diagnosis in situations with an unusual morphologic picture. The presence of young cells, with blastic physiognomy was interpreted initially as an acute leukemia (possible monoblastic) but the flow cytometric analysis demonstrated the diagnosis of a lymphoproliferative disorder with large B cells suggesting a NH malignant lymphoma. The immunochemistry of the bone marrow confirmed this diagnosis.

Aproximatelly 1/3 of Malignant non-Hodgkin's lymphomas with large B cells and bone marrow involvement presents malignant cells in the peripheral blood.

P16. THE CLINICAL COURSE AND RESPONSIVITY TO TREATMENT FOR CHRONIC MALIGNANT LYMPHOPROLIFERATIVE DISEASES ASSOCIATED WITH AUTOIMMUNE PHENOMENA.

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INTRODUCTION:

Among the hematologic malignancies, autoimmune phenomena are most commonly associated with B-cell chronic lymphoproliferation . The most frequent manifestation is autoimmune hemolytic anemia (AIHA),it is rarely associated with autoimmune thyroiditis / multinodular goiters, Sjogren's syndrome, rheumatoid arthritis, polyneuropathy. In this presentation we analyzed which is the moment when autoimmune phenomena were associated with lymphoproliferative disease but also clinical and biological parameters with prognostic significance of this combination.

PATIENTS and METHODS:

A group of 42 patients with immune complications associated with chronic lymphoproliferation were taken in a study that lasted between 2002-2013.The diagnosis was confirmed by histopathology and immunohistochemistry of lymph node biopsy or osteo-medulare biopsy..The diagnosis of the autoimmune manifestations was established combinning the clinical with the laboratory data. Patients were followed up in terms of histological subtype, lymphoproliferative disease stage, degree of anemia at the time of diagnosis of autoimmune phenomena, association with adverse prognostic factors such as increased number of lymphocytes, the level of beta micro2globulina / hipogamaglobulinemia at diagnosis, the clinical and laboratory evolution, the degree of the patients.response to the complex treatment .

RESULTS and CONCLUSIONS:

Chronic lymphoproliferative diseases are relatively frequently associated with autoimmune phenomena. Except for 2 patients, the diagnosis was made simultaneously, or as a complication in the course of malignant disease evolution. Most autoimmune complications are hematologic, autoimmune hemolytic anemia is the most common (80%) with reticulocytosis present in 60% cases .Rare it was associated autoimmune thrombocytopenia, autoimmune thyroiditis / multinodular goiter, Sdr.Sjogren, cryoglobulinemia.

Regarding histopathological and immuno-histochemical , the chronic lymphocytic leukemia and small B-cell lymphoproliferation were the majority lymphoproliferation associated with autoimmune complications

Autoimmune diagnosis was associated in 75% of patients with advanced disease stage, 25% of patients associated with medullary determination; it is possible that anemia and / or thrombocytopenia have both dual mechanism, autoimmunity and secondary bone marrow infiltration :60% of patients were presented with Hb. <8g/dl.at the time of diagnosis, 25% with Tr <100.000/mm3 (of them, 6 cases were considered as autoimmune trombocytopenia).

At the diagnosis the autoimmunity was associated with known adverse prognostic factors: important leucocitosis , important peripheral lymphocytosis , beta2microglobulina and LDH increased (indirect sign of extravascular hemolysis but also marker of active disease), and hypogammaglobulinemia.

Regarding the treatment , all patients received injectable corticosteroid during hospitalization, ~ 50% followed by po corticosteroid.at hospital discharge. It remains the most common drug used in combination with autoimmune phenomena, which was added to chemo-or immunotherapy with Rituximab (30%). Regarding anemia, half the patients group was required substitution with ipacked red blood cell izogr.izoRh (CER)., 25% were associated with erythropoietin and a small part - with po iron

In terms of survival, was seen getting a favorable response to patients treatment about 98% complete and partial remissions (2 cases of 39-RC) and only 3 patients with progressive disease. We believe that the association of the autoimmunity did not significantly affect evolution and response to treatment of the chronic limfoproliferative disease, and should be considered an independent prognostic factor, noting the importance of research of the cytopenias etiology of in patients with possible bone marrow infiltration, so that treatment involved the lower risks but the best response of both malignancies.

P17. JAK2V617F MUTATION IN ESSENTIAL THROMBOCYTHEMIA.

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Essential thrombocythemia (ET) is a clonal myeloproliferative disease involving a hematopoietic stem cell and, manifesting predominantly as thrombocythosis, and it is associated with thrombohemorrhagic complications and myeloid transformation to diseases such as myelofibrosis and acute myeloid leukemia. In 2005 a unique acquired clonal mutation in JAK2 was found. This mutation was observed in the majority of polycythemia vera patients and in about half of ET or primary myelofibrosis patients. We analyzed 104 cases of ET, from a single institution to determine the prevalence of JAK2V617F mutation and the clinical correlations. Mutation screening was performed on genomic DNA from peripheral blood from all 104 patients. The JAK2V617F mutation was found in 52,4% of cases. 7,2% were homozygous for the mutant allele (>75%). Patients with JAK2V617F positive had higher haematocrit, leucocytes levels and advanced age.

P18. THERAPEUTIC RESULTS FOR PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA.

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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. The treatment of MM has undergone significant developments in recent years. The development of new agents with potent anti-tumor activity has considerably improved the survival of MM patients.

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

Patients and Methods. 42 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. 42 patients were evaluable for efficacy, 63% had refractory disease and 37% were relapsed. The median age was 61 years (37-76), 54% were male, 46% female. Median time from diagnosis was 15 months (2-115) and median number of prior therapy lines was 1 (1-5): 70% had undergone conventional chemotherapy, 17% Alkerane and Dexamethasone and 13% were autografted. Overall response rate of 62% was observed, 30% of patients achieved a complete response (CR), 23% a very good partial response(VGPR), 30% a partial response (PR). Stable disease (SD) was observed in 15%. The median progression free survival (PFS) was 16,8 months. The most common grade 3-4 toxic effects were neutropenia 13%, thrombocytopenia 15%, anemia 8%, infections 10%, 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.

P19. EVALUATION OF TREATMENT RESPONSE IN ELDERLY PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA.

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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 52 consecutive cases of ALL of elderly age collected in the last ten years. Median age was 64 years (range 61-86).
Methods. L2/L1 FAB classification: 46/8; Median WBC was 15xl09L (range 2-195); Male/Female ratio was: 20/32. Forty-four (84,6%) belonged to B cell lineage (pre-pre-B 11, common 28, pre B-5) and 8 (15,3%) to T cell lineage (pre-T staged). Philadelphia chromosome was present in l3 patients (29,5%).

Out of the 52 revisited patients, 36 patients (median age 65 years, range 61-75, good performance status and without co-morbidity factors), received an intensive treatment such as ALL protocols. In the remaining 16 older patients (median age 78 years (range 61-86) 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 (19,2 %) of the group treated with curative intent died during the induction phase; 26 patients (50%) achieved complet remission (CR) and, at present, 4 patients are alive at 12, 48, 50 and 59 months Out of 16 patients receiving less intensive and supportive treatment only 4 (25%) achieved a short CR: other patients had an early relapse and dead after 4, 6, 8 and 12 months.

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.

P20. SPLENECTOMY IN CURRENT TREATMENT OF IMMUNE THROMBOCYTOPENIC PURPURA.

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Introduction. Splenectomy is the treatment used in patients with immune thrombocitopenic purpura (ITP) which do not respond to medical treatment.
Aim. To evaluate the results of splenectomy in ITP patients.

Material and method. During May I 2002 - December 2012 we performed 94 splenectomies in patients with ITP. The majority were performed through exploratory laparotomy with splenectomy or laparoscopic. Intraoperatory, the surgeon had to verify the existence of eventual accessory spleens that have to be extirpated and to avoid splenic rupture that might bring later to development of splenosis phenomena.

Results. Splenectomy went without complications in all 84 patients. There was no intraoperative death. Postoperative complications were observed in 11% of patients. We obtained a significantly decrease of intraoperative medium time from 186,2 minutes in the first 50 cases, to 133,3 minutes in the last 44 cases. Postoperative hospitalization media was 5,3 days. Accessory spleens were found in 8% of patients. After splenectomy 73,5% patients had an excellent platelet response, in 19,5% there was an higher increase of platelet count and 7% of patients had partial response. Preoperative results in corticosteroids therapy did not affect postoperative remission rate. The most reliable indicators of splenectomy efficiency were thrombocytes value ≥100,000/μL immediately postoperative.

Conclusions. Splenectomy is a safe technique with satisfactory remission rate in patients with ITP that do not respond to medical treatment. Postoperative immediately platelets value ≥ 100,000/μL are positive prognostic factor for postsplenectomy long therm remission in patients with ITP.

P21. RETROSPECTIVE STUDY OF CAUSES IN IRON DEFICIENCY ANEMIA IN HEMATOLOGY CLINIC.

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Background. Iron deficiency anemia (IDA) is the common nutritional deficiency worldwide and occurs in 3,5-5,5% of adult men and postmenopausal women. The studies concerning various causes of IDA in adult men are rare, although it is assumed that chronic gastrointestinal blood accounts for the majority.
Aim of the study is to evaluate retrospectively adult men with IDA.

Methods. One hundred ninety-five male with IDA participated at this study from January 2002 to december 2012. Anemia was defined as Hg<13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Mot patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive.The abdominal CT scan were performed according to clinician's recomandation.

Results. The median age was 58 (range 24 to 86) years old. 159 of 195 (81,5%) men with IDA had symptoms such as fatigue, dizzines, or digestive complaints. The history of prior gastrectomy, hemorrhoid, that probably had caused IDA were reported in 29 (14,8%), 38 (19,4%), patients, respectively. FOBT was positive in only 57 (29,2%) subjects. 152 (77,9%) patients underwent EGD. The most common findings from EGD were gastritis (42 patients) and peptic ulcer (35 patients). Sixty-five (33,3%) patients were found to have upper gastrointestinal disorders (15 patients with erosive gastritis, 16 gastric ulcer, 13 duodenal ulcer, 21 gastric cancer. Eighty-one (41,5%) patients underwent colonoscopy. Evaluation with colonoscopy showed 42 clinically important lesions that probably caused IDA; colon cancer in 15 (7.69%) patients, colon polyp in 13 (6,66%) patients and hemorrhoid in 14 (7,17 %) patients. Concerning malignant lesions which are responsible for IDA, 24 malignant lesions were found in patients older than 50 years accounting for 19.5% (24/123 patients) and patients younger than 50 years were 16.6 % (12/72 patients).
Conclusions: This study demonstrated that gastrointestinal blood loss is the main cause of IDA in adult men, and that there is a high rate of malignancy in men older than 50 years, emphasizing a complete and rigorous gastrointestinal examination in this group of patients.

P22. VIDAZA THERAPHY IN MYELODYSPLASTIC SYNDROMES: CASE PRESENTATION.

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We report the case C.R. of 70 years old male with medical history: permanent atrial fibrillation, Heart Failure NYHA II, high blood pressure, prostate hyperplasia. He was admitted in January 2012 to Gastroenterology Fundeni Clinic for investigations of anemic syndrome. The laboratory investigations showed anemia and thrombocytopenia and severe inflammatory syndrome (Fbg 574 mg/dl, VSH 60 mm/1h). Upper endoscopy and colonoscopy did not identify the source of bleeding. In February 2011 the patient presented to Haematology Fundeni Clinic with altered general status, asthenia, palpitations, dizziness, dyspnea at medium effort. Hematologic findings: Hb 9g/dl Ht 29% VEM 93.3 fl HEM 31.4pg CHEM 33.78 g/dl Ret 1.2% L 11.010/µl Mbl 1 Pro 1 Mi 10 Mt 8 N 10 S 54 Li 10 Mo 5 hypogranular granulocytes Plt 78.000/µl.

Bone marrow aspirate- hypercellularity; SG 56% Mbl 3-5%, hypogranular granulocytes; SE 42% common forms macrmegaloblastoid; erythroblasts in mitosis, basophilic stippling, bi and multinucleate forms, megakaryocytic dysplasia with polymorphic forms, round and separeated nucleus.

Bone marrow biopsy- pancytosis, SG deviation to the left, ALIP absent, macromegaloblastoid forms, hyperplasia megakaryocytic with MK small, dysplastic, hypolobulate or denudated. Conclusion- myelodysplastic syndrome.
Cytogenetic analysis- 10 of 11 metaphases showed absence of chromosome Y. During 5 months the patient experienced repeated episodes of infections: interstitial pneumonia, acute enterocolitis difficile Clostridum positive, acute prostatis, perianal abcess and lesions of erythema. The patient received repeated red cell and platelet packed transfusions, treatment with antibiotics.

Due to increased ferritin 1000 ng/dl (secondary to repeated red cell packed transfusions) the patient received treatment with Deferasirox. The therapy also included human recombinate erythropoietin (Epoetinum Beta). A new evalution after 6 months with bone marrow biopsy showed increased percentage of myeloblasts (7%) and progression of myeloid dysplasia. A new assessment of cytogenetic analysis showed absence of y chromosome and monosomy 22 (in 3 metaphases). New assessment included the patient in myelodysplastic syndromes AREB I (WHO). IPSS score included the patient in intermediate risk class. From June 2012 he started treatment with Azacitidine 75 mg/m2/day sc 7 days every 21 days, in total 14 cycles. Last hematologic evaluation in July 2013: Hb 13.5 g/dl L 1000/µl S 38 E 4 B 1 L 52 M 3 Plt 140.000/µl. In conclusion therapy with Vidaza determined transfusion independence and quality of life.

P23. FIRST-LINE TREATMENT IN ADVANCED HODGKIN'S LYMPHOMA.

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Introduction. Hodgkin's lymphoma is a malignant cell proliferation of localized or disseminated, lymphoreticular system, initially affecting the lymph nodes, the spleen, the liver and the bone marrow. The standard treatment is ABVD polychemotherapy but other polichemotherapies such as BEACOPP can be used. Hodgkin Lymphoma results from clonal transformation of the B cells, that leads to the appearance of binucleate pathognomonic Reed-Sternberg cells. Chemotherapy with or without radiation therapy leads to complete remission of over 75-80% of patients.

Goal. The evaluation of the response to polychemotherapy of the patients with advanced Hodgkin's lymphoma.

Methods. We performed a retrospective analytic case study for 80 patients between May 2008 and April 2013 diagnosed with Hodgkin's lymphoma from the Department of Hematology, Timisoara. The main method of diagnosis was the biopsy followed by the histopathological and immunohistochemistry stain. The staging was made with the computerized tomography. Polychemotherapy and the number of cycles were decided according to the histologic grade and the stage of the disease. The patients data, the medical history and the laboratory tests were extracted from the observation sheet of the patient.

Results. The average age of the patients was 31 years, from the 80 patients, 60 (75%) were males and 20 were (25%) females. The most common symptoms were enlarged lymph nodes in the cervical area. In our survey 68 (85%) of patients had enlarged lymph node groups, splenomegaly was present at 25 (31.25%) patients (followed by hepatomegaly with decreased percentage.

A total of 72 (90%) patients followed the polychemotherapie ABVD and 8 (10%) polychemotherapy BEACOPP. From the point of view of the staging 2 (2.5%) stage IV Bx, 9 (11.25) IV (B) , 4 (5%) IV A,10 (12.5%) were staged III Bx, 16 (20%) III B, 14 (17,5%) III A, 3 (3.75%) II Bx ,9 (11.25%) II B, 10 (12.5%) II A ,3 (3,75%) I B. According to the histological 47 (58.75%) presented nodular sclerosis, 26 (32.5%) mixt cellularity and 7 (8.75%) lymphocytic depletion. There were applied an average of 6 cycles of ABVD polychemotherapy and 6 polychemotherapy BEACOPP cycles. Until April 2013; 60 (75%) of the patients are in complete remission, 25 (12.5%), partial remission (3.75) progressive disease, 4 (5%) died and 3 (3.75) from the evidences.

Conclusions. This study demonstrates the effectiveness of polychemotherapy ABVD in advanced Hodgkin's Lymphoma .

P24. COST-BENEFIT STUDY OF THE TREATMENT OF THE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA.

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Introduction. Multiple myeloma is a malignancy of the plasma cells that produce monoclonal immunoglobulins that invade and destroy adjacent bone tissue. The treatment involves combined chemotherapy depending on the stage of the disease.

The aim of the study is to evaluate the cost-benefits of the treatment of the patients with newly diagnosed multiple myeloma from the Department of Hematology, Timisoara

Methods. We carried out a retrospective analytical case study for 105 patients with multiple myeloma from January 2008 until June 2013. Multiple myeloma was diagnosed by the presence of monoclonal immunoglobulin in the blood and excretion of light chains in urine (Kappa or Lambda), as plasma cells in the bone marrow more than 10%, and lytic bone lesions.

The clinical examination, complete blood count, biochemical tests, x-ray (skeleton) were performed on all patients. CT was performed according to the clinician's recommendation.

Results. The average age of the patients was 60 years including 64% men and 36% women. Out of the 105 patients, 95 % presented bone pain, bone injuries and fractures, 35% patients had kidney failure, anemia, nausea, constipation, neurological symptoms. A total of 40 patients (38,09%) were treated with Alkeran and Dexamethasone; 30 patients (28,57%) VAD polychemotherapy followed by Velcade with Dexamethasone; 20 patients (19,04%) Alkeran with Dexamethasone followed by Velcade and 15 patients (14,28%) Velcade with Dexamethasone. The costs of treatment for a patient with Alkeran and Dexamethasone is about 1150 Euros (8 treatments), VAD 1820 Euros (8 treatments) and Velcade 27280 Euro (6 treatments). In our study 65% of patients currently present a complete remission.

Conclusion. This study demonstrates that the treatment with Velcade is the most expensive, it is the most effective and has the fewest side effects.

P25. A CASE REPORT OF BLASTIC PLASMOCYTOID DENDRITIC CELL NEOPLASM (BPDCN): IS ASCT A OPTION?

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Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematopoietic malignancy, formerly known as blastic NK cell lymphoma or CD4+/CD56+ hematodermic neoplasm. Further studies demonstrated the myeloid origin of the tumor cells. Most patients with BPDCN present with cutaneous lesions with or without bone marrow involvement and leukemic dissemination. Immunohistochemical studies are of critical importance, because of demonstration of CD4 and CD56, together with markers more restricted to plasmocytoid dendritic cells, CD123, BDCA-2, TCL1. This is a case report about a young man presented with multiple cutaneous bruise-like deep red mixted lesions widespread to his face, scalp, trunk and upper limb, supradiaphragmatic lymphadenopathy and bone marrow involvement. Histopathology of a skin biopsy specimen and flow-citometry exam of peripheral blood define BPDCN. The patient received SMILE regime and achieved complete remission. To consolidate the response to chemotherapy, the patient has received autologous bone marrow transplant. Six months after autologous transplant, the patient relapsed and he received salvage chemotherapy as DeVic regimen with achieving a second complete response, but only for 4 weeks. Now he is receiving a new chemotherapy protocol and he is waiting for allogeneic HCT, the only possible treatment for a long-lasting remission.

P.26. REGRESSION OF CARDIAC AMYLOID DEPOSITS AFTER CHEMOTHERAPY IN ONE PATIENT WITH AL AMYLOIDOSIS: A CASE REPORT.

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Free light chain type of amyloidosis (AL amyloidosis) represents a neoplastic disease due to deposits of light chains as amyloid fibrils in different organs. The prognosis is poor and depends on the number and severity of organ involvment especially cardiac involvement. According to the published data, the presence of cardiac involvement at diagnosis and/ or enlarged interventricular sept (> 15mm) represents a poor prognosis factor with a median survival of 6 months.

We report a case of 57 year old woman admitted in March 2008 for progressive dyspnea, angor, oedema of legs and hyposalivation, symptoms with abrupt onset and evolution. The clinical examination revealed medium general status, no signs of orthostatic hypotension and ascendent paresthesia to both legs.
The lab results showed Hb=12.6 g/dl, Hct=37.7 %, Wbc=9100/mmc, Plt=282.000/mmc, (Segmented=74, Eosinophyls=1, Basophyls=1, Lymphocytes=18, Monocytes=6), no Jolly bodies; coagulation tests were in normal ranges with the exception of serum level of FX= 57%. The serum protein electrophoresis did not revealed any monoclonal component; serum levels of Ig were in normal ranges; free kappa= 16 mg/l, free lambda= 62.4 mg/l, raport free kappa/ lambda=0.25 (VN=0.26-1.65). serum protein immunofixation revealed compact band identified with anti- lambda light chain antiserum; proteinuria was 6 grams/24

hours. The bone marrow exam (aspiration and trephine): normocellular bone marrow with 5% lymfoplasmocytes infiltration. The abdominal fat tissue aspirate was Congo red positive. The cardiac assessment showed that there were ECG and cardioechogram signs of infiltrative cardiomiopathy (SIV= 17) NT-proBNP 781 pg/mls; EMG: sensitive polineuropathy; Fibroscan: F3 Metavir.
The clinical setting and lab results were suggestive for lambda light chain type primary amyloidosis cu multiple organ involvement (cardiac, renal, liver and peripheral nervous system).

The patient received 7 cycles of MP regimen and at the end of the treatment the lab results were Hb= 12 g/dl, Hct =36%, MCV= 102 fl Wbc=5370/mmc, Plt=106.000/mmc, (Segmented=55, Eosynophyle=2, Basophyle=1, Lymphocytes=32, Monocytes=10); raport free kappa/lambda= 1.48; cardioechography: diastolic disfunction type delayed relaxation, SIV= 12.5).

At 16 months after end of treatment, the patient presented progression of sensitive neuropathy. The patient received 5 cycles of MP regimen (Melphalan was given intravenously) with remission of neuropathy.
The evaluation from 2013 (after 6 years since diagnosis) showed: Hb=9.3 g/dl, Hct= 28.7 %, MCV=116 fl, Wbc=3200/mmc, Plt=50.000/mmc, , (Segmented=74, Eosinophyls=1, Lymphocytes=17, Monocytes=8), frequent macrocytes, granulocytes with hypersegmented nucleus.

Free kappa=14 mg/l, free lambda=11.1 mg/l, rapport free kappa/lambda =1.27; proteinuria was absent. The bone marrow exam (aspiration and trephine) showed normocellular marrow; granulocyte line was normal, hypogranular myelocites and neutrophils with pelger nucleus; hyperplasic erythrocyte line with basophilic megaloblasts; small size megakaryocytes.
Cardioechography: SIV=13, FE= 60%; Fibroscan: F3 Metavir;

The clinical setting was suggestive for late trilineal dysplasia due to chemotherapy and the lab tests confirmed the suspicion.

This case report ilustrates a case of lambda light chain type of systemic amyloidosis with important cardiac involvement which had a good response with intravenous Melphalan but in the same time, developed multilineal dysplasia as late side effect.

This work was supported by the grant CEEX 74/2006 from the Romanian Ministry of Research and Technology.

P27. TREATMENT WITH HYPOMETHYLATING AGENTS IN CASES WITH ACUTE MYELOID LEUKEMIA SECONDARY TO MYELODYSPLASIA – THE COLŢEA HEMATOLOGY CLINIC EXPERIENCE.

Andrei Coliţă, Elena Coleş, Mădălina Oprea, Ana Maria Ivănescu, Cecilia Ghimici, Geanina Ofiţeru, Andrei Turbatu, Anca Roxana Lupu
Colţea Clinical Hopital, Bucharest

Hypomethylating agents have an important role in the therapy of patients with high-risk or transformed myelodisplastic syndrome (MDS). These agents (azacitidine and decitabine) are used in the treatment of elderly and frail patients, and lead to hematological improvement and transfusion independency in about 50% of cases and prolongation of survival in patients receiving azacitidine.

In the Colţea Hematology Clinic we used azacitidine in 4 patients, older than 60 years, with acute myeloid leukemia (AML) secondary to MDS. In 3 cases, azacitidine was used as first line therapy resulting in 1 complete remission and 1 partial response. The number of treatment cycles ranged from 1 to 6.

In the 4th case we used azacitidine as postremission therapy with maintenance of remission for 6 month. The most frequent toxicity consisted in moderate/severe thrombocytopenia.

We also used decitabine in a patient with relapsed secondary AML but without achieving remission.
In our opinion, hypomethylating agents are a valuable therapeutic option agents in patients with AML secondary to MDS or high-risk MDS on condition of formulating precise indications and use in selected cases.

P28. ACUTE BASOPHILIC LEUKEMIA- CASE REPORT.

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Acute basophilic leukemia is a type of acute myeloid leukemia in which the differentiation is to basophils. This is a very rare disease, with a small number of reported cases, comprising <1% of all cases of AML.

The patients may have cutaneous involvement, organomegaly, lytic lesions and symptoms related to hyperhistaminemia. Since this is a rare type of acute leukemia, there is little information on therapy and overall survival and the cases reported had poor prognosis.

We report the case of 61 year old male who in July 2013 was admitted for pallor, progressive asthenia and fatigability. The clinical exam showed good general status, afebril, medium pallor, general oedema, pruritic and erythematous plaques on the calves, no organomegaly. Lab tests showed Hb=10.6g/dL, MCV= 96.6fL, WBC=66240/mmc, Plt= 9000/mmc (Blasts= 42,Promylocyte=1,Myelocyte=4,Metamyelocute=2,Bands=1,Segmented=5,Basophyls=40,Lymphocytes=5) The cytochemical tests: POX positive 40% in peripheral blood, positive Auer rods.

The peripheral blood cyto-flow exam showed a population representing 70% cells, with small-medium internal complexity, fully expressing CD117, CD34, cyMPO, CD33, CD38, CD13 low, CD123, CD71low, CD16 + / - and which is divided into two subpopulations: one subpopulation (50%) that co-express HLA-DR, CD19, and a subpopulation (25-30%) with SSC, CD45 more positive, which coexpress CD203c, CD22, CD2, CD25 (markers of patological mast cell).

The bone marrow aspirate was dry tap and a trephine biopsy was obtained. The bone marrow immunohistochemestry tests revealed CD25 negative, CD34 positive (40%) and CD68 positive in histiocytes but also in frequent mononuclear groups.The bone marrow tryptase was positive. Molecular biology tests revealed AML1-ETO positivity, the other tested transcrips (including bcr-abl and JAK2) were negative.
Serum protein electrophoresis shows compact peak in the beta2 globulins area. serum levels of Igs were normal(especially IgE) except IgG who was increased. Serum protein immunofixation: compact band identified with antibodies against gamma heavy chain and kappa light chain.

The patient received cytoreductive therapy with Hydroxycarbamide, and after reducing the riks of tumour lysis syndrome, 1 cycle of "3 +7"regimen was administered.

Bone marrow aspirate from day 7 shows low cellularity and the one from day 14 shows blasts 6-7% (myeloblasts), basophils <1%.
At present, the pacient is receiving consolidation treatment.

Our case report represents the challenge of accurate diagnosis and treatment of this rare disease. This rare form of AML requires differential diagnosis with chronic mieloproliferative disease, acute mast cell leukemia, systemic mastocytosis, myeloid mast cell leukemia or systemic mastocytosis accompanied by acute myeloid leukemia.

P29. **DIAGNOSTIC DIFFICULTIES IN ACUTE LEUKEMIAS.**

Mihaela Tevet [1], Dana Georgescu [1], Marius Balea [1], Horia Bumbea [2], Doina Barbu [3], Adrian Trifa [4], Mihaela Popescu [1], Rodica Gogulescu [5]

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Background:

Laboratory diagnosis of acute leukemia in hematologic modern practice is based on guidelines that require the availability of immunophenotypic examinations, cytogenetic and molecular biology exams. The majority of cases of acute leukemia belong to a specific lineage origin, either lymphoid or myeloid, classification based on morphologic features and cytochemical and immunophenotypic profile of the blast cells. A minority of acute leukemias however, show no clear evidence of differentiation along a single lineage. These are now classified under acute leukemias of ambiguous lineage and there are acute leukemia blasts that express antigens of both myeloid and lymphoid. There are acute leukemia in which there are two distinct populations of blasts, each expressing antigens of a different lineage, referred to as "bilineal" leukemias or a single blast population expressing antigens of multiple lineages, referred to as "biphenotypic" acute leukemias.

Methods:

We report 7 cases of acute leukemia evaluated in our clinic during 2013 who had difficulty in diagnosis and the screening line. These were assessed by morphological, immunohistochemical, immuno-phenotypic, cytogenetic and molecular biology exams.

Results:

We report two cases of acute myelogenous leukemia associated with myelodysplasia in patients younger than 30 years with no history of exposure to toxic radio / chemotherapy, one being framed initially as a secondary leukemoid reaction and the other one as a myelodysplastic syndrome. A case of B-cell acute lymphoblastic leukemia in a female patient aged 49, known to our clinic with a diagnosis of myelodysplasia with excess blasts of myeloid line. Another case was of a

male patient aged 34 with pulmonary tuberculosis framed initially as hairy cell leukemia and subsequent investigations concluded that the diagnosis was billinial acute leukemia. Also we report a case recorded in our clinic with the diagnosis of acute myeloid leukemia FAB M0, which is in remission for 5 years and who relapsed with acute erythroleukemia. Also, a patient with pancytopenia and colonic polyps, which was suspected of colon cancer and the biopsy diagnosis was myeloid sarcoma and bone marrow puncture put the diagnosis of acute myeloid leukemia FAB M1. And finally, a patient from the dermatology department where he was initially addmited for bullous pemphigoid, but skin biopsy ruled the diagnosis of monoblastic sarcoma.

Conclusions:

Given the polymorphism of acute leukemias, we emphasize the overwhelming importance that the modern methods of diagnosis have in accurately identifying the phenotypic line and the prognostic factors in acute leukemia.

P30. **RARE EXTRANODAL DETERMINATIONS IN NON HODGKIN LYMPHOMAS**

Mihaela Tevet [1]***, Dana Georgescu*** [1]***, Marius Balea*** [1]***, Camelia Dobrea*** [2]***, Oana Patranoiu*** [1]

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Background:

Primary extranodal, extralymphatic Hodgkin lymphomas (PEEHLs) are a rare occurrence. When they are encountered, they become diagnostic challenges. The most common extralymphatic sites were the gastrointestinal tract and the lung, rare determinations are cited in virtually any tissue or organ. Prognosis could not be correlated with the specific sites of involvement. Patients with bulky disease (greater than 10 cm) or more than three sites of involvement had a significantly lower survival.

Methods:

We report ten cases of lymphoma with rarely encountered extralimfatic onset diagnosed in our clinic in the 2012-2013 period. We have not included in this report, cases of chronic lymphoproliferation with gastric or lung involvement. We use current imaging methods (MRI, CT scan) and all determinations were biopsied and we performed histopathological and immunohistochemical examinations required for precise framing the lymphoproliferative disorder.

Results:

The ten patients diagnosed in our clinic with primitive extranodal lymphoma had the following locations: bone (three cases), breast (one case), pancreatitis (two cases), kidney (one case), frontal sinus (one case), maxillary sinus (one case) and eyeballs (one case). Of these, seven cases were classified as diffuse large cell NHL, one of them was marginal NHL phenotype and two cases were diffuse small cell NHL. We could not revealed the presence of hepatitis virus or of EBV or CMV antibody positivity in any of these patients. Eight patients achieved complete response with conventional chemotherapy, two of them associated with radiotherapy and one partial response was obtained and the patient is currently being carried autotransplantation.

Conclusions:

Based on the patients admitted in our clinique, the most common subtype of primitive extranodal NHL was diffuse large cell B subtype. We noticed that it was no correlation reported with the presence of viruses known to have lymphotropism. Response to chemotherapy was good but no statistical conclusions can be drawn given the small number of cases.

P31. **AN UNUSUAL INITIAL PRESENTATION OF CML – CASE STUDY.**

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Chronic myeloid leukemia (CML) is a myeloproliferative disease arising at the level of a pluripotent stem cell and consistently associated with the BCR-ABL fusion gene.

CML most commonly manifest in a chronic phase of the disease with leukocytosis owing to neutrophils in different stage of maturation, basophilia, thrombocytosis or thrombocytopenia, anemia, splenomegaly, and the demonstration of the Ph chromosome is the ultimate confirmation of the diagnosis.

We describe the case of a female patient presenting with isolated marked thrombocytosis resembling Essential Thrombocytemia (ET). The clinical and morphological initial findings were typical for ET: asymptomatic patient, no splenomegaly, no leukocytosis, no immature myeloid cells on smear, no anemia, only persistent raised platelet count (2.200.000x109/L).

We performed the PCR test for mutated JAK2 and the result was negative. Also, the analysis of peripheral blood cells by FISH for t(9,22) came negative. Bone

marrow biopsy specimen was suggestive for 5q- syndrome. To confirm this hypothesis we performed the conventional cytogenetic study. Unexpectedly, it showed the presence of the Philadelphia chromosome, and the absence of other genetic anomalies. The diagnosis of CML was confirmed by PCR testing positive for BCR-ABL p210 type b3a2 transcript.

The particularities of this CML patient case are thrombocytemic onset mimicking ET, the unusual finding of negativity for BCR-ABL fusion gene by FISH concomitant with the presence of Ph chromosome by cytogenetic analysis, demonstrating that in some cases the diagnosis of CML may be a challenging one.

TRANSFUSION HEMATOLOGY EDUCATIONAL SESSION

E2. EUROPEAN GUIDELINES ON GOOD PRACTICE TO SUPPORT IMPLEMENTATION OF QUALITY SYSTEMS IN BLOOD ESTABLISHMENTS

A. Dobrotă
Regional Blood Transfusion Center Constanța

Introduction: Directive 2005/62/EC promotes Community standards and specifications for the elaboration and implementation of a quality system for blood establishments and hospital blood banks.

Given the diversity in the organization of various transfusion systems in the EU, the movement of citizens within it, as well as import / export of blood, art. 2.2 of the Directive foresees a best practice guide for the interpretation of these standards and specifications, so as to achieve an equivalent level of quality and safety in Member States and non-EU countries involved in export activities to the EU.

Objective: The presentation introduces the first version of the Guide on good practice.

Methods: In 2010 a EC-CoE joint project has been launched, whose goal was to develop European Guidelines on good practice for the implementation of quality management systems in BEs. A working group was appointed consisting of professionals with expertise in transfusion specific activity, quality systems and GMP-EU.The activities have started in 2011.

The work plan included individual work at home activities coordinated by the project manager, as well as six workshops. According to the objectives approved, the Guide integrates into a consolidated text the Annex of Directive 2005/62/EC, the principles of GMP-EU (according to art. 47 of Directive 2001/83/EC) and Chapter 1 Standards of the Guide for the preparation, use and quality assurance of blood components (16th ed).

Results: The first version of Guidelines on good practice was published in 2013 in the 17th edition of the Guide for the preparation, use and quality assurance of blood components, in Chapter 1 Standards. An updated version will be published after the 2013 public consultation in the 18 th edition of the CoE Guide.

Conclusions: The occurrence of the first version of the Guidelines on good practice to support implementation of quality system in BEs and HBBs provides practitioners and responsible persons an opportunity to review and update the quality system as a measure either to ensure compliance with legal

requirements, and to improve the quality and safety in transfusion activity.

E3. **EXTERNAL AUDIT EXPERIENCE – BLOOD BANK PLOIEȘTI**

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

Introduction Quality management system requires mandatory auditing both internal and external. All organizations that have implemented Quality Management System fully enjoy the benefits of internal and especially external audit.

Material and methods Following a questionnaire proposed by EDQM in June 2012 and sent for completion by transfusion centers in Romania, Ploiesti Blood Center received an audit visit by EDQM. Visit Romania has been proposed in some random a center, namely, the blood Ploiesti was sent an invitation to participate in a European educational which I responded positively.

I was heavily involved in its preparation, and from this audit we had a lot to learn. I accepted the invitation as a new and useful challenge. I got visitation and audit program was held during two days in the morning and afternoon. I was asked to send prior audit quality management system documents: Quality Manual, list for general and specific procedures for operating list in electronic format.

The audit was conducted by the three experts, the compartments: blood collection, blood testing, blood processing, storage and distribution of labile blood products, control documents of the Quality Management System. Experience of such an audit is unique. It is very useful for revealing strengths and especially the weaknesses of the organization that are difficult to see inside. On this occasion it teaches many aspects of quality assurance audits are essentially a self-learning by attention to detail.

Results After the audit report was submitted in final and have made recommendations which were of great benefit organization audited.

The report was made by EDQM flattering for the blood Ploiesti and expert conclusions were that can assure the quality of a transfusion center just full staff involvement and willingness to commit to work, even if it is understaffed and unmotivated material.

Conclusions Blood Bank Ploiesti recommend everyone to participate in an educational program and be audited by EDQM.

E4. **MONITORING AND CONTROL OF THE QUALITY MANAGEMENT SYSTEM IN BLOOD ESTABLISHMENTS: INSPECTION, SELF INSPECTION**

A. Dobrotă
Regional Blood Transfusion Center of Constanța

Introduction: Directive 2002/98/EC setting up standards of quality and safety for all specific activities of a BE foresees compliance control actions organized under the authority of the competent authority, through inspections and other appropriate measures, depending on the situation.

Monitoring and control of the quality management system in BE are key elements to ensure its functionality and improvement. Directive 2005/62/EC establishes that mandatory self-inspections must be organized by the BEs. Order 946/2005 establishes that it is mandatory for public institutions to organize an internal managerial control system .To ensure smooth and functional implementation of all these requirements corroborated and achieve as outcome a real improvement of medical activity is a challenge for the management team.

Objectives: Presentation of inspection and self-inspection scope and purpose and their benefits in terms of monitoring and improvement of the implemented quality system. Topics to be developed: types of inspection, how to prepare for inspection, attitudes during inspection, inspection reports; elaboration of self inspection plan, organization and valuing of its results, integration in the overall internal control measures plan.

Methods: National regulations-Law 282/2005, Order 1225/2006, 607/2013, 1132/2007, 1228/2006 - establish general requirements for inspection and self inspection. On this basis, a general national procedure will be developed (for inspection) and specific local procedure for each BE may be developed. There are various international guidelines and recommendations on these control activities. Taking into account the organizational and criteria diversity for both inspection and self-inspection among MS, the EC initiated a project to develop a guide for inspection and self inspection: EUBIS. Romanian Ministry of Health translated the guide into Romanian. This may be used as a documentation source for organizing inspection and self-inspection under a common national model; in case of self –inspection, it should be adapted to specific conditions of each institution, based on a risk assessment approach.

Results: Based on EUBIS guide and additional information on the practical aspects, the responsible persons in BEs may prepare self- inspection plans and organize such activities as a tool to assess the QMS effectiveness and efficiency.

Conclusions: Inspection and Self- inspection programs developed based on objective risk analysis and root-cause analysis (if applicable) can provide the competent authority and BEs management documented evidence of the level of quality and safety transfusion activities either nationwide, or institutional.

E5. INTERNAL QUALITY CONTROL IN IMMUNE-HEMATOLOGY LAB (BTC AND HOSPITAL TRANSFUSION UNITS)

V. Halmagi*, C. Bichis**
*Blood Bank Deva , **Blood Bank Hunedoara

Introduction. Quality management is an integrated system of quality assurance to influence individual and collective, each representing a component for quality assurance. Good laboratory practice, quality control and audits management program is about errors and accidents. Risk resulting from hemolysis immunological incompatibility anti-eritrocitary antibody present in the recipient and is the most common and serious. Prevent risk and transfusion safety requires immunohematology tests: determination of ABO blood group, Rh, RAI, direct and indirect Coombs test, direct compatibility.

Purpose. Internal quality control laboratory to detect anomalies and errors immunohematology be rectified immediately. It includes measures to verify all phases of activity and is composed of: -Control equipment -Control reagents -Control technique Elements involved in obtaining reliable results, the quality of this analysis are: -Selection of reagents and their validation techniques -Validation of their reception -Prepared secondary-control reagents (red test) -Internal-controls daily These internal quality checks are made daily. They are samples from the series similar work samples, standardized and delivered by specialized companies or performed in the laboratory. These internal controls are designed to detect anomalies due to equipment, reagents and difficulties of determining the ABO group and Rh, allo and autoantibodies in patients. The paper presents the main difficulty in determining the ABO Rh group.

Conclusion. Internal quality control system is part of haemovigilance and transfusion safety.

E6. LEGISLATIVE BENCHMARKS AND NATIONAL STANDARDS RELATED TO THE HOSPITAL TRANSFUSION ACTIVITY

A. Dobrotă
Regional Blood Transfusion Center Constanța

Introduction: The importance, magnitude and increasing complexity of transfusion therapy in modern medical practice, as good as evidence of a residual risk that can not yet be reduced to zero imposed a set of Community requirements (standards and specifications) regarding the organization and the scope of activity for HBBs. The transfusion activity in the clinical service is regulated by national legislation.

Objective: Review of the legislative framework and standards that regulate the hospital transfusion activity, supported by explanations on correct interpretation, implementation and continuous improvement.

Methods: This presentation reviews the EU (Directive 2002/98/EC, Directive 2004/33/EC, Directive 2005/61/EC, Directive 2005/62/EC) and national (Law 282/2005, Order 607 / 2013, 1214/2006, 1224/2006, 1132/2007, 1227/2006 updated, 1228/2006 updated, 1237/2007 updated, 1226/ 2006 updated, 1343/2007) requirements, emphasizing the logical sequence of steps recommended for hospital managers and physicians in charge with HBB coordination to accuratly and effectivly implement these requirements, for the benefit of patients. Risk- assessment based solutions are proposed, supported by examples from practice.

Information on various relevant international guidelines and recommendations for quality assurance and transfusion safety in the hospital are provided.

Results: The presentation provides an algorithm for the implementation of organizational, technical and quality assurance requirements for hospital transfusion activity, adaptable to the type of hospital, in order to ensure a real improvement in this activity and not just a formal compliance to a new set of legal regulations.

Conclusions: Awareness of hospital managers and treating physicians on the real role of transfusion therapy as part of overall curative or supportive measures is a precondition for a successful qualitative and safe transfusion activity, in accordance with the current requirements and standards. Otherwise, the action taken is limited to a superficial, quick and often partial response to formal and legal requirements, leaving space for errors and incidents that may endanger the patient's life.

E7. THE TRANSFUZIONAL STAGES AT THE HOSPITAL

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Blood transfusion and blood unstable products (LBP) constitutes a therapeutic support which can save or can improve the living conditions of a patient, but, at the same time there is a procedure which is never devoid of risks. To reduce to a minimum the transfuzional risks

act, rules are laid down and principia of good practice Transfusion Haematology, regulated at international and national level. At the hospital there are laid down 3 steps you can take to ensure the safety transfuzion, at each stage responsibilities returning personnel in the sector concerned.

In step 1, the responsibility lies with clinical people and staff working in the sector concerned, consisting in determining transfusion indications, sampling and accurate identification of the samples of blood and fill in the forms of request of blood.

In the second step, the responsibility lies with BTU staff who carry out pretransfuzional tests, liases with CTS general, verify the conditions for the receipt of the products requested, complete documents for the award of the products indicated patient concerned.

In the third step, the transfuzional process takes place in clinical sector level, the responsibility is for doctors clinicians, ATI and staff transfuzor. In this step is decisive appearance check unit assigned to it, to preserve the consistency of the identity of the assigned unity and the patient, the group 0AB at bedside, logging and monitoring transfusion purposes, management of transfusional reactions, monitoring after transfusion the patient.

All of these stages in the transfusional process at the hospital are firmly established by standard operational procedures, their observance by ensuring a high degree of safety transfusion haematology.

TRANSFUSION HEMATOLOGY ORAL PRESENTATIONS

C1. BLOOD DONOR'S CLINICAL AND LABORATORY EXAMINATION – ROLE IN INCREASING THE SECURITY AND SAFETY OF TRANSFUSION. BLOOD BANK BUCHAREST EXPERIENCE

F. Neagu, M. Popa, C. Ruxandu, I. Cristea, Cârstea, A. Olteanu, D. Gosa
Bucharest Blood Bank, Roumanie

According to the legislation in the field of blood transfusion, every donation is necessarily preceded by a medical examination of the donor, consisting of medical history and clinical examination. Talk to your doctor is preceded by completing a written questionnaire default. Examination by a doctor is targeted both to detect blood-borne diseases, to protect the recipient, and to detect diseases for temporary delayed donation, in the interest of the donor.

Donors deemed suitable for donation, the medical examination shall be subject before donation biological controls designed to ensure both their protection and the quality of blood products prepared from donated blood.
In conclusion, a correct and complete information to donors, their advice on donating blood, their self-evaluation by medical questionnaire and subsequent evaluation by clinical examination and biological control before donation steps were required and sufficient for correct assessment of donor suitability.

In the last 2 years, but due to the deterioration of living standards, the health of the population and socio-economic context, it was found that, in parallel with increased blood donors present in Blood Bank Bucharest attracted by a possible source of income has increased and the number of potential donors with poor social status, with poor, even severe in some cases, health status, those who conceal, deliberately various ailments or certain addictions (alcohol, drugs, etc..) and people who consider donating useful therapy to its own conditions.

In this case, the Blood Bank Bucharest, requiring changes to clinical and laboratory methods, increasing human and material resources in the blood transfusion services, clinical and laboratory investigations need to broaden, deepen before donation biological control and, not least, initiate special campaigns promotion of blood donation, in order of population awareness and accountability, promoting the collection phones, in order to change the donor's profile and the organization of training courses for professionals in the medical

system.

In particular, but in the blood, clinical and biological control before donation have gained importance, the decisive steps in donor suitability decision, and thus in maintaining safety and security transfusion and consultant doctor must improve , to diversify and exploit the possibilities of investigation available to ensure the protection of the donor and the quality of blood products prepared from donated blood.

C2.THE IMPORTANCE AND IMPACT PARTNERSHIP BUCHAREST BLOOD BANK WITH DIFFERENT ORGANIZATIONS IN PROMOTING BLOOD VOLUNTARY DONATION

F. Neagu, M. Popa, C. Ruxandu, I. Cristea, Cârstea, A. Olteanu, D. Goșa
Bucharest Blood Bank, Roumanie

Modern blood transfusion practice was founded on the principies of voluntary donation, anonymity of donor and recipient and volunteer donor, absence of any profit from the units involved in blood transfusion services.

Voluntary and unpaid blood donation is a factor that can contribute to raising the standards of safety for blood and blood components and thus the protection of human health.

In Romania, by law, be required to provide biological support blood donation through the allocation of food stamps used by the donor for the purchase of food and soft drinks. While it was found that due to the deterioration of living standards and socio-economic context, getting food vouchers became, for some donors, a possible source of income, thereby distorting the real purpose of humanitarian act, but having the effect of increasing the number of blood donors.

For this period, this result was expected, and achieved the stated purpose of some broader campaign to promote blood donation, on the other hand, however, need to comply with EU recommendations, requires a new approach to the promotion policy and strategy supported by strong partners involved politically, economically and socially, by blood transfusion services.

C3.DETERMINATION OF HAEMOGLOBIN TROUGH A NON-INVASIVE METHOD

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

Introduction The level of hemoglobin Quickly Can be detected in predonation, through a non-invasive method, painless and with no risk of infection. Potential Blood Donors Will no longer have to bear the painful sting of the fingertip. Haemo SPECT ® is a device for measuring extremely suitable for use as a fast and efficient way to intake of Blood Donors.

Material and method

HAEMOSPECT ® is a mobile device, portable, easily Useful to be used everywhere, dedicated to non-invasive measurement of hemoglobin. Ensure measurement without bloodshed in the level of hemoglobin, it is easy to use thanks to the digiclip. That provide measurement reading in a few seconds. It is hygienic, easy-to-use, and reliable without any risk of infection. It is equipped with batteries, therefore, can be used anywhere in the establishments to collect. It is friendly to the environment because it works without supplies, without any replacement of sensors being so extremely economically. The principle of operation of the sensor head is placed on the skin of the donor. A beam of white light from xenon is projected in the donor tissue. A portion of the Projected Light wave is absorbed by the various components of tissue, while some of it is reflected. A guide transmitted reflected light wavelengths, due to the physical condition, back to the device. The by reflection contains information specific to the tissue. This light is guided to the assessment, through the fiber optics, optical sensor That Makes evaluation year and THEN is evaluated by the program. The resulting time algorithm is processed using year and is visualized on the display of your device. The device is powered by batteries. A right smart charger is included in the device, so the Batteries That Will not be overloaded. With Fully Charged batteries, the device can measure at Least Five hours in continuous operation.

Results In the period 15. 02-15-03 were tested in the laboratory of predonation Blood Transfusion Center, with HAEMOSPECT a total of 1,600 aiming Donors and the blood hemoglobin value was determined in parallel using Nihon sampled.

Conclusions Results of the screening Hematology Analyzer in venous hemoglobin with HAEMOSPECT lab for predonare is Useful in screening Donors, is a very well tolerated by the donor, but may not have the precision and accuracy of the determination of venous blood on hematology analyzer.

C4. QUALITY CONTROL OF LABILE BLOOD PRODUCTS, THE FINAL HAEMOLYSIS RED CELL CONCENTRATES

G. Hanganu, M. Catană, D. Gheorghe, M. Coman
Blood Bank Ploiești

Introduction

The purpose of quality control of labile blood products in terms of measuring hemolysis seeks compliance labile blood products, offering accurate data processing and storage of products containing erythrocytes correct them, by studying free hemoglobin released into plasma at the end of life, with meeting the limits of the standard values.

Material and methods

This method was applied in laboratory hematology control for labile blood products. Low hemoglobin value was determined in plasma bags reached its expiry by hemoglobin device Hold / Low Hb Photometer, HemoCue. They controlled 1% of each type of labile blood product or at least 4 units per product type / month. Erythrocyte concentrates resuspended reached expiry date were checked before being removed from circulation and destroyed after harvesting work samples directly from the bag. Resuspended red cell concentration was evaluated at the end of storage hemolysis and red cell concentrate resuspended leukocytes depleted, hemolysis after the filtration step, the fresh frozen plasma was evaluated in plasma free hemoglobin (samples were obtained from randomly selected bags)

CER.UA were studied 40, 30 CE.DL, 30 PPC. Free plasma Hb free in CER.UA and CE.DL must be <0.8% of total Hb. Free plasma Hb in PPC must be <0.05g/dl. Only if this condition is met, resuspended red cell concentrate / leukocytes depleted can be labeled "conform" selection and processing, as well as PFF, processing and selection. Residual values over Low Hb; Hb> 0.8% show a deficit defective processing and preservation products. The results are not included in this limit sun labeled as non-conform.

Results

In all the 100 products studied was found compliance hemolysis was below 0.8%.

Conclusions

Processing and storage of labile blood products for the period studied was in accordance with European standards.

C5. SECURITY AND CREATING SELF-SUFFICIENT TRANSFUSION BLOOD COMPONENTS HUMAN IN BRASOV COUNTY

L. Florea
CTS Brașov

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 a national organization; the blood donor must be volunteers and unpaid; there must be a thorough testing and processing of all units collected; the rational clinical 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-sufficiency locally.

I have studied the statistical data gathered in Blood Bank Brasov over the past 5 years regarding the number of outlets of blood taken from new donors in and the recurrent ones. In parallel, 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.

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, the lack of an organized blood transfusion, lack of 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 sufficiency is sometimes hard to bi accomplished: having in mind that only 17-18 % of the people are blood donors and that requests for blood production are significant. Deficiency of certain blood components is ranging between 80-90%.

C6. BLOOD DONATION AND THE MODIFICATION OF THE DONOR'S PERCEPTION UPON HIS OWN HEALTH

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The long experience that we have had with blood donors permitted us to observe the strong positive-reflexive relation between the blood donation process

and the perception of the donor upon his own state of health.

A questionnaire applied on 500 donors revealed the following:

Once the donor had his first blood donations, he becomes more and more aware of his own state of health, seeing it as an asset, asset that he didn't realize to have before his blood donations.

This revelation is followed in a natural way by a positive change in the donor's behavior, as to maintain the state of health, now in a conscious way.

The changes, according to the questionnaire, refer to the adaptation or accommodation of the donor to a keep fit program, to giving up all behaviors that could endanger health- drinking, smoking, etc.

From the data obtained from the questionnaire we could follow up the group of population with the highest modification in their perception upon their own state of health, that is the group of people between 20-28 years old. The adaptation to a risk free behavior and to nutrition hygiene was of 87% for this age group.

At the age group of 35-48 years old, the changes were smaller in percentage, only 68% from the people taking the questionnaire had a positive modification in nutrition hygiene and behavior.

The big picture indicates though very clearly that the relation between blood donation and health is obvious and positive.

C7. **RAPID DETERMINATION OF SCREENING-HCV ANTIBODIES IN ORAL FLUID**

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Introduction Determination of HCV antibodies in oral fluid using Quick Time HCV Rapid Antibody Test.

Quick Time Rapid Antibody Test is a disposable test for anti-HCV screening. It is an immunological test for the qualitative detection of Ig G antibodies in oral fluids especially, but also in whole blood obtained by capillary puncture or by venipuncture, plasma and serum.

Material and methods Quick Time Rapid Antibody Test is a test manual is read in 20 minutes. The test contains recombinant protein core, NS3 region of NS4 region and goat anti human Ig G in the control line, which are immobilized on nitrocellulose membranes. The test also contains phosphate buffered saline. Test specimen: oral fluids: the patient should not be eating, drinking, brush your teeth, or chewing gum 20 minutes before the test. Collect saliva and immediately in the developing solution. Blood, plasma, serum: spatula is inserted in the developing solution within 60 minutes after adding the sample. The actual test: the components are brought to room temperature. Place the rack on the bench reusable. Open the developer solution vial. Wipe the upper and lower gum pad without touching the palate, cheeks or tongue. Insert the device in solution development. The wait for 20 minutes. The read test. A single band = negative test, two lanes = positive test.

During 10.01.2013 - 30.06 .2013 Ploiesti in the blood were tested a total of 20 tests Time Quick Rapid Antibody Test on new donors, and patients comparing the results of Quick Time Rapid Antibody test results obtained by the method ELISA.

Results Only patients with elevated reactivity of anti-HCV present positivity in Quick Time Rapid Antibody test.

Conclusions Quick Time Rapid Antibody Test is a screening test that can be used in a family medicine practice, its use in transfusion centers were excluded.

C8. **PRELIMINARY RESULTS OF MINI-POOL(MP) NAT TESTING OF BLOOD DONATIONS FOR TRANSFUSION TRANSMITTED INFECTIONS(TTI)**

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BACKGROUND: Introduction of nucleic acid amplification techniques (NAT) worldwide, as a complement to serological testing, aims at reducing the residual risk of TTI, due to serological window or silent chronic infections. In Romania, blood donation screening for TTI relies on state-of the art serological methods, but the residual risk, although considerably lowered as compared to the moment of the introduction of specific screening, remains well above the level registered in western EU. Local prevalences and incidences and previous occazional detection of viremic seronegative donations point to the need of introducing NAT for screening donations. We report here the results of MP NAT testing on a lot of blood donations as compared to the serological screening .

METHODS: 2496 plasma samples from blood donations collected during september-october 2012 at CTSMB, were sent to LCR-VTS and extracted in 416 MP of 6 donations, for West Nile Virus testing. The remaining extractions were amplified with Arthus HIV, HBV and HCV kits (Qiagen) respectively. Repeat extractions were done from the reactive MP and from the component individual(ID) samples , and the amplifications were performed in the same run. The serologically reactive samples resulted from the current screening of these donations by CTSMB were also refered to LCR-VTS for serological confirmation and

viral load quantification.

RESULTS: 18/416 MP tested NAT reactive as compared to 22/2496 serologically reactive reported donations. HIV: One MP was NAT positive (1/2496) containing the only positive donation confirmed by serological testing and ID NAT. HBV:11 positive MP were detected while 12 ID donations tested positive by NAT out of the 13 serologically confirmed.One serologically confirmed donation had a low viral load witch became nondetectable upon dilution in MP and the other was not detectable even by ID NAT. HCV:7 serologically reactive donations were reported;one was a nonspecific reactive and 6 were confirmed by imunoblot, out of witch only 5 were detected by MP and ID NAT; 1 NAT positive MP contained 1NAT positive serologically negative donation, coresponding to a window-period donation from a first time donor.

CONCLUSIONS: The additional detection by NAT of a window-period donation points to the need of introducing NAT as a complementary tool to serological screening of blood donations, supported also by previous findings of viremias among serologically negative repository smples from repeat blood donors who seroconverted between consecutive donations. On the other hand, serologically positive HBV and HCV nonreplicating infections are frequently detected worldwide, especially among blood donors and further investigation is needed to evaluate the extent of this fenonmenon in our donor population. 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. Further reduction of the residual risk of TTI would occur only through introducing the NAT testing of all donations and improving standards for donor selection.

C9. **THE IMPORTANCE OF ALT (ALANINE ALAMINOTRANSFERASE) DETERMINATION IN BLOOD DONOR SCREENING, ESPECIALLY WHEN NAT/PCR TESTING IS NOT AVAILABLE**

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Introduction Determination of ALT serum level is the most inexpensive and most noninvazive method of assessing liver activity. All the A--- E Hepathitis have in common the increase of ALT serum level.

The ALT level is directly proportional to the extension of liver lesions, but does not correspond to the phase of viremia growth .Recently appeared articles show that some international specialized organization reached the conclusion that, in case the blood donor screening is performed by NAT/PCR, ALT testing might be given up.

Study's objective The importance of ALT determination in blood donors screening, testing in terms of NAT / PCR absence

Materials and methods

Methods to determine the ALT serum level: colorimetry and dry biochemistry

Reagents:- Sentinel with reference range between 1 -75 U / liter

-Vitros with reference range between 17 – 72 U/ liter (for males) and 17 – 52 U/ liter (for females)

The samples with high levels of ALT, but with negative viral markers, are sent to LCR-VTS (INTS) for aditional investigations: anti HBc (core) antibodies; anti HBs (surface) antibodies; Hbe antigen; anti HBe antibodies; anti HCV antibodies

The results received from LCR will be the base of the decision to exclude or not the blood donor.

Casuistry: The ALT serum level determination for every blood donation.

Results: statistical data for the period 2010-2012 will be presented, with the following content:

Blood donors rejected for: High levels of ALT (above the maximum accepted level), re-tested on LCR-VTS for hepatic markers and found postive for at least one of these markers.

Conclusions Even if ALT determination is considered a “surrogate” test, we consider that the determination of ALT serum level in blood donors screening is of high importance, especially when NAT / PCR testing is not available.

The ALT determination proved its efficiency in sorting all the blood donors (new, occasional and loyal) and contributed to:

- Increase the safety of blood supply;
- Reduced the B hepathitities occurrence associated with blood transfusion;

Also, the ALT determination is very helpful in detecting potential subliminal viremia , which otherwise would pass unnoticed.

C10. **STUDY ON FREQUENCY BLOOD TRANSMITTED INFECTIONS IN DONORS BLOOD CRAIOVA REGIONAL BLOOD TRANSFUSION CENTER IN 2008-2012**

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INTRODUCTION

Transfusion activity is one of the most important links in the performance of medical, transfusion of blood or blood components accounting for a large proportion of patients vital intervention.

This being so it is understandable to be granted a

special interest in the selection of the maximum requirement to become or are already donors.

Material. and method This paper is based on a retrospective study, a total of 57,366 donors aged 20-64 years divided in three categories, new donors, loyal and casual in a period of 5 years ie 2008-2012.

Results. Our results show that the most common disease in the category of dual transmission, but sexual and blood is represented by Hepatitis B disease incidence has been rising continuously since 2008 (156 cases) to 2012 (158 cases), the peak being reached in the years 2009 - 242 cases and 2010 - 207 cases. The other double transmitted infections were the Hepatitis C, with higher incidence in women, syphilis and not least human immunodeficiency virus infection, which after more than 20 years we have met among donors. Age group results show that the share is held in both men and women subjects aged between 20 and 44 years. Serologic testing performed in all 3 categories of new donors, loyal and casual showed that all positive results were recorded in the "new donors".

Conclusions. In the end we remark that in order to avoid problems related to the transmission of various diseases associated with donor blood, the imperative to preserve a large number of loyal donors, using rational "the book" of blood transfusions, promoting auto transfusions and not least new techniques mandatory donor screening, knowing that this therapeutically act is the bearer of a lot of risks.

C11. **HLA TESTING FOR THE SELECTION OF A COMPATIBLE HEMATOPOIETIC STEM CELLS DONOR – STRATEGIE, DIFFICULTIES, RESULTS**

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Introduction: Stem cells transplantation is one of therapeutic potential curative procedure frequently used in malignant diseases treatment.. One of the most important conditions for a successful hematopoietic grafting is the selection of a HLA matched donor.

Materials and Methods: About 60% of the last 10 years National HLA Laboratory" s activity was dedicated to the selection of donors for patients that needed a hematopoietic stem cells allo-transplantation. The working protocol suffered several changes according to the typing methods available in our laboratory. The present protocol was established according to the Bone Marrow Transplantation Center requirements, in compliance with European Federation of Immunogenetics (EFI) standards for histocompatibility testing. The tests are performed in 2 steps. The first step is to test HLA-A,B,DRB1 alleles of patients together with all available first degree relatives. Only DNA methods, SSP or SSO 2 digits are used. All patient-donor compatible pairs, even those with clearly haplotype segregation, are tested for HLA-A,B,C,DRB1,DQB1 alleles, on new fresh blood samples, using molecular biology 4 digits typing kits. Also, for patients without a suitable family donor, our protocol includes performing a second HLA typing using new fresh blood samples, by molecular biology 4 digits typing methods. The most common difficulties in performing the HLA tests and the selection of a compatible donor were: wrong sapling, leucopenia, unknown exclusion of paternity , crossing-over, rare HLA alleles or haplotypes.

Results and conclusions: Our statistical data for 2008-1012 show that about 120 families have been tested every year, with a significant growth trend in 2013. The tests result" s analyses shoved that about 28% of the patients tested in our laboratory had a potential compatible donor in their family. In the majority of the cases it was a HLA genotypic identical sibling and in 2 of the cases, other relatives such as parents had 100% allelic compatibility. Regarding the selection of an unrelated compatible donor, 35 extended or verification typing requests have been received in our lab – there were 15 romanian donors and 20 foreign donors. Those tests confirmed the HLA compatibility for 21 patients-donors pairs. Between the potential compatible donors selected from Romanian National Registry, 3 donors have been confirmed – 2 for a Romanian patient and 1 for a foreign patient.

C12. **RELEVANCE DETERMINING HLA MARKERS TO CELIAC DISEASE IN PATIENTS WITH NEGATIVE SEROLOGY.**

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Introduction: Celiac disease is an inflammatory disorder with autoimmune component, wich involves the interaction of a protein in gluten and genetic, immune and environmental factors. Data from the literature demonstrates a strong association between HLA DQ locus (α gene and β gene) and individual predisposition in response to the ingestion of gluten or other similar proteins. The wide spectrum of clinical symptomes corelated with to digestive and extradigestive serologic tests could give the diagnostic orientation in only 1 to 7 cases.

Material and method: The aim of this study was to correlate clinical the symptoms, the response to diet with HLA markers in patients with negative serologic tests and without intestinal biopsy. For this purpose, in the period 2012 – 2013 HLA markers (HLA DQ A1 and DQB1) for 221 pedriatic patients have been tested. The methods used to test HLA were molecular biology techniques PCR-SSP low and high resolution. A total of 42 subjects (19%) HLA with markers present (DQ2, DQ8) had no positive serologic tests for specific antibodies.

Conclusions: Although serology was negative, the patient's clinical symptoms could be correlated with HLA markers and a favorable response to gluten-free diet. The presence of HLA DQ2, DQ8 antibodies in the absence of serological and intestinal biopsy, together with a favorable response to gluten-free diet, suggests the diagnosis of celiac disease.

C13. **PROSPECTIVE STUDY ON THE Rh D VARIANTS IN BUCAREST BLOOD DONORS POPULATION**

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Objective:

The aim of this study was to monitor and to compare the different RhD variants and antibodies and their frequency during the last year in CTSMB

Material/Method:

Equipments: semiautomatic analyzer Zenyx (Biotest), fully automated system Qwalys (Diagast), Ortho BoiVue System, Dia Med System.

The negative test was carried out in comparison with ID DiaMed Anti Ig G cards with Anti D weak serum, technique based on the Coombs test .

A number of 10540 samples were taken and processed for Rh D tested routinely with different monoclonal AntiD (Ig M and IgM + Ig G) All negative or weak reactions (1+ - 3+) were confirmed with polyclonal anti D ser and molecular biology testing .

For the detection and identification of antibodies were used DiaMed and Ortho Bio Vue panels

Results

- 15 samples (0.14%) were weak D and partial D from various category
- the antigens ranged between 7.07% D and 87.29% E
- in the Rh - negative the more functional variants were: dd cc ee 87,62 %// dd Cc ee kk 6,44 % // dd cc Ee kk 1,11 % // dd cc ee KK 4,23 % // dd Cc ee KK 0,50 % // dd CcEe kk 0,10 %
- Correlation weak D variant Rh D blood group looks like this: Cc ee kk 0.10% A / / 0.10% B / / 0.31% O / / Cc Ee A KK 0.10% - 37.6% of the samples showed positive DAI anti Rh system antigens, in 15.5 % cases we detected antiD antibodies.

Conclusions: Setting Rh phenotype is absolutely necessary in the transfusion.

The RhD category is very important in order to establish the transfusion strategy.

Using appropriate reagent and methods it is possible to detect the variants D week.

Besides the classic phenotype Rh (dd ee cc kk) there are other functional variants in percentage of 12,38 % with possible transfusion implicatzii.

It is necessary to investigate the anti –D alloimunization rate of the variants to provide a better immune transfusion service

C14. **AQUIRED B ANTIGEN – CASE REPORT**

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Background: During the common AB0 phenotyping, it is possible to appear some discordant results between erytrocytic and seric tests, due to a lot of causes.

Aim: Carring out multiple immunohematologyc tests to indicate a group of 0AB system, which has presented the discrepancies results between eryrthrocytic and serum-sample.

Casuistry, methods: patient, man 66 years old, undifferentiated carcinoma digestive abnormalities associated with infection with B Coli, HDH and severe anemia (Hb. 4g%).

For this purposes of determining the routine blood group AB presents himself as poor AB with an antiB in his serum; determination of RhD factor does not have any problem. For the specification of 0AB are undertaken reactions of agglutination of erythrocytes with anti A1, antiH, antiB poli and monoclonaly, normal and acidified reagents. Determine secretory status and salivary gland substance from the group.

Results Group A1, RhD negative, with acquired B antigen. Compatibility testings were without problems with A1 RhD negative blood.

Conclusions: In the case of digestive carcinoma association with infection with B. Coli, bacterial deacetylases converts the antigen A1 in antigen B like, so that it is seen discrepancies between sample Beth-Vincent and Simonin tests in determining the AB0 group.

C15. THE IMPORTANCE OF IMMUNOHEMATOLOGY COMPLETE TEST TO AVOID ACCIDENTS AND ALOIMUNIZATION POSTTRANSFUSION

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Introduction: Immunohematology Security is an important level of security transfusion in Blood Bank Brasov. It performs a series of Immunohematology tests, both blood donors and the recipients (OAB group, RhD, research irregular antibodies, RhK phenotype, major compatibility tests).

Casuistry, methods and reagents. Samples from blood donors and blood product recipients.
Testing done by micromethod of hemagglutination in gel DiaMed and OrthoBioVue and by hemagglutination macrometode liquid phase.

Results. In the period January 2011 - January 2013, from 25 176 donations were tested for DAI 16228; were also tested 420 recipients.

Irregular anti-erythrocyte antibodies to blood donors 0.29% - 0.21% - 0.08% women and men, with specificity in the system Rh (anti-D, anti-D + C, anti-E, anti-C, anti-Cw) - 44.7% , in Lewis system 38.3% to 10.6% Kell system, Duffy system in 2.12%, 4.2% in MNSS system.

Frequency irregular antibodies in recipients = 15.23% - 10.23% women and 5% men, with specificity in the system Rh (anti-D + C antidepressant, anti-E, anti-C, anti-Cw, anti-c anti-D + E, anti-c + E) - 56.25%, in Lewis system 7,18% 6,25% in the Kell system, Duffy system in 1.56% to 1.56% Kidd system , the system MNSS 4.68% blend of 21.87% allo and auto-antibodies (anti-D + auto-C-C + self-car is self-c + e self-self-c, self-e anti-E + self-c).

Conclusions: Conducting tests according to the algorithm working Immunohematology both donors and the recipients, ensuring a high degree of transfusion safety (accident prevention and alloimunization).

C16. THE ROLE OF THE MULTIDISCIPLINARY TEAM IN THE MANAGEMENT OF PATIENTS WITH AIHA

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Introduction Autoimmune hemolytic anemias are a group of diseases characterized by the presence of autoantibodies that bind on patient's self red cells leading to their premature destruction. When hemolysis exceeds the BM ability to compensate erythrocyte mass destruction, anemia occurs. Although AIHA is relatively rare in children, it may begin as an hematologic emergency whose prognosis is influenced by the quality of interdisciplinary collaboration for rapid diagnosis and therapeutic intervention.

Aim of the paper The paper presents the case of a 13 y old girl who is admitted as a great hematologic emergency. The severity of anemia and her brutal onset, apparently in full health condition was a challenge for the medical team involved, representing three medical institutions: hospital clinical service, blood transfusion center and clinical laboratory providing outsourced laboratory services to hospital. The added-value of direct comunication between the various specialists involved for the quality of medical care in emergency situations is emphasized by the presentation.

Case presentation 13 years old patient is hospitalized as an emergency in pediatric ICU for generalized hypoxia, lethargy, intense sclerocorneal and skin jaundice, pallor, occurring several hours before. Blood samples tested in the laboratory, HBB (pretransfusional protocol) and RBTC (for Immunohematology Diagnosis and pretransfusional protocol) showed the following relevant initial results: Hg 3.6 g%, Ht 7.3%, reticulocytes 2.11%, frequent sferocytes and microsferocytes, DBR, 2mg/dl, IBR 6.3 mg / dl, LDH 1312mg/dl, low haptoglobin, DAT polispecific positive. ABO different results between in HBB and RBTC. Treatment with corticosteroids is innitiated. As the patient's condition required urgent substitutive therapy, decision is taken to launch the procedure for transfusion in major emergency: packed red cells O negative free of immune antiA/B Abs were issued by HBB; the occurrence of mild low back pain complaints stopped the transfusion. In parallel, investigations continue to determine the type of AIHA (RBTC) and etiology (SYNEVO), as well as selection of compatible packed red cells (RBTC). Immunohematologic profile: discordance BV-S, D positive, DAT positive polispecific, monospecific IgG positive, C3c positive, C3d positive, IgM positive, negative IAT. Subsequent transfusions without adverse reactions. Virological tests, HBV, HCV ann Mycoplasma negative, anti EBV IgM positive. Favorable outcome after the first 24 hours of hospitalisation.

Conclusions Cooperation and direct communication between the 3 specialists allowed accurate and rapid diagnosis and treatment initialisation in a short period of time, which resulted in rapid restoration of patient's vital functions and a favorable prognosis.

C17. **DEATH CAUSES IN ROUMANIAN MAJOR THALASSAEMIA PATIENTS BEFORE AND DURING IRON CHELATION THERAPY**

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Introduction Major thalassaemia is a hereditary disease, manifested as a very severe anemia depending of regular blood transfusions therapy. As a consequence of those transfusions, patients with thalassaemia major becomes iron overloaded. With multiple organ failure and death. Iron chelation therapy prevents iron accumulation and is mandatory when serum ferritin levels reached 1000ng/ml.

Material and method Our study group includes patients with major β thalassaemia registered at NITH level in chronic transfusion and iron chelation therapy, more then 50% of Romanian patients. New oral chelation and hyper transfusion regime leads to some changes in medical aspects of disease.

Results and discutions: Before the end of 1995, the main death causes was cardiac failure (75.7%), followed by hepatic and infectious mortality. Introduction of National Programme for treatment of hemophilia and thalassaemia in 1997 and new oral chelator in 2008 leads to significant decrease of number cardiac death to 33%.

Conclusions: a correct and sustained iron chelation therapy reduced very much cardiac causes death. Cardiac, infectious and liver complications still remains the main death causes in major thalassaemia in Romania, as all over the world.

C18. **DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS DURING PREGNANCY**

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Introduction: Hereditary spherocytosis is a hereditary hemolytic anemia, the red blood cells defect consisting in a structural change of the erythrocyte membrane.
Aim: The study presents the cases of hereditary spherocitosis (oligo-symptomatic forms) in anemic patients pregnancy.

We have studies 25 anemic pregnant patients, sent to us by various clinics of obstetrics and gynecology.
These patients, between 20 and 36 weeks of pregnancy, had a well-tolerated anemia, with levels of hemoglobin ranging between 8 and 11,8 g./dl. Most of them had bad a slight anemia before pregnancy never been investigated. The pregnant ladies have been followed from clinical and hematological point of view, all along the pregnancy and also after giving birth.We have followed the levels of hemoglobin and hematocrit, the value of MCV (always normal), the aspect of the peripherical blood film and the level of serum iron) / normal or slightly increased). At first, we considered these cases to be common pregnancy anemias (iron deficiency and folic acid deficiency); In the blood films, we have found traits suggesting hereditary spherocytosis (spherocytes, "fat discs", polychromasia and sometimes basophilic strippling). The diagnosis hypothesis was confirmed by osmotic fragility and autohemolysis tests. We mention that all these cases were very well compensated there has never been a patent hemolysis and we noticed especially folic acid deficiency, due to increased requirements of the fetus. We increased folate supplements with doses of 15 – 20 mg. daily.

Conclusion: Through this study, we intend to draw attention to the fact that often a "common" anemia of pregnancy, can hide a form of hereditary spherocytosis.

C19. **PARTICULAR ASPECTS OF ANEMIA IN GERIATRIC PATIENTS**

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Introduction: Anemic syndrome in old age is a very common disorder, with multiple causes and often mixed.
Aim: Throuth this study we aimed to identity different aetiological categories of anemia in geriatric patients.
We have studied 170 patients, between 60 and 92 years of age, that we have followed in the Hematological Diagnosis Laboratory in the last two years (2012 – 2013); we have not included in this group patients with β – thalassemia minor which were already in our evidence.

We have followed the level of hemoglobin and hematocrit, the aspect of the peripheral blood film, the values of MCV, MCH and MCHC, serum iron and TIBC and we have also explored level and renal function. For patients with severe anemia, we required gastro-intestinal tract explorations and bone marrow puncture in specialised clinics.

We have performed at least 3 clinical and hematological check-ups per years. We have found a slight female prevalence (56%) and different degrees of anemia (the hemoglobin level ranging from 6.5 to 11.5 g./dl.)

We have established the following etiological types of anemia (in order of their frequency) 1. iron deficiency anemia (almost half of the cases) due

especially to gastrointestinal bleeding ; 2. myelodisplastic syndromes (age – related myelodisplasia, sometimes associated with age-related bone marrow failure); 3. macrocytic and megaloblastic anemia (due to cobalamin and/or folic acid deficiency); 4. anemia of chronic inflammation; 5. hemolytic anemia (autoimmune or due to membrane disorder).

We have frequently found cases with two or three etiologies (for example: iron deficiency + chronic inflammation; or myeloblastic anemia + MDS + iron deficiency) and even cases with several etiologies (iron deficiency + MDS + chronic inflammation + anemia in uremia) and this is a special trait of anemia in elderly patients.

Conclusion: Throuth this study we intend to emphasize the complexity of anemic syndrome in old age, and contact with other age-related diseases.

C20. **FATAL COLD AGGLUTININ DISEASE IN A PATIENT WITH SURGERY FOR GASTRIC CARCINOMA: A CASE REPORT**

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Introduction Cold agglutinin disease (CAD) is a condition in which auto-antibodies, usually immunoglobulin M directed against the I/i and H antigens, cause red blood cell agglutination at decreased body temperature. Autoimmune and lympho-proliferative disorders, Mycoplasma pneumoniae and other infections can be associated with the production of cold agglutinins. The association between CAD and gastric carcinoma is very rare. Peri-operative hypothermia is common in all patients and may be associated with significant hemolytic risk in a patient suffering from cold agglutinin disease. Several factors play a role in determining the ability of a cold agglutinin to induce a haemolytic anaemia such as antibody concentration and thermal amplitude.

Case presentation A 82-year-old male patient was diagnosed in Surgery Department UEH with signet ring cell gastric carcinoma. Gastrectomia was proposed due to hydration and feeding difficulties. Laboratory data showed mild anemia (10,5 g/dl) without reticulocytosis. Blood samples were also sent to Blood Transfusion Unit. We encountered difficulties in determining patient blood group as strong agglutination was present in both Beth Vincent and in Simonin tests as well as in control tests. Blood group performed at 37 degrees was A with Rh phenotype CCDee.

We also found a direct positive Coombs test against anti-C3 and low titer of cold agglutinin (1:128 at 4 degrees C) with thermal amplitude (1:2 at 22 degrees C). Antibody screening was negativ. These blood tests revealed the presence of cold agglutinin syndrome.

The physician in charge was informed about the the risk of intra-operative hemolysis due to immuno-hematological patient's status. The following measures were recommneded in view of surgery: to prewarm the operating room to 31~32degrees C as well as intravenous fluids and to cover the patient lower body and the upper extremities with warming blankets.

Subtotal gastrectomy and lymphadenectomy were done and during surgery a fulminant hemolytic reaction with hemoglobinemia, hemoglobinuria appeared. Patient developed a fatal cardiovascular shock although 7 compatibles blood units were administrated.

Conclusion In this report we describe a complication caused by recipient cold agglutinins and the activation of the complement system in the context of peri-operative hypothermia, responsible for hemolysis and consequent fatal cardiovascular shock. The role of Blood transfusion Unit was important for the diagnose and for the selection of compatible blood.

NURSES SESSION

A1. PREVENTION AND TREATMENT OF PRESSURE SORES

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Pressure sores are tissue injuries caused by insufficient irrigating due to long term compression between bones prominences and a solid surface.

Pressure sores prevention consists of a set of specific measures:
- Supervision of the skin in the predisposed areas, at bedridden patients
- Avoid keeping the patient for a long time in the same position
- Rigorous hygiene of linens and avoidance of creases and seams
- Avoid excessive heat and moisture
- The use of complementary tools
- Balanced nutrition and hydration
- Stimulating blood vascularization through massage

Pressure sores treatment involves reducing the risk factors, mobilization, wound care and surgical treatment.