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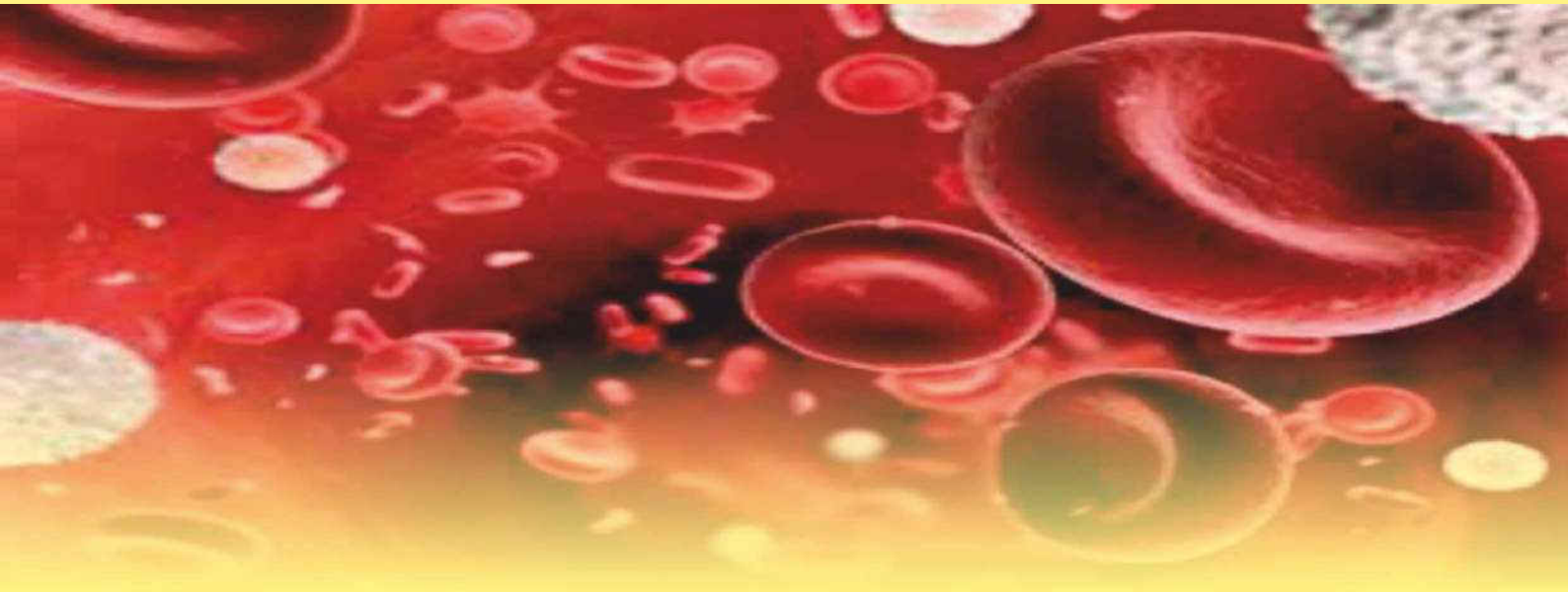

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CONTENTS

ROMANIAN SOCIETY OF HAEMATOLOGY

NATIONAL SOCIETY OF BLOOD TRANSFUSION FROM ROMANIA

CLINICAL HAEMATOLOGY SECTION
EDUCATIONAL SESSION

SPLENIC MARGINAL ZONE NHL.

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SMZL is a distinctive form of indolent lymphoma originating in the spleen, characterized by prominent splenomegaly and variable involvement of lymph nodes, bone marrow, peripheral blood, and other organs. The WHO definition is a B-cell neoplasm .It is an uncommon form of NHL, accounting for less than 1% of new cases. The immunophenotype of SMZL is similar to NMZL and MALT lymphoma Further evidence supporting SMZL as a distinct entity is a unique gene expression profile when compared to other indolent B-cell lymphomas. Association with hepatitis C infection has been reported.Patients often have modest cytopenias that are primarily due to splenic sequestration with a smaller contribution from marrow infiltration. Usually a bone marrow biopsy is the best initial diagnostic test and will often establish the diagnosis. Occasionally the distinction with other lymphoproliferative disorders, such as hairy cell leukemia (HCL), can be challenging. Flow cytometry of circulating lymphoma cells or the marrow can be helpful as SMZL is typically CD25 negative and CD103 negative, unlike HCL. If no blood or marrow involvement is present, the diagnosis is best established by splenectomy. The prognosis is usually good after a diagnosis of SMZL. Splenectomy is usually the treatment of choice for SMZL. In the absence of comparative trials, it is difficult to know if any particular regimens should be preferred, but it appears as though alkylating agents may be less active than purine analogues, and amongst the purine analogues, 2-chlorodeoxyadenosine may be less active than fludarabine and pentostatin. Interestingly, single-agent rituximab has been reported to be highly active in SMZL with an ORR of 100% and a CR rate of 71% .

MONITORING PATIENTS WITH CHRONIC MYELOID LEUKEMIA: BETWEEN PRACTICE AND THEORY.

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Chronic myeloid leukemia (CML) is a malignancy in which huge progress has been made - the introduction of therapy with tyrosine kinase inhibitors (TKIs) have dramatically improved survival rates at 10 years. Unfortunately, CML patients who have evolution towards accelerated phase (AP) and blast phase (FB) have a poor prognosis - modern therapy for these patients brought no progress, except for those eligible for bone marrow allogeneic transplantation. For this reason, the only possible strategy, at this time, is the decrease the risk of progression to FA / FB.
The clinician who treats patients with CML has some questions to answer:
- What is the first line therapy? At this moment there are available three commercially preparations of TKIs: imatinib, nilotinib, dasatinib. There are studies that indicate a marginal benefit for second-generation TKIs on the overall survival and progression free survival. But the cost difference is important: the second generation of TKIs are more expensive by 30% to 100% compared to imatinib. The introduction of generic imatinib makes this difference be very high.
- What is the optimum time to change the therapeutic line? What is the best tool for monitoring of treatment response? How deep must be the response to treatment? Can we talk about stopping TKI therapy, outside of clinical trials? Can we talk about treatment free remission ? NCCN and ELN guidelines recommend evaluating at 3 and 6 months of starting therapy with TKI. These guidelines recommend early switch of TKI if the transcript bcr / abl not fall below 10%. There are enough data for this strategy? What about patients who achieved cytogenetic complete remission but have not achieved major molecular response?
This presentation aims to answer these questions.

This work was supported by the grant PN 41-087 / 2007 from the Romanian Ministry of Research and Technology

THE MONOCLONAL GAMMOPATHY WITH UNDETERMINED SIGNIFICATION AND OTHER PREMYELOMA STATES: SOME PATHOGENESIS DATA AND FOLLOW UP.

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Multiple myeloma (MM) is a plasma-cell neoplasm that accounts for 10% of hematologic diseases. It involves adults with a median age at onset of 69 years. Fewer than 2% of patients are younger than age 40 at diagnosis.

MM evolves from premalignant conditions named monoclonal gammopathy of undetermined significance (MGUS) and smouldering multiple myeloma (SMM). These precursor diseases of MM are asymptomatic but have in common with the MM the same genetic events, the presence of a serum monoclonal protein and of clonal marrow plasmocytes in the bone marrow. MGUS and its derivative, the MM, are in fact multiple subtypes accordingly to the immunoglobulin isotype of the monoclonal protein. The most well characterized MGUS are IgG (69%), IgA (11%) and biclonal (3%). They are followed by light chain MGUS and, in a smaller proportion the IgD, the IgE and the nonsecretory variety. About 17% of all MGUS is the IgM subtype, an apart subcathegory which engenders the rare IgM myeloma variety and a different disease: Waldenström's macroglobulinemia. The risk of progression to MM from MGUS is 1% per year and for SMM is 10% in the first 10 years after the diagnosis. These risks of progression are not uniform. The cases MGUS are stratified by some risk factors (e.g. non-IgG isotype, serum M protein concentration and free light chains ratio) each is worth for 1 point. At 20 years of follow up, the risk of progression for patients with MGUS with 0, 1, 2 or 3 factors is 5%, 21%, 37% and 58 % respectively. For SMM, the risk factors include BM plasma cells > 10% (1 pt.), serum M protein concentration > 3g/dL (1 pt.) and a skewed FLC-ratio. The cumulative risk of progression at 10 years is 50%, 65%, 84% respectively. The clonal progression of genetic and molecular abnormalities seconded by the tissular depositions of the monoclonal protein are the common pathogenic factors of these three disease states suggesting a spectrum continuum process. The consensus on the atitude is the watch and wait strategy or, at the most the limit the therapy within the frame of clinical trials.

RECENT ADVANCES IN DIAGNOSIS, MOLECULAR PATHOLOGY AND THERAPY OF WALDENSTROM MACROGLOBULINEMIA.

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Waldenström macroglobulinemia (WM) is a rare lymphoid neoplasm resulting from the accumulation, predominently in the bone marrow (BM), of a clonal population of lymphocytes, lymphoplasmocytic cells and plasma cell wich secrete a monoclonal immunoglobulin M (IgM). According to WHO classification, WM is classified as a lymphoplasmocytic lymphoma (LPL/WM). The origin of the malignant clone is thought to be an arrested B cell that has undergone somatic hypermutation in germinal center without differentiating to plasma cell. The most common cytogenetic abnormality is 6q deletion detected in 50% of cases with WM and is associated with an adverse prognosis. Recently, whole genome sequencing has discover two activating somatic mutations: MYD88 L265P (in chromosome 3p22.2) and WHIM-like CXCR4, associated with significant differences in clinical presentation and survival. MYD88 L265P are found in 90-95% of WM patients and may help to differentiate WM from other lymphoproliferative disorders with overlapping feature (SMZL, LLC, IgM-MM). MYD88 L265P is a molecular biomarker of WM. Risk factors for developing WM: pre-existing IgM-MGUS, familial hystory, immunological factors. The diagnostic criteria for WM: IgM monoclonal gammapathy of any concentration; bone marrow infiltration by small lymphocytes showing plasmocytoid or plasma cell differentiation; intertrabecular pattern of bone marrow infiltration; surface IgM+ CD5± CD10- CD19+ CD20+ CD22+ CD23- CD25+ CD27+ FMC7+ CD103- CD138- immunophenotype. The clinical manifestations include those related to clonal cell infiltration of bone marrow, limph node, liver, spleen. Manifestations related to the IgM monoclonal protein include hyperviscosity, cryoglobulinemia, antibody mediated disorders (neuropathy, hemolitic anemia, Schnitzer syndrome), amyloidosis. Prognosis of WM depends on 5 major factors (IPSS-WM): age, hemoglobin level, platelet count, B2 microglobulin and monoclonal IgM concentration. Treatment decision are based on the presence of symptoms, patients factors (age, performance status), diseases factors (citopenias, significance adenopathy or organomegaly, symptomatic hyperviscosity, neuropathy, amyloidosis, cryoglolinemia, cold aglutinin disease, evidence of disease transformation). Current treatment options include alkylating agent, purine analogues, monoclonal antibody (Rituximab), proteasomal inhibitor. Plasma exchange is a treatment indicated for acute management of hyperviscosity syndrome. Agents under study: everolimus, carfilzomib. High-dose chemotherapy with autologous stem cell rescue in primary refractory or relapsed disease shoud be considered for eligible patients. Novel target therapeutic strategies directed against MYD88 signaling (ibrutinib) and CXCR4 (plerixafor) are in clinical investigations.

UPDATE IN IMMUNE THROMBOCYTOPENIC PURPURA.

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Abstract: Primary immune thrombocytopenia (ITP) is an acquired immune-mediated disorder characterized by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100 × 109/L, and the absence of any initiating or underlying cause of the thrombocytopenia.
Once regarded as idiopathic, immune thrombocytopenia (ITP) is now known to have a complex pathogenesis: presence of antibodies against multiple platelet antigens leading to reduced platelet survival as well as impaired platelet production.
The incidence in adults is approximately equal for the sexes, except for the middle age where female gender is more prevalent. Categories of ITP have also been established in order to facilitate management decisions, as follows: newly diagnosed (under 3 months of evolution), persistent (3-12 months' duration), chronic (≥ 12 months' duration) and severe ITP.
Whereas ITP in adults typically has an insidious onset followed by a chronic course, ITP in children is usually transitional, with at least 2/3 recovering spontaneously within 6 months. Signs and symptoms vary widely: some have no symptoms or minimal bruising, whereas others experience serious bleeding. The severity of thrombocytopenia correlates to some extent but not entirely with the bleeding risk, which is also influenced by additional individual factors as: age, lifestyle factors, comorbidities, etc.
Multiple therapies with different mechanisms of action are available to treat ITP, and the treatment should be individualized according to clinical course, ITP onset, hemorrhagic risk and/or individual features (age, pregnancy, diabetes, etc).
While the first line therapy is based on corticosteroids and immunoglobulins, the guidelines for managing ITP in adults has changed with the advent of new agents (thrombopoietin receptor agonists and rituximab) as options for second-line therapy. Splenectomy continues to provide the highest cure rate (60%-70% at 5+ years) with no significant complications. Each approach has advantages and disadvantages, therefore treatment needs to be individualized.

NON-HODGKIN LYMPHOMA OF GASTRO-INTESTINAL TRACT-CLINICAL PRESENTATION, EVOLUTION AND TREATMENT.

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Introduction.Extranodal lymphomas arise anywhere outside the lymph node region: from sites with primary lymphoid organs (spleen, thymus,Waldeyer ring); from organs or tissues devoid of lymphoid tissue (brain, soft tissue); or from organs with a significant lymphoid tissue component (gastrointestinal tract). In the gastrointestinal tract, lymphoid elements occur in the lamina propria and submucosa,but primary or secondary lymphomatous neoplasms may occur in any portion of the gastrointestinal tract(1).
Secondary gastrointestinal involvement is common because of the frequent origination of lymphomas in the mesenteric or retroperitoneal nodes. The primary lymphomas of the gastrointestinal tract usually involve only one site.The most commonly involve the stomach but can involve any part of the gastrointestinal tract from the esophagus to the rectum.
Incidence and Pathogenesis.The incidence of primary gastrointestinal NHL is approximately one in 100,000 individuals per year. There is a male predilection, with a male-female ratio of 3:2. A number of risk factors other than HIV infection have been identified in the pathogenesis of gastrointestinal lymphoma: Helicobacter pylori(H pylori) infection, celiac disease, inflammatory bowel disease, immunosuppression after solid organ transplantation(1). Although there is no lymphoid tissue in the gastric mucosa, chronic H pylori infection is associated with the development of lymphoid tissue in the lamina propria. Most low-grade primary gastric lymphomas arise from this mucosa-associated lymphoid tissue (MALT) and are classified as MALT lymphomas. It has been suggested that high-grade lymphomas result from transformation of the low-grade tumor (2). Immunoproliferative small intestine disease, a special form of MALT lymphoma, is also suspected to have an infectious etiology (3). Celiac disease has been noted as a risk factor for small bowel adenocarcinomas, esophageal cancer, melanoma, and NHL (4).Celiac disease is often associated with enteropathy type T-cell lymphoma.Patients with HIV-induced immunodeficiency are at high risk for developing a B-cell phenotype intestinal lymphoma with unusual morphologic features, a high grade of malignancy, and a poor prognosis (5).
Esophagus.Esophageal lymphoma occurs secondary to cervical and mediastinal lymph node invasion or contiguous spread from gastric lymphoma. Primary esophageal lymphomas are predominantly B-cell type.The predominant appearance is that of submucosal infiltration, but may also manifest with a polypoid mass ulceration, or nodularity (6).

Perforation and fistulization may be demonstrated.Barium studies better demonstrate subtle mucosal and submucosal abnormalities and CT better defines the extent of local disease and the disease stage.

Stomach.Primary gastric lymphoma represents 1%–5% of gastric malignancies (7) and is the most common type of extranodal lymphoma, accounting for 50%–70% of all primary gastrointestinal lymphomas. It is recognized that chronic H pylori gastritis is associated with the development of low-grade MALT lymphoma.Primary gastric lymphoma originates as a low-grade MALT lymphoma,and transforms into intermediate or high-grade large cell lymphoma if not diagnosed or treated in time (8).

When it is diagnosed at an early stage has a good prognosis, and eradication of H pylori with antibiotic therapy has resulted in regression of early stage tumors. The double-contrast barium studies may reveal ulcerative, polypoid, or infiltrative patterns, which are the same as those of gastric carcinomas.Together with barium studies gastroscopie in all gastrointestinal lymphoma;demonstrats a lot of pathological aspects and offers possibility to do biopsy very important for diagnosis.The diagnosis of lymphoma may be suggested by the presence of multiple polypoid tumors with central ulceration ("bull' seye" appearance), giant cavitating lesions, or extensive infiltration with gastric fold thickening . The latter finding may be distinguished from linitis plastica on the basis of the preservation of gastric distensibility. That have been described:single or multiple ulcers of varying size; single or multiple masses with or without an ulcer, along with thickened folds; rugal thickening, commonly converging to an ulcer or a mass; mucosal nodularity of varying size, either focal or diffuse; and coarse areae gastricae(1). Low-grade MALT lymphoma has a wider spectrum of appearances than does high-grade MALT lymphoma, in which a mass-forming lesion or severe fold thickening is present (2).Preservation of the perigastric fat planes at CT is more likely to be seen in lymphoma than in adenocarcinoma, in the presence of a bulky tumor (9).The stomach remains pliable even with extensive lymphomatous infiltration, and the lumen is preserved, making gastric outlet obstruction a rather uncommon feature (10). Adenopathy is seen in both adenocarcinoma and lymphoma, but if it extends below the renal hila or the lymph nodes are bulky, lymphoma is more likely (11). Complications such as obstruction, perforation, or fistulization can occur and can be detected with CT and barium studies.

Small Bowel.Lymphoma is the most common malignancy of the small bowel (12), and its incidence related to B-cell hyperactivation in HIVpositive patients has increased. The small bowel lymphoma accounting for 20%– 30% of all primary gastrointestinal lymphomas. The distal ileum is the most common site of small bowel B cell lymphoma because of the greater amount of lymphoid tissue in this portion of the bowel.

Small bowel B-cell lymphoma may appear as a circumferential bulky mass in the intestinal wall with extension into the small bowel mesentery and regional lymph nodes.

The tumor may involve a long segment of bowel and may ulcerate and perforate into the adjacent mesentery.Aneurysmal dilatation of the lumen may be seen due to replacement of the muscularis propria and destruction of the autonomic nerve plexus by lymphoma.A focal, polypoid, homogeneous intraluminal mass without wall thickening or lymphadenopathy has been described (13).

Barium studies show single or multiple polypoid lesions,diffuse or segmental ulcerative or infiltrative change, or diffuse or focal nodularity. Peritoneal lymphomatosis from primary gastrointestinal lymphoma is rare compared with carcinomatosis.The prevalence of malabsorption and intestinal recurrence is high in enteropathy-associated Tcell lymphoma. The peripheral T-cell lymphoma is seen in the small intestine, particularly the jejunum and has a higher prevalence of multifocal involvement and bowel perforation(1).

Large Bowel.Primary lymphoma of the large bowel accounts for 0.4% of all tumors of the colon, and colorectal lymphomas constitute 6%–12% of gastrointestinal lymphomas (14). Primary lymphoma often affects the cecum and rectum than other parts of the large bowel (15). The primary colorectal lymphoma comprises low-grade B-cell lymphoma arising from MALT, mantle cell lymphoma, and T-cell lymphoma (14). Most colorectal lymphomas are NHL, usually of B-cell origin. Mantle cell lymphoma is an aggressive disease that manifests as multiple polyps (lymphomatous polyposis). Low-grade B-cell lymphoma arising from MALT has an indolent course and might also manifest as multiple polyps. Peripheral T-cell lymphoma of the colon manifests as either a diffuse or a focal segmental lesion with extensive mucosal ulceration at double-contrast barium enema examination (1). The colonic perforation frequently occurs.There are the following findings in lymphoma: polypoid masses,frequently near the ileocecal valve; circumferential infiltration,a cavitary mass excavating into the mesentery; endoexoenteric tumors; mucosal nodularity; and fold thickening (14).The focal strictures, aneurysmal dilatation, or ulcerative forms with fistula formation may be seen. Features that help differentiate lymphoma from adenocarcinoma include extension into the terminal ileum, well-defined margins with preservation of fat planes, no invasion into adjacent structures, and perforation with no desmoplastic

reaction (16). Primary rectal lymphoma is a rare type of gastrointestinal lymphoma and is clinically indistinguishable from rectal carcinoma.Primary lymphoma of the appendix is very rare,it is more common to see cecal lymphoma extending to the base of the appendix .Patients typically present with acute symptoms that are suggestive of acute appendicitis.

Treatment. There is still no consensus on the optimal treatment for primary gastrointestinal lymphoma. Nowadays surgery is limited to rare cases and radiotherapy – combined or not with chemotherapy – represents an effective therapeutic option ensuring long-term, organ-salvage benefits mainly in aggressive histological subtypes. In the MALT lymphomas associated with H-pylori infection,antibiotics alone can induce lasting remissions.A global therapeutic approach has changed over the last 10 years: innovative, conservative options to reduce treatment toxicity, thus preventing systemic relapses, have made their appearance and are on the rise.

Conclusions. Gastrointestinal lymphoma is an uncommon disease with a wide variety of imaging appearances. Primary gastric lymphoma is a rare cancer of the stomach with an indeterminate prognosis.The features such as a bulky mass or diffuse infiltration with preservation of fat planes and no obstruction, multiple site involvement, and bulky lymphadenopathy are suggestive of lymphoma.

References.

1. Sangeet Ghai, MD, FRCR ● John Pattison, FRCR ● Sandeep Ghai, MD Martin E. O'Malley, MD ● Korosh Khalili, MD ● Mark Stephens,MRCPath. Primary Gastrointestinal Lymphoma: Spectrum of Imaging Findings with Pathologic Correlation[1] RadioGraphics 2007; 27,5,1371–1388
2. An SK, Han JK, Kim YH, et al. Gastric mucosa associated lymphoid tissue lymphoma: spectrum of findings at double-contrast gastrointestinal examination with pathologic correlation.Radio-Graphics 2001;21:1491–1504.
3. Isaacson PG. Gastrointestinal lymphomas of Tand B-cell types. Mod Pathol 1999;12:151–158.
4. Green PH, Fleischauer AT, Bhagat G, Goyal R, Jabri B, Neugut AI. Risk of malignancy in patients with celiac disease. Am J Med 2003;115:191–195.
5. Wang CY, Snow JL, Daniel Su WP. Lymphoma associated with human immunodeficiency virus infection. Mayo Clin Proc 1995;70:665–672.
6. Carnovale RL, Goldstein HM, Zornoza J, Dodd GD. Radiologic manifestations of esophageal lymphoma. AJR Am J Roentgenol 1977;128:751–754.
7. Gossios K, Katsimbri P, Tsianos E. CT features of gastric lymphoma. Eur Radiol 2000;10:425–430.
8. Yoo CC, Levine MS, Furth EE, et al. Gastric mucosa-associated lymphoid tissue lymphoma: radiographic findings in six patients. Radiology 1998;208:239–243.
9. Miller FH, Kochman ML, Talamonti MS, Ghahremani GG, Gore RM. Gastric cancer: radiologic staging. Radiol Clin North Am 1997;35:331–349.
10. Ciftci AO, Tanyel FC, Kotiloglu E, Hicsonmez A.Gastric lymphoma causing gastric outlet obstruction. J Pediatr Surg 1996;31:1424–1426.
11. Buy JN, Moss A. Computed tomography of gastric lymphoma. AJR Am J Roentgenol 1982;138:859 865.
12. Serour F, Dona G, Birkenfield S, Balassiano M, Krispin M. Primary neoplasms of the small bowel. J Surg Oncol 1992;49:29–34.
13.Balthazar EJ, Noordhoorn M, Megibow AJ, Gordon RB. CT of small-bowel lymphoma in immunocompetent patients and patients with AIDS:comparison of findings. AJR Am J Roentgenol 1997;168:675–680.
14. Lee HJ, Han JK, Kim TK, et al. Primary colorectal lymphoma: a spectrum of imaging findings with pathologic correlation. Eur Radiol 2002;12:2242–2249.
15. Dodd GD. Lymphoma of the hollow abdominal viscera. Radiol Clin North Am 1990;28:771–783.
16. Wyatt SH, Fishman EK, Hruban RH, Siegelman SS. CT of primary colonic lymphoma. Clin Imaging 1994;18:131–141.

FROM DISSEMINATION PATTERN TO CLINICAL AND THERAPEUTIC ASPECTS OF NON-HODGKIN LYMPHOMAS

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Non-Hodgkin lymphoma (NHL) is a heterogenous and highly disseminated disease, but the mechanisms of its **growth and dissemination** are not well understood. Normal lymphocyte trafficking is essential for the regulation of systemic immune processes, as wel as lymphocyte differentiation and development. Most mature lymphocytes recirculate continuously fom blood to tissue and back to the blood again. This recirculation is regulated by lymphocyte-endothelial interactions and mediated by **adhesion molecules and selected chemokines**. Such interactions may be maintened in lymphoma trafficking paterns. Normal ***lymphocyte homing*** (that is a multistep process) and recirculation molecules are implicated in lymphoma dissemination and invasion. NHL represent the malignant counterparts of lymphocytes arrested at a specific stage of maturation, and lymphoma dissemination is a reflection of conserved physiological behavior. Understanding the molecular mechanisms underlying this behavior may provide novel targets for treatment of

lymphoma patients.
The homing signature of a lymphocyte is dependent on differention stage and antigen experience. The conserved homing programs mediate the dissemination of NHL. NHL related to small recirculating lymphocytes (small-lymphocytic lymphoma/chronic lymphocytic leukemia and mantle-cell lymphoma) usually show systemic dissemination at presentation, whereas NHL related to lymphocytes undergoing active proliferation and differentiation (diffuse large B-cell lymphoma, Burkitt lymphoma) often are initially localized. **The dissemination patterns reflect basic rules of lymphocyte homing, explaining the strikingly tissue-specific dissemination** (e.g., mucosal lymphomas, cutaneous lymphomas, and multiple myeloma). The best characterized pathways of lymphocyte homing are those mediating homing to the gut-associated lymphoid tissue and skin, as both intestine and skin represent barrier tissues exposed to high antigen load.
Cell migration is essential during differentiation. In the tissue microenvironment, different cell types exhibit distinct migration strategies. 1.Mesenchymal migration: Mesenchymal cells display an adhesive phenotype and develop a spindle shape. The elongated morphology is dependent on integrin-mediated adhesion and the presence of traction forces on both cell poles. Simultaneous with integrin and actin concentration at focal contacts, the cells recruit surface proteases to these substrate contact sites to digest and remodel the extracellular matrix, thus generating matrix defects that allow cell migration. Other cells may follow along the generated matrix defect creating a moving cell chain. 2. Cluster/cohort migration: Migrating cancer cell collectives use an integrin- and protease-dependent migration mode similar to mesenchymal migration, but the migrating cells within the cohorts are interconnected by cadherins and gap-junctional communication. 3. Ameboid migration: Lymphoid cells display a characteristic "ameboid" type of migration, in which integrin-mediated adhesion is dispensable and cell movement is driven by short-lived relatively weak interactions with the substrate. The lack of focal contacts and high deformability of lymphocytes allow movement at high velocity, while the fast deformability of lymphocytes allows them to overcome matrix barriers by physical mechanisms, independent of proteolytic matrix degradation.
Lymphocyte interaction with endothelium. In the postcapillary venules, selectin-sialomucin interactions (or interactions mediated by integrin α4β1 or α4β7) mediate "rolling" of lymphocytes on the endothelium. Chemokines, presented by heparan sulfate proteoglycans expressed on the endothelium, bind to chemokine receptors, which are G protein–coupled receptors, leading to increased affinity/avidity integrins on the surface of lymphocytes. Interaction of these integrins with their ligands results in stable adhesion of lymphocytes to endothelium and in diapedesis, involving engagement with junction adhesion molecules (JAMs) and PECAM-1 (CD31).
There is a specific recruitment of tumor cells by locally produced chemokines and activated endothelium, with tumor dissemination to sites of trauma and inflamation in lymphoma patients. Extranodal lymphoma arising in the gut-associated lymphoid tissues or the skin show a strong preference to disseminate to mucosal sites and skin, respectively, and they may eventually disseminate to lymph nodes.
Lymphocyte trafficking and the tissue-specific dissemination of T-cell lymphomas. Lymphocyte migration is strictly regulated by adhesion molecules and chemokine receptors on lymphocytes and their ligands expressed by the endothelium. Naive T lymphocytes can home and recirculate via all secondary lymphoid tissues because they express both α4β7 (for mucosal homing) and L-selectin (for homing to peripheral lymph nodes). Migration of activated T lymphocytes to sites of inflammation involves several receptor-ligand pairs, including selectin-sialomucin, α4β1-VCAM-1, α4β1-CS-1, and CD44-hyaluronate interactions. Upon antigen priming by dendritic cells, T lymphocytes become memory cells and acquire a "homing signature," that is, a specific adhesion and chemokine receptor make-up, which enables them to selectively home to specific tissue environments, thereby increasing the efficacy of immunosurveillance. The T-NHLs related to lymphocyte populations with tissue-specific homing properties usually display tissue-specific dissemination patterns and express homing receptors corresponding to the tissue of origin.
The heterogenous group of peripheral T-cell lymphomas are derived from memory T cells, and comprise several well-defined entities with distinctive molecular, pathological, and clinical characteristics. The tissue of primary presentation, dissemination pattern, pathological and molecular data are important criteria for the classification of these tumors into distinctive clinicopathological entities. There is a differential expression pattern of chemokine receptors on specific lymphoma subtypes. Expression of Th1 chemokine receptors in peripheral T-cell lymphoma is related to a favorable prognosis. Nodal T-cell lymphomas express L-selectin, but lack the skin-homing receptor CLA as well as the mucosal-homing receptor α4β7. In cutaneous T-cell lymphoma the expression of CLA and CCR4 permit these cells to home effectivelly to the skin. The tumor cells of mycosis fungoides and Sezary syndrome show different homing signature, which correspond to distinctive

dissemination patterns. A loss of skin-specific chemokine receptors is seen during mycosis fungoides progression. Intestinal T-cell lymphomas are most often enteropathy associated, and express the mucosal-homing receptor α4β7.

Adhesion molecule and chemokine receptor expression profiles of B-lymphocyte subsets and related lymphoid malignancies. Naive B lymphocytes coexpress L-selectin and α4β7, enabling them to migrate to the mucosa as well as to peripheral lymph nodes. Germinal center reactions in Peyer patches lead to generation of α4β7-expressing memory B lymphocytes, which subsequently can differentiate into IgA-secreting plasma cells. Most memory B cells arising in lymph nodes, on the other hand, differentiate into IgG-secreting plasma cells. These cells express CXCR4 and the integrins α4β1 and LFA-1, which can mediate homing to the bone marrow, where these cells become long-lived plasma cells. The B-cell malignancies are related to lymphocyte populations with tissue-specific homing properties.

B lymphocytes adapt their homing signature to thei specific maturational stage, and this is largely conserved in B-cell lymphomas, controlling their dissemiantion. Ectopic chemokine expression at site of chronic inflammation with lymphoid neogenesis is a key factor in the selective homing of malignant B cells to these sites. B-cell migration to the skin and other extralymphoid sites occurs exclusively in the context of chronic inflammation driven by locally persistent antigen or autoantigens. Differention of a B-cell to a plasma cell is accompanied by a coordinated change in chemokine receptor expression. Multiple factors in the bone marrow microenvironment may modulate multiple myeloma cell homing. Various chemokines and growth factors produced in the bone marrow stimulate integrin-mediated adhesion and can contribute to resistance of multiple myeloma cells to treatment.

The expression of adhesion molecules on lymphoma cells has been linked to tumor spread and poor outcome. Some adhesion molecules facilitate lymphocytes binding to the vascular endothelium with subsequent migration to the nodal areas. Serum CD44 has been correlated with disseminated disease and shortened survival in NHL. Tumor angiogenesis is critical for local growth and distant spread of NHL. Targeting adhesion and chemokine receptors, that are part of the homing signature of malignant lymhocytes, with monoclonal antibodies or small-molecules drugs, may prove a successful novel means of therapeutic intervention in lymphoma patients.

Selected references:

1. Blonska M., Zhu Y., Chuang HH. et al. Jun-regulated genes promote interaction of diffuse large B-cell lymphoma with the microenvironment. Blood. 2015;125(6):981-991.
2. De Boer JP, Hiddink RF, Raderer M et al. Dissemination patterns in non-gastric MALT lymphoma. Haematologica 2008;93(2):201-206.
3. Ito K., Smith BR, Parashurama N et al. Unexpected dissemination patterns in lymphoma progression revealed by serial imaging within a murine lymph node. Cancer Res 2012;72(23):611-618.
4. Drillenburg P, Pals ST. Cell adhesion receptors in lymphoma dissemination. Blood. 2000;95(6):1900-1910.
5. Massberg S, KhandogaAG, von Andrian UH. Hematopoietic cell trafficking and chemokines. In Hematology: basic principles and practice/ ed. by Hoffman R, Benz EJ jr, Silberstein LE et al.- 6th ed.,Saunders Elsevier 2013:105-116.
6. Pals ST, Horst E, Ossekoppele GJ et al. Expression of lymphocyte homing receptor as a mechanism of dissemination in non-Hodgkin's lymphoma. Blood. 1989;73(4):885-888.
7. Pals ST, de Gorter DJJ, Spaargaren M. Lymphoma dissemination: the other face of lymphocyte homing. Blood.2007;110(9):3102-3111.
8. Salmi M, Jalkanen S. Lymphocyte homing to the gut:attraction, adhesion, and commitment. Immunol Rev. 2005;206:100-113.

TRANSFORMATION – MODALITY TO EVOLVE IN HEMATOLOGICAL NEOPLASMS

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UMF GrTPopa Iaşi, IRO-Iaşi

The ability of cancer to evolve and adapt is a principal challenge to therapy in general, and to the paradigm of targeted therapy in particular.

- ***In aplastic anemia (AA)***, immunosuppressive therapy (IST) induces remissions in 50%-70% of patients. Apart from relapse and refractoriness to IST, evolution of clonal diseases, including paroxysmal nocturnal hemoglobinuria and myelodysplastic syndrome (MDS), are the most serious long-term complications. Evolution of MDS occurs either early or late in the course of the disease and constitute a strong argument for definitive therapy with BM transplantation if possible.
- ***Myelodysplastic syndromes (MDS)***, one of the most prevalent hematological disorders, constitute a heterogeneous class of stem cell malignancies, characterized by ineffective hematopoiesis in one or more bone marrow (BM) lineages. About one-third

of patients with MDS progress to secondary acute myeloid leukemia (sAML). The prognosis of patients who undergo transformation from MDS into sAML is generally grave; most patients are resistant to currently available treatment options and the long-term survival rate among treated patients is <10%.

- ***Myeloproliferative neoplasms (MPNs)*** are clonal hematological diseases in which cells of the myelo-erythroid lineage are overproduced and patients are predisposed to leukemic transformation. Hematopoietic stem cells (HSCs) are the suspected disease initiating cells and these cells must acquire a clonal advantage relative to non-mutant HSCs in order to perpetuate disease.
- ***Acute myeloid leukaemia (AML)*** is an aggressive malignancy characterised by a block in myeloid differentiation and uncontrolled proliferation of abnormal myeloid progenitors that accumulate in the bone marrow and blood. Some cases develop from other haematopoietic disorders or follow genotoxic therapy for unrelated malignancies. AML represents an excellent model for understanding the principles of cancer evolution
- ***Richter syndrome (RS)*** is the development of secondary aggressive lymphoma in the setting of underlying chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). Most frequently CLL transforms into diffuse large B-cell lymphoma (DLBCL) (90%) and rarely (10%) into Hodgkin lymphoma, termed Hodgkin variant of Richter syndrome (HvRS). There are described two biologically different conditions: CLL transformation to a clonally related DLBCL, that accounts for the majority of cases; development of a DLBCL unrelated to the CLL clone. RS is generally characterized by an aggressive clinical course and poor prognosis.

This ability to evolve is fueled by the co-existence of multiple, genetically heterogeneous subpopulations within the cancer cell population. Increasing evidence has supported the idea that these subpopulations are selected in a Darwinian fashion (Nowell, 1976), by which the genetic landscape of the tumor is continuously reshaped. Recent studies reveal the complex evolutionary trajectories occurring across individual hematological malignancies. They also suggest that while clonal evolution may contribute to resistance to therapy, treatment may also hasten the evolutionary process. New insights into this process challenge us to understand the impact of treatment on clonal evolution, and inspire the development of novel prognostic and therapeutic strategies. This presentation try to summarise the new insights in this process of transformation.

MANAGEMENT OF ACUTE PROMYELOCYTIC LEUKEMIA: "THE GOLDEN HOUR"

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Management of acute promyelocytic leukemia (APL) is the perfect example for how translational research changed clinical practice in hematology-oncology. Over the last three decades, APL transformed from one of the worst diagnosis in hematologic malignancies, a nightmare for patients and physicians alike to a highly curable disease, a gratifying experience for leukemia doctors. In 1977, Rowley et al. identified a unique genetic abnormality, "t(15;17) (q22?;q21)" as the hallmark of APL blasts. In the 1980's, inspired by traditional Chinese remedies, Huang et al. determined that pharmacological levels of retinoids can terminally differentiate APL blasts with emergence of neutrophils. The subsequent integration of all-trans retinoic acid (ATRA) into APL treatment paradigms became the first targeted therapy in hematology, twenty years before the use of Imatinib in chronic myeloid leukemia. Unfortunately, single agent ATRA induces "remission without cure" in APL and these studies first proposed the existence of "minimal residual disease" (MRD) as malignant cells that are present even though the patient is in morphological remission. Since then, MRD became a term routinely used in hematological malignancies and oncology in general. The mechanism by which MRD persists in APL patients treated with single agent ATRA remains an area of active research. One hypothesis, proposed by our group this year suggests that the bone marrow microenvironment metabolize ATRA and thus, protects some APL cells. If so, this may explain why liposomal ATRA can single handedly cure some APL patients. Nevertheless, over the last two decades, the addition of arsenic trioxide (ATO) to ATRA was able to eliminate MRD, decrease relapse in APL and improved cure rates even without cytotoxic chemotherapy.

From a clinical standpoint, current challenges in APL gravitate around rapid diagnosis and initiation of ATRA, management of coagulopathy and differentiation syndrome. To this end, we will use two clinical vignettes to showcase our approach at Johns Hopkins towards patients with this disease. Briefly, any patient suspect of APL has a peripheral blood smear reviewed by a hematologist within 30 minutes. Presence of circulating malignant promyelocytes, sometimes

hypogranular, results in initiation of ATRA therapy. Peripheral blood flow cytometry is used to further support a diagnosis of APL based on a combination of cell surface markers (CD33 bright, HLA-DR negative, CD34 negative), though various staining patterns could be identified more so if Flt3 mutations are present. A hematopathologist together with a hematologist review these results within hours from admission. In most cases, the diagnosis is confirmed by FISH for t(15;17) within 36-48h and later on by classical karyotype and RT-PCR for PML-RARα.

The initial management of patients with APL is geared towards treating infectious complications and diffuse intravascular coagulation (DIC). Regarding management of DIC, in addition to initiation of ATRA therapy which should address the underlying pathophysiology of uncontrolled fibrinolysis, supportive measures include transfusion of platelets and fibrinogen rich products (preferable cryofibrinogen). There are currently no clear indications for the use of unfractionated heparin, tranexamic acid or rhThrombomodulin, though these products will be discussed with potential risks and benefits.

Regarding APL-directed therapy, based on Lo Coco et al. 2013, patients with WBC over 10000/mm3 at presentation are treated with ATO+ATRA while those with more than 10000/mm3 are treated with ATRA and Idarubicin based on Gore S et al. Even though this classification is rather arbitrary, the lack of cytotoxic chemotherapy in ATO+ATRA regimen does bring some interesting challenges (i.e. hyperleucocytosis and differentiation syndrome) that will be discussed. Nevertheless, in spite of potential complications, these patients have lower risk of severe infections and thus, decreased hospitalization and better outcomes.

Finally, since the cure rate in APL reaches over 90% in some cases, it remains unclear the role of monitoring MRD during consolidation. Patients with relapsed APL, if they were not previously treated with ATO will receive ATRA+ATO. If refractory to ATO based therapy, they can be treated with either cytotoxic therapy followed by autologous/allogeneic BMT or by anti-CD33 (Gemtuzumab). While not approved by FDA in US, Tamibarotene is approved in Japan and is available for compassionate use or as part of a clinical trial. Lastly, based on our most recent laboratory data, a clinical trial using a novel synthetic retinoid for treatment of relapsed refractory APL and non-APL AML is currently being developed at Johns Hopkins.

PRIMARY CUTANEOUS LYMPHOMA.

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Approximately 25 percent of all NHL cases will present at an extranodal site without systemic involvement. The skin is the second most common primary extranodal site, second in frequency only to the gastrointestinal tract. The overall incidence of primary cutaneous lymphomas in Western countries is estimated to be 0.5 to 1 case per 100,000 people annually.The clinical and histologic diagnosis of cutaneous lymphoproliferative disorders is one of the most vexing issues in dermatology and dermatopathology, despite significant advances in their classification, pathogenesis, and treatment. The average delay between initial presentation and the ultimate diagnosis of mycosis fungoides, the most common primary cutaneous T cell lymphoma, is six years. During this period, patients typically undergo numerous skin biopsies, which, in the absence of definite histopathologic criteria for early mycosis fungoides, may be interpreted by the pathologist as “atypical lymphocytic infiltrate” or “atypical lymphocytic proliferation.” This nonspecific but potentially serious diagnosis is often a source of anxiety and frustration for both the patient and the clinician. — The term “atypical lymphocytic infiltrate” describes the histologic finding of a dermal infiltrate of atypical lymphocytes admixed with cytologically banal, reactive-appearing lymphocytes in a pattern that is suggestive of lymphoma or leukemia and is generally used when the pathologist cannot reliably differentiate a reactive from a malignant lymphoproliferative disorder on histopathologic. However, despite histologic clues, immunohistochemical staining patterns, and molecular data, pathologists are often left without a definitive diagnosis. In such situations, the best approach is to describe the salient histopathologic features and provide the clinician with an extensive differential diagnosis based upon the synthesis of the various findings. In addition, a direct discussion of the case with the clinician may be extremely helpful to both parties, especially if the possibility of a lymphoproliferative disorder was not clinically suspected.

Folowing these steps will eventually lead sooner or later to diagnosis and classificatin into B or T cells lymphoproliferations.

Regarding B cells lymphoproliferative disorders with primary cutaneous involve, we consider three aspects (primary cutaneous follicle centet lymphoma, primary cutaneous large B cell lymphoma, leg type and ptimary cutaneous marginal zone lymphoma in which initial staging is mandatory the absence of any other systemic involve/ lession.

These clinical entities suggest similarities with various steps in B cells onthogenesis, similarities sustained by clinical, hystological, imunophenotype and molecular aspects for each lession. The prognosis, evolution and therapeutic approach varies.

Skin-based lymphoma of T cell origin includes besides mycosis fungoides, and Sezary syndrome, also CD 30+ lymphoptoliferative disorders, (L/LTA), extranodal NK/T cell lymphoma, nasal type, subcutaneous panniculitis -like T cell lymphoma and primary cutanous aggresive, epidermotropic CD8+ T cell lymphoma, cutaneous □□□ T cell lymphoma, primary cutaneous CD4+ small/medium size lymphoma.
In conclusion primary cutaneous lymphoproliferative disorders are not so rare, are difficult to diagnose and includes aggresive form which needs rapid diagnosis and treatment, among with indollent forms that take years of evolution untill diagnosis.

ANCTHE IMPORTANCE OF CHRONOBIOLOGY IN THE TREATMENT OF HEMATOLOGICAL MALIGNIES.

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Chronobiology is the cantitative study of the temporal relationships of biologic phenomena.
Biophisical and biochemical processes vary with respect to time in a regular and predictible periodic manner across several rythmic frecvencies. Endogenous biological ritms have been demonstrated at all biological levels, from yeasts and nucleated unicells to man and at all levels of biological organization.
The existence of molecular time-keeping mecanism, „clock" gene was first inferred for Drosophila melanogaster which has been named per (period).
There is evidence to suggest that suprachiasmatic nucleus (SCN) of the hypothalamus is a site of critically important circadian pacemaker cells in mammals.
The protooncogene C-fos may be a molecular component of photic pathway necessary for entrainment of mammals to the light/dark cycle.
At least three major biologic rytms have been defined.
- The circadian rhytm (20-28h) the solar day
- The circatrigintan rhytm (30±7 days), the lunar month
- The circannual rythm (12±2 months), the year.

The basic properties of biological rythms are similar in plants and animals.
The rhytms are endogenous and genetic in origin, persist without time clues and are regularly influenced by cyclic variations of certain environmental factors called syncronizers.
Under constant conditions the endogenous circadian period lenghts of the various species are not precisely 24h (more than 24h, but less than 25h).
If their circadian pace makers were not reset by the daily schedule, the timing of their endogenous rhytms would be delayed with respect to clock time each day.
In man and many other species the most powerfull synchronizers of the circadian rhytms are the diurnal alteration of lyght (activity) and darkness (rest) and our 24-h life routine.
There are two general categories of circadian organization wich bear most directly upon the practice of oncology:
- the circadian aspects of drug handling
- circadian organization of cell division in normal and malignant tissues

1. Pharmacokinetics of many anticancer drugs show a circadian temporal variation depending on the time of their administration.
The temporal variations that have been documented in drug absorbtion and distrubtion, metabolism and excretion could explain this to a large extent.
Hemodinamic circadian ritms are also potentially relevant to drug delivery and metabolism.
Hepatic blood flow have a significant circadian variation with the maximum at 8 a.m.
This would affect the metabolism of drugs, that exhibit hepatic blood flow dependent clearence.
Tumor blood flow and therefore drug delivery are two fold higher during daily activity span of nocturnally active rats.
2. Most normal tissues are either more or less sensitive to effects of drugs at specific times of day.
The variable sensitivity can be explained and quantified in terms of bioperiodic changes in the concentration of receptors of a given sistem for a given drug.
In other cases, a circadian variation of cellular defense mecanisms may play a part.
Cell proliferation rytms in the gastrointestinal tracts and bone marrow are relevant to the oncologist since these are common targets for toxicity of antineoplastic agents.
In the gastrointestinal tract the highest DNA syntethic activity is between 5-9 a.m. each morning.
In bone marrow are blood cells undergoes strong regular temporal variations – circadian and seasonal.
The percentage of cells in DNA syntesis in the bone marrow presents a large variation along the circadian time scale for each 24h profile 29 to 339% with the highest DNA syntetic activity between 7 a.m. and 4 p.m.
Two clinical trials have demonstrated an asinchrony in DNA syntesis between tumor tissue and normal tissue.
In nonHodgkin's lymphoma the within day variation in S-phase values observed in individual patients ranged from 21 to 353% and the majority of peak values were found late in the evening or during the night.
This peak is 12h out of phase with the circadian variation in S-phase in normal bone marrow.
The avaiability of portable infusion pumps capable of delivering single or multiple drugs each with their optimal circadian scheduling has made thechnical application and testing of these principles plausible.

Clinical benefit of this strategy depends upon a certain cytokinetic and metabolic asynchrony between the circadian susceptibility patterns of the normal tissues at risk for drug toxicity and the tumor.
If this asynchrony exist then at the time when the normal tissue are less vulnerable to the toxic effect of a drug, the tumor may not be protected to the same extent.
This would allow more dose intensive treatment to be given without increased toxicity.
A similar argument can be made for the toxic biological response modifiers (IL2, TNF, IFN). Whereas those that are nontoxic (EPO, G-CSF) might achieve a better therapeutic effect with a lower and less costly dose if given at the optimal time

SYSTEMIC MASTOCYTOSIS.

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Systemic mastocytosis represents a group of disorders characterized through excessive accumulation of mast cells in tissues and organs accompanied by clinical symptoms derived from degranulation and releasing of mast cell specific mediators such as histamine, tryptase, prostaglandins . Clinical aspects are heterogeneous, according to the tumoral burden and visceral infiltration.
WHO classification describe two categories of disease:
1. Cutaneous mastocytosis- mast cell infiltration is limited to the skin, without any systemic involvement, seric tryptase level being normal;
2. Systemic mastocytosis, with large spread of clinical aspects, depending of the grade of visceral infiltration (hepatomegaly, splenomegaly, adenopathies, lung involvement), with or without skin involvement.
Systemic mastocytosis(SM) is a rare disease, with an uncertain incidence. SM affects both genders and any age. Children have frequently cutaneous form, adults the systemic one. The molecular abnormality described in SM is the somatic mutationof protooncogene c-kit, which encodes a tyrosinekinase receptor for srem cell growth factor; this mutation takes place in codon 816, with valine replaced by aspartate (Asp816Val). The result is an independent activation of c-kit receptor, with autophosphorilation and activation of STAT5, P13K,AKT paths. The final consequence is uncontrolled proliferation and resistence to apoptosis of mast cells from skin and organs. These molecular abnormalities are translated in aberrant expression on the membrane of mast cell of the receptor for interleukin5 and for c-kit. The abnormal mast cell has an aberrant coexpression of CD117, CD25, CD2 and cytoplasmic tryptase.[1,2,3]
Clinical aspects are extremely polymorphous, according to the presence and severity of skin and visceral infiltrates with mast cells and with the presence of degranulation symptoms(skin involvement-Darier'sign, café au lait spots, bullous eruptions , patchy or diffuse rash; hepatomegaly, splenomegaly, adenopathies, with or without signs of organ failure for systemic form of disease; symptoms of activation syndrome, triggered by alcohol,stress, infections, interventional measures, heat or temperature variation,etc, such as: fever, rash, diarrhea, collapse, hypotension, abdominal cramps, angioedema, more or less life threatening)[2,3.4,5]
Diagnosis can be difficult and require an experienced interdisciplinary team(allergologist, dermatologist, hematologist and hematopathologist), because of corroboration of clinical, hematological and histopathological aspects of diagnostic, in order to establish if the WHO criteria of diagnosis are fulfilled and to frame the disorder in the clinical form. The diagnosis is made in the presence of a major and a minor criteria or in the mandatory presence of 3 minor criteria.[1,3,6,7]
Major criteria :
1. Multifocal infiltration with ≥15 mast cells in bone marrow and/or extracutaneous tissue; cells are positive for blue toluidine and for the immunohistochemical test for tryptase,
Minor criteria:
1. 25% mast cells with atypical morphology(spindle shaped) in bone marrow or extracutaneous tissue;
2. Coexpression of CD25, CD117, CD2, on flowcytometry or immunohistochemistry
3. Detection of c-kit mutation (D816V) in blood or bone marrow
4. Seric tryptase level >20 ng/ml not detected if it is associated a clonal myeloid disorder)

The presence of B (burden) signs > 30% mast cells in bone marrow or serum tryptase >200 ng/ml; hypercellularity of bone marrow, organomegaly, but without signs of organ failure) does not represent an indication for treatment. C (cytoreductive) signs must be correlated with impaired visceral function due to mast cell infiltration and their presence represent a sign for starting cytoreductive therapy (cytopenias, hepatosplenomegaly accompanied by hypoalbuminemia, portal hypertension, hypersplenism, osteolytic lesions, fractures of pathological bone, cachexia, malabsorbption)[8,9]
Treatment of SM has two objectives: improving the quality of life through reducing symptoms. Degranulation syndrome can be prevented by patient

education- avoid triggers for mast cell activation (alcohol, heat, emotional stress, infections). Prophylaxis of degranulation is made with H1, H2 inhibitors, corticosteroids and/or addition of mast cell membrane stabilizers (cromoglicate) . The treatment of aggressive systemic form is challenge. There is no standard of treatment. First line of treatment consist in Interferon administration, associated with cortico-steroids in order to minimize degranulation syndrome. Second line treatment is represented by Cladribine reserved for interferon resistant or intolerant patients). Tyrosine kinase inhibitors (Dasatinib) is more effective than Imatinib, that does not act on D816V mutation, but it can be used in mast cell disease associated with clonal myeloproliferative disease hypereosinophilic syndrome PDGFR/FIP1L1). In clinical trials inhibitors of c-kit such as Rapamycin and analogue Geldanamicin, or anti CD25 monoclonal antibodies can be used for aggressive ,malignant forms of disease. For these aggressive forms, including mast cell leukemia, the chemotherapy followed by allotransplant could be an option.[8,9,10]

The prognosis of SM is variable, depending of clinical aspects, age and form. There is a possibility of continuum progression toward an aggressive form, so clinical observation is indicated.

It is mandatory to collaborate with other specialists for managing such a difficult and heterogeneous disease.

Bibliography:

1. Horny HP, Metcalfe DD, Bennett JM, Bain B, Akin C, Escribano L, Valent P- Mastocytosis, 54-61, in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed, 2008, IARC, edited by Swerdlow S, Campo E, Lee Harris N, Jaffe E, Pileri S, Stein H, Thiele J, Vardiman J.
2. Worobec A, Metcalfe D, chapt 88, pag 2054-2067- Systemic Mastocytosis in Wintrobe's Clinical Hematology, 12th ed, 2009, Lippincott Williams &Wilkins.
3. Molderings G, Brettner S, HomannJ, Afrin LB- Mast cell activation disease: a concise practical guide for diagnostic workup and therapeutic options, Journ of Hematology and Oncology, 2011, 4:10.
4. Scwartz LB- Clinical utility of tryptase levels in systemic mastocytosis and associated hematologic disorders, Leuk Res. 2001, 25, 553-562.
5. KudaH, MorinagaS, Shimoshata Y et al- Solitary mast cell tumour of the lung, Cancer, 1988, 61, 2089-2094.
6. Furitsu T, Tsujimura T, Tono T et al- Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia line causing ligand independent activation of c-kit product.; J. Clin Invest.1993,92,1736-1744.
7. TefferiA, Lasho TL, Brockman SR,Elliott MA, Dispenzieri A, Pardanani A- A FIP1L1-PDGFRA and c-kit D816V mutation based clonality studies in systemic mast cell disease associated with eosinophilia; Haematologica,2004,89,871-873.
8. Valent P, Sperr WP, Akin C- How I treat patients with advanced systemic mastpcytosis- Blood, 2010,116,5812-5817.
9. Valent P, Akin C, Sperr WR, Horny HP, Metcalfe D-Smouldering mastocytosis with slow progression; Int Arch.Allergy Immunol.2002, 127(2),137-139.
10. Quintas-Cardama A, Aribi A, Cortes J, Giles F, Kantarjian H, Vertovsek S- Novel approaches in the treatment of systemic mastocytosis; Cancer, oct1, 2006, vol107, no7.,1429-1437.

SOLITARY PLASMAYTOMA – NOVELTIES IN DIAGNOSIS AND TREATMENT.

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Solitary plasmacytoma represents under 10% of plasma cell neoplasia and is characterized by the presence of a tumor with monoclonal plasmacytes, localized, without systemic involvement, similar to multiple myeloma. The plasmacyte tumor can be localized in the bones (solitary bone plasmacytoma) or in soft tissues, especially in the digestive and upper respiratory tract.

Solitary plasmacytoma is a heterogeneous disease, sometimes it is presented as a strictly localized lesion, and it may progress to multiple myeloma in 2-3years. The diagnostic criteria have changed over the last years. Current examinations have increased the precision of diagnosis. Thus multiparameter flow cytometry and molecular detection of heavy and light chain of immunoglobulin allow the highlighting of monoclonal plasmacytes in patients in which these were not evidenced through optical microscopy.

Due to the usage of MRI, the risk of a misdiagnosis of solitary plasmacytoma instead of multiple myeloma has decreased significantly. Still, in solitary plasmacytoma the risk of progression persists in about 40-50% of cases.

The elected treatment is local radiotherapy with or without surgical excision which ensures a high rate of local control and a high rate of survival, without signs of disease and a good general survival.

CNS LYMPHOMAS – NEWS IN PATHOGENESIS, DIAGNOSIS AND TREATMENT.

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CNS lymphomas, both primary and secondary, continue to represent a diagnostic, therapeutic and prognostic challenge, despite the significant progress that has been achieved in the past years.
Primary CNS (PCNS) lymphomas are limited to the brain parenchyma, intraocular compartment, cranial nerves, leptomeninges and spinal cord. In secondary CNS lymphomas there is concomitant systemic and CNS localization.
Approximate 90% of PCNS lymphomas are DLBCL.
CNS involvement in NHL is rare and that's why there is limited randomized data regarding the best therapeutic strategies. There is still an important fraction of CNS lymphomas patients who do not receive optimal therapy.
The goal of these presentation is to overview the literature regarding pathogenesis, diagnosis and therapeutic strategies of CNS lymphomas as well as an attempt to elaborate our own treatment protocol. We also present our experience with CNS lymphomas

ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS.

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Acute myeloid leukemia (AML) is a disease that affects mostly elderly patients; over a third of newly diagnosed patients were aged at least 75 years and the median age at diagnosis is 67 years.
AML in elderly patients has different biological and clinical characteristics compared to younger patients that lead to a more severe evolution, expressed by a 5-year survival rate of up to 39% in patients <65 years, which decreases to values of <2% in patients over 75 years.
In patients aged > 60 years, AML often occurs secondary to myelodysplasia or chemotherapy / radiotherapy administered for other malignancies and leukemia cells have different characteristics from those of younger patients: frequent expression of immaturity antigens (e.g. CD34), increased incidence of cytogenetic abnormalities associated with poor prognosis, chemoresistance determined both by frequent expression of P-glycoprotein / MDR1 and certain specific gene expression profiles (eg. Inhibition of certain tumor suppressor genes). To the poor outcome also contributes a series of age-related factors: low performance status, subclinical organ dysfunction, associated comorbidities, impaired bone marrow reserve with poor tolerance to myelosuppressive chemotherapy.
The first step in initiating therapy is to evaluate the patient's capacity to support therapy based on performance status, comorbidity scores and geriatric assessment (fit, vulnerable or frail patients).
The main therapeutic options include: intensive chemotherapy (based on combinations of Ara-C and anthracyclines), hypomethylating agents, low-dose Ara-C, investigational agents and supportive treatment with hydroxyurea and transfusions.
The results of several studies show that intensive chemotherapy, even in patients up to 80 years, lead to higher response rates and better survival compared to low-dose or palliative therapy. The commonly used chemotherapy is the classical "7 + 3" regimen. The results vary greatly depending on the type of cytogenetic abnormalities, age and performance status with complete remission rates (CR) ranging between 20-60%. Over time various studies proposed modalities to improve therapy outcome. Some studies have reported improved CR rates and survival by increasing daunorubicin dose in patients with normal cardiac function; other studies have reported better results by associating chemotherapy with retinoic acid (ATRA) or gentuzumab ozogamicin or using clofarabine.
Frail patients who cannot tolerate intensive chemotherapy are treated with less aggressive regimens: low-dose Ara-C, hypomethylating agents (azacitidine, decitabine) or other treatments that are being evaluated: low dose mercaptopurine valproic acid, ozogamicin gentuzumab, or clofarabine + low dose Ara-C.
For elderly patients achieving CR there isn't currently a standard postremission treatment. Studies published so far indicate a better survival in fit patients receiving reduced intensity allogeneic hematopoietic stem cells transplantation. In patients who are not eligible for transplantation the indication is for "consolidation" treatment using low doses of the same drugs as in the induction therapy or hypomethylating agents. For patients who achieved remission with hypomethylating agents the general recommendation is to continue this type of therapy indefinitely, until disease progression.
In the current era it is recommended that the majority of elderly patients receive antileukemic treatment in order to improve the survival and quality of life. The choice of therapy should take into account clinical status and comorbidities as well as the biological features of the disease.

DIAGNOSIS BY IMMUNOPHENOTYPING IN LYMPHOPROLIFERATIVE DISORDERS WITH PERIPHERAL BLOOD INVOLVEMENT (LEUKEMIC LYMPHOMA) - INDICATIONS, PARTICULARITIES.

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Lymphoproliferative disorders with leukemic discharge in peripheral blood are characterized by lymphocytosis in peripheral blood, which is a mark present in all these cases. Diagnosis of these disorders involves the analysis of lymphocytosis, besides clinical examination and anamnesis, the patient first approach, applying methods to identify the nature of lymphocytes, to define whether there is a clonal expansion of lymphocytic and type of lymphocyte.
Immunophenotyping is thus the only method that can meet these criteria, and therefore the method of choice in the diagnosis of this lymphocytosis associated with lymphoma and allows the distinguish from reactive lymphocytosis (infectious, congenital, tobacco, etc.)
Lymphomas with peripheral blood involvement are:
B-cell lymphomas:
Small cell lymphocytic lymphoma (SLL) ● Mantle cell lymphoma (MCL) ● Marginal splenic lymphoma (SMZL), MALT ● Follicular Lymphoma (FL) - rare
T-cell lymphomas:
Sezary Syndrome (SS) ● Lymphoma / adult T-cell leukemia (ATLL) ● Peripheral T-cell lymphoma (PTCL) - rare
The mechanism of discharge is related to the presence of adhesion molecules and interactions with the microenvironment of malignant clonal cell. In this respect we have for example a massive discharge of malignant lymphocyte after therapy with inhibitors of intercellular signaling pathways that "pull" these cells from the microenvironment that protects them. Also, the similarity of intercellular signaling, for example through integrin CD40 in lymphoma with peripheral discharge and CLL, sustain these mechanisms.
Identification of B cell lymphomas permits the identification of mantle cell lymphoma, co-expressing CD5, and using pathognomonic markers CD23 and CD200 and combination of CD79b and CD43 allows a clear differentiation of CLL or SLL. Marginal zone lymphoma (SMZL and MALT) does not express CD5 and have immunophenotype of marginal zone / mature B cell. Splenic lymphoma requires differentiation from hairy cell leukemia, which has specific markers CD103 and CD25. Follicular lymphoma is distinguished by coexpression of CD10 and bcl-2, but requires differentiation from Burkitt and diffuse large cell lymphoma, which have in advanced stages peripheral blood discharge.
T-cell lymphoproliferative are difficult to identify because must be differentiated from reactive lymphocytosis, one of which as infectious mononucleosis may have even atypical, but transient, immunophenotype.
Sezary syndrome is defined by the presence of at least 1000 Sezary cells / mmc, usually helper cells and ATLL has usually memory helper T cell immunophenotype, but there could be different versions of immunophenotype. Rarely, we can have other peripheral T-cell lymphomas with peripheral blood discharge, and requires the differentiation of reactive lymphocytosis.
Immunophenotyping of lymphocytes is an easy and extremely useful method indicated in lymphocytosis analysis and allows fast and accurate diagnosis of chronic lymphoproliferative disorders.

PATHOGENETIC AND THERAPEUTIC IMPLICATIONS OF HEPATITIS VIRUSES IN NON-HODGKIN LYMPHOMAS.

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The number of viruses known to have pathogenetical and therapeutical implications in non-Hodgkin lymphomas(NHL) has increased significantly during the last years. Some of these viruses have direct oncogenic effect, for exemple Epstein-Barr virus, Human T-cell lymphotropic virus-1 or human herpesvirus -8, while others cause lymphoma due to chronic antigenic stimulation (like hepatitis C virus) or due to immunosuppression associated with the virus, which is the case of human immunodeficiency virus. Beside their role in pathogenesis, lymphoma-associated viral infections have major therapeutical implications. Treatment of these lymphomas is difficult because of the risk of reactivation after immunosuppressive treatment which could cause severe organ-damage (like severe hepatitis in case of hepatitis virus B or C reactivation), resulting sometimes in reduction of the scheduled doses or even treatment discontinuation. These patients should be monitored carefully and antiviral therapy should be associated to chemotherapy. In other cases, especially in indolent NHL (for example splenic marginal zone lymphoma associated with hepatitis C virus), antiviral therapy alone could lead to regression of lymphoma. Recognizing this category of NHL is important because correct management of them could improve survival of the patients.
Key words: non-Hodgkin lymphoma, viruses, reactivation, chemotherapy

CLINICAL HAEMATOLOGY SECTION
BONE MARROW TRANSPLANTATION SESSION

THE FIRST 18 MONTHS OF ACTIVITY IN THE BONE MARROW TRANSPLANTATION DEPARTMENT OF THE COLTEA HOSPITAL.

Andrei Colita, Cecilia Ghimici, Raluca Manolache, Madalina Oprea, Doina Barbu, Florentina Gradinaru, Andrei Turbatu, Anca Roxana Lupu
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The Department of Bone Marrow Transplantation in Coltea Hospital was accredited in April 2013 and since 2014 operates within the National Programme for Organ, Tissue and Human Cells Transplantation.
The first transplant was performed in December 2013 by own funds while the other procedures were performed after obtaining the financing from the National Programme.
Until 1st of July 2015 we harvested peripheric hematopoietic stem cells from 28 patients of which in 22 cases we performed autologous peripheral stem cells.
Peripheral stem cell harvesting was performed initially at the Fundeni Institute using a Cobe Spectra machine (first two cases) and later in our compartment using Optia Spectra equipment.
Patients undergoing apheresis were diagnosed with multiple myeloma (MM) in 13 cases and lymphoproliferative diseases in 15 cases: Hodgkin;s lymphoma'(HL) 9 cases and non-Hodgkin's lymphoma (NHL) - 9 cases.
Mobilization regimens consisted of cyclophosphamide and filgrastim for myeloma cases, respectively DHAP, IGEV or Etoposide associated with filgrastim for patients with lymphoma. In one case of NHL treated with multiple lines of chemotherapy and radiotherapy we associated Plerixafor. In another case of NHL, with an allergy to filgrastim, we used IGEV regime for mobilization without adding growth factor, but we added Plerixafor in evening previous to the apheresis.
The number of harvested CD34 cells ranged from 2.014 to19.3 x 106 / kg. Graft cryopreservation was performed in the Stem Cell Bank of the Fundeni Institute
The patients who underwent autologous transplantatio (22) were diagnosed with MM in 10 cases , LH in 9 cases and NHL in 3 cases. Conditioning regimens consisted of Melphalan 200 for myeloma cases and BEAM for lymphoma patients. The infused grafts had cellularity ranging from 2.57 to 19,3 x 106 CD34cells/kg and the median engraftment duration was 11 days for neutrophils and 14.5 days for platelets.
Follow-up duration ranges from 15 to 565 days. Of the transplanted patients 21 are alive, 2 cases of MM and 2 of LH have relapsed, and in one case a second neoplasia was diagnosed NHL; the remaining 16 cases are in continous remission.

STEM CELL TRANSPLANTATION IN CHILDHOOD ACUTE MYELOPROLIFERATIVE SYNDROMES.

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Background. Stem cell transplantation (SCT) represents a standard therapeutical indication for high risk acute myeloblastic leukemia (AML) in first complete remission (CR), for all AML patients in 2nd CR and the only potential curative option for patients with juvenile myelo-monocitic leukemia (JMML).
Aim. To evaluate the indications and results of SCT in children with acute myeloproliferative syndromes treated in Pediatric BMT Department, Fundeni Clinical Institute, Bucharest.
Patients and methods. We present a retrospective analysis of children with acute myeloproliferative syndromes and SCT followed in Pediatric BMT Department, Fundeni Clinical Institute between 2001-2015. Transplant indication has been established according to EBMT (Europeean Group for Bone and Marrow Transplantation) recommendations. All AML patients received standard myeloablative conditioning with busulfan and cyclophospamide (Bu-Cy) and patients with JMML received busulfan-cyclo-phospahmide-melphalan (Bu-Cy-Mel). Graft versus host disease (GVHD) prophylaxis consisted in ciclosporine (CSA) and methotrexate (MTX) for sibling donors, CSA + MTX and antithymocytic globulin (ATG) for unrelated donors. All patients received antibacterial, antifungal, antiviral prophylaxis according to the internal protocols. Posttransplant evaluations included hematological, biochemical tests,

viral DNA, donor chimerism with STR (short tandem repeats) on days +30, +60, +90. The IBMTR score has been used for acute GVHD staging and HIH score for chronic GVHD.
Results. We present a consecutive series of 14 children with acute myeloproliferative syndromes treated in Fundeni Clinical Institute, age range 10 moths – 15 years, male/female: 1. Transplant indications were: AML, 1st CR- 5 cases, AML, 2nd CR- 4 cases, JMML- 4 cases, myelodisplastic syndrome – refractory anemia with blast excess in transformation (AREB-t)- 1 case. The procedure has been performed using sibling donors in11/14 cases and from registry donors in 3/14 cases. In 12/14 cases there were 10/10 HLA matching and in 2/14 cases there were 9/10. The stem cell source used was peripheral blood (10/14), bone marrow (3/14) and bone marrow + cord blood (1/14). All patients engrafted after 12-20 days, without severe infectious complications during the first 30 days after transplant. The chimerism analysis showed complete donor chimerism in 13/14 cases on day +30. The follow-up showed 4 severe viral complications in 4 cases (CMV, BKV, HHV6), GVHD in 6 cases. Eight patients are in complete remission and very good clinical condition after long term follow-up: 6 months – 14 years. We registered 6 deaths: relapse after 2 years: 1 case, severe GVHD: 3 cases, CMV encephalitis: 1 case, aspiration pneumonia (infant): 1 case.

Discussions /Conclusions. SCT represents a therapeutical option for children with acute myeloproliferative syndromes associated with a better chance for cure compared with standard chemotherapy. The main complications associated with unfavorable prognosis are severe GVHD and viral infections.

CHALLENGES IN IMPLEMENTATION OF THE GLOBAL REGISTRATION IDENTIFIER FOR DONORS (GRID.)

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This document sets out the challenges that professional organizations are facing and their efforts to develop a global standardization of coding and labelling for cellular therapy products, to assure the traceability and to reduce the risk of misidentification of donors or their donations due to the lack of global uniqueness of identifiers.

- The Single European Code (SEC) was foreseen in Article 10 of the Directive 2006/17/UE and is compulsory to be used starting with 2014. In September 2015, was approved an EU joint project called Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation - VISTART (National Agency of Transplant and National Registry of Hematopoietic Stem Cell Voluntary Donors are part of consortium). The task of this consortium is to provide technical support and guidance to EU Tissue and Cell Competent Authorities and to Tissue Establishments across the EU in the implementation of the SEC for tissues and cells together with the ISBT 128 standard. Each of the transplant centres, HLA testing laboratories, apheresis and collection centres, donor centres, should have in place a unique donation and product code for all HPC products produced in and imported to EU countries.
- The cellular therapy accreditation bodies: AABB, JACIE, FACT and WMDA all require accredited cellular therapy facilities to use ISBT 128 standard terminology and to have an ISBT 128 implementation plan in place. Global standardization using ISBT 128 is essential to support secure and reliable traceability on a global basis for the large proportion of cell therapy products which are distributed across national borders.
- In this context, WMDA has a Memorandum of Understanding with ICCBBA to assign and manage the list of issuing organization numbers and support the development of associated standards documents. Representatives from WMDA and ICCBBA have been working together to develop the structure of the Global Registration Identifier for Donors (GRID). The goal of the project is to create a system for assigning a globally unique identifier to potential hematopoietic stem cell registry donors. Given the global nature of the work done by hematopoietic stem cell donor registries, a system to uniquely identify potential donors on a global scale is needed to facilitate communication and prevent errors in identification of donors. A standard machine-readable format for a GRID that can be used by electronic process control systems, as well as a standard format for the human-readable version, has been developed.

The WMDA has a GRID Implementation Working Group who is tasked with assisting with the delivery and the implementation of this concept within the unrelated donor community. They will provide support and guidance to interconnected registration organizations on all operational matters related to the GRID.

Key words: global standardisation, coding, labelling, unrelated donors, unrelated hematopoietic stem cell transplantation.

RETROSPECTIVE ANALYSIS OF THE RESULTS OF AUTOLOGOUS BONE MARROW IN MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE IN 137 PATIENTS.

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According to the current treatment guidelines, autologous bone marrow (ASCT) is a standard first-line treatment for patients younger than 65 years. In our Institute ASCT it is a routine procedure since 2002. This study is a retrospective analysis of the results obtained on a lot of 137 patients who performed ASCT. The study was conducted in patients transplanted between 2003 - 2014 and were included only patients whom we had complete information.

Results and Discussion: Most patients were aged ≤ 65 years and only 3 patients were older than 65 years (until 70 years). The gender distribution was almost equal. Regarding therapy performed before transplantation, most of the patients received bortezomib-based therapy (VelDex, CyBorD, PAD, VTD) or VAD type (mostly patients in the early years). The median time from diagnosis until the harvesting hematopoietic stem cells (HSCs) was 7 months. The median time from harvest HSC until the transplant procedure was 5 months. The response to treatment before ASCT was, partial remission (PR) for 68 patients (50%), ≥VGPR for 34 patients (24.81%), CR + nCR for 29 patients (21.16%). At 100 days post ASCT improving response was achieved in 23 patients (16.8%). Thus, 10 patients (7.3%) switched from PR to VGPR, 9 patients (6.6%) from PR to CR and 4 patients (2.9%) from VGPR to CR. Overall survival (OS) was 77 months median. In terms of progression free survival (PFS) it had a median of 32 months. The results for OS and PFS are comparable to those reported in the literature, but events free survival (EFS) post ASCT disease was only 12 months - half of that reported in other studies. One explanation for this would be the long period between diagnosis and harvest CSH, respectively between harvesting and transplantation. Post-transplant, 23% of patients died with a median survival of 44 months.

Conclusion: ASCT is a standard procedure that can be performed in Romania with results comparable to those of other international centers.

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FIRST TWO YEARS EXPERIENCE IN UNRELATED STEM CELL TRANSPLANT AT FUNDENI CLINICAL INSTITUTE.

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An essential component of allogeneic stem cell tarnsplantation is to identify a human leukocyte antigen (HLA) matched bone marrow donor. For those patients who has transplant indication but without a suitable family sibling donor, the RNDVCSH provide a service to locate a HLA-matched unrelated donor.

Fundeni Clinical Institute started the unrelated transplant program in 2013. This presentation bring into your attention our results within the first two years (july 2013- july 2015). In this period we had 29 unrelated stem cell transplant cases in adult patients. The diagnosis transplanted: AML (9), CML (7), ALL (B, Ph+, biphenotypic) (8), severe aplastic anemia (3) and MDS (2). The median age was 37,7 years (20 – 61). Seventeen patient had myeloablative conditioning with BuCyATG. Fourteen patients had 10/10 HLA-matched donors and 15 had 9/10 mismatched donors (with one allele or one antigen mismatch). The transplant related mortality was 4/29 (13,79%) and one patient died at 5 months after transplant because of relapse. OS=82,7%.

The first 29 cases of unrelated transplants in adult patients is compared with our first 29 cases of sibling transplants (juin 2004 -february 2010), when the diagnosis were: AML (14), CML(1), ALL (B and biphenotypic) (7), SAA (2), MMM (2) and Hodgkin disease (3). The median survival was 30,5 months (11 days -11 years) and 27,5% are long time survivors. TRM was 48,2%.

ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR HEPATOSPLENIC T CELL LYMPHOMA: A RETROSPECTIVE STUDY OF THE EBMT LYMPHOMA WORKING PARTY.

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Hepatosplenic T-cell lymphoma (HSTL) is an extremely aggressive and rare subtype of peripheral T cell lymphoma (PTCL). Although anecdotal cases of successful hematopoietic stem cell transplantation HSCT for HSTL have been described, structured analyses of the efficacy of autologous or allogeneic HSCT in HSTL have not been published until this study.

The objective of this study was to analyze the outcome of patients who underwent allogeneic (alloHSCT) or autologous HSCT (autoHSCT) for HSTL.

This is a registry-based retrospective multicenter study including patients 18 years or above with histologically verified γδHSTL who underwent alloHSCT or autoHSCT between January 2004 and January 2013 and were reported to the European Society for Bone and Marrow Transplantation (EBMT). Baseline patient, disease and transplant data were collected from MED-A forms. 76 patients were identified in the EBMT database. Additional information upon center request was provided for 36 them. Eleven of these had to be excluded after histopathology review, leaving 25 patients in the final study sample (alloHSCT 18, autoHSCT 7). With a median follow-up of 36 months, 2 patients relapsed after alloHSCT, resulting in a 3-year progression-free survival of 48%.

This study shows that allotransplantation can provide long-term disease control in patients with hepatosplenic T cell lymphoma. Preliminary evidence suggests that graft-versus lymphoma activities are contributing to disease eradication in this otherwise inevitably fatal lymphoma subset.

HAPLOIDENTICAL STEM CELL TRANSPLANTATION – AN ALTERNATIVE FOR PATIENTS WITHOUT AN UNRELATED COMPATIBLE DONOR; THE FIRST SUCCESSFUL APPLICATION IN ROMANIA.

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Hematopoietic stem cell transplant (HSCT) is a standard treatment for different malignant and non-malignant diseases, being performed more than 30,000 HSCT each year in Western Europe. Two-thirds of patients requiring an allogeneic stem cell transplant, haven't an available HLA compatible donor. An unrelated voluntary donor is identified in only 50-60% of cases. Haploidentical donors (parents, children and siblings- 50% compatible) are alternatives for these patients that can not find a donor. Transplant outcomes using haploidentical donors and posttransplant Cyclophosphamide have improved past several years and are comparable with outcomes of matched unrelated donors. These encouraging early results extended this form of transplantation worldwide.

In this paper, we present the case of a 33-year-old women, who received an haploidentical transplant after relapsed after multiple chemotherapy regimens, including autologous hematopoietic stem cell. This haploidentical transplant represent the first successfull procedure of this type in Romania (patient in complete remission, PET negative at 8 months after transplant).

INTEGRATED APPROACH OF PATIENTS WITH ACUTE LEUKEMIA FROM FUNDENI CLINICAL INSTITUTE HEMATOLOGY AND BONE MARROW TRANSPLANT CENTER – EXPERIENCE OF THE LAST TWO YEARS.

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The identification of patients with acute leukemia who needs allogeneic transplantation is made using an integrated risk profile that incorporates cytogenetics exam, molecular genetic data and asseement of clinical response. Other factors influencing therapeutic decisions are: patient's age, comorbidities, performance status. Allogeneic hematopoietic stem-cell transplantation (HSCT) is a potentially curative treatment of acute leukemia, but it is necessary to evaluate the individual patient's risks for relapse and nonrelapse mortality (NRM). The intensity of conditioning chemotherapy regimen used has been shown to influence the disease-free survival.
In this presentation we have included patients with acute leukemia who were diagnosed and treated in our center from 2013 until june 2015. Patients were stratified according to age (<59 vs >59 years) and disease risk category. For 29 patients (16 with acute myeloid leukemia and 13 with acute lymphoblastic leukemia) who underwent allogeneic stem cell transplant in our center, we have proposed to assess the relationship between overall survival (OS)/leukemia-free survival after HSCT and the following parameters: time from diagnosis to stem cell transplant, intensity of conditioning regimen, post-transplant complications (i.e. acute/chronic graft-versus-host disease (GVHD)), time to engraftment, type of donor (unrelated vs. related donor) and chimerism. Most patients received a standard myeloabaltive conditioning regimen (MAC) and the most common post-transplant complication was bacterial/viral infection. At the time of presentation, 62% of these patients are alive.
This presentation highlights some very important issues for diagnosis and treatment of acute leukemia: the interval from diagnosis to allogeneic transplant, role of MRD monitoring before HCT, differences between a reduced intensity conditioning (RIC) regimen and standard myeloablative conditioning regimen (MAC).

Keywords: Acute myeloid leukemia, Allogeneic hematopoietic cell transplantation, Reduced intensity conditioning, Myeloablative conditioning.

VIRAL INFECTIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION – EXPERIENCE OF THE BONE MARROW TRANSPLANTATION CENTER TIMISOARA.

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Introduction: Viral diseases continue to negatively influence the outcome of allogeneic hematopoietic stem cell transplantation (HSCT). In the last years, pre-emptive therapy guided through the results of molecular monitoring, managed to reduce the incidence and severity of life-threating viral diseases. Therefore we aimed to assess the incidence and outcome of viral reactivations after allogeneic HSCT as well as the safety and efficacy of pre-emptive therapeutical approaches.
Material and methods: In a prospective study, we have consecutively included all the HSCT procedures (n=58) performed between January 2008 and December 2014 in the Bone Marrow Transplantation Center Timisoara, Romania. Antiviral prophylaxis consisted mainly of Acyclovir (n=55; 94.82%), whereas 3 patients (5.17%), considered at high-risk for CMV reactivation, received Ganciclovir (GCV) prophylactically. Quantitative RT-PCR was performed weekly or once in two weeks in peripheral blood for Herpesviridae family members (HSV1, HSV2, CMV, EBV, VZV, HHV-6 and HHV-7) and in urine for polyoma-BK. Thresholds for pre-emptive measures were the following: 500 copies/mL for CMV, 1000 copies/mL for EBV and HHV- 6, positivity of RT-PCR (100 copies/mL) for the rest of the Herpesviridae viruses and polyoma-BK.
Results: CMV was the most frequent pathogen observed, with an incidence rate of CMV reactivation of 27.58%. Results of pre-emptive therapy with GCV were the following: clearance of CMV viremia after an average time of 25 days, low recurrence rate of CMV reactivation (18.75%) and a relatively high-rate of GCV-associated neutropenia (37.5%). HHV-6 reactivation was detected in 10.34% of the HSCT procedures and responded favorably to GCV pre-emptive therapy (given due to concomitant CMV reactivation) with the exception of one patient who required administration of Foscarnet. In 13.79% of the HSCT procedures, EBV was detected through RT-PCR, all of the patients with EBV reactivation responding to reduction of immunosuppression,

without concomitant administration of Rituximab. Polyoma-BK was pozitive in the urine of 19 patients (32.75%), at a mean of 43.69±36.47 days after infusion of hematopoietic stem cells, 10 of them (52.63%) developing polyoma-BK hemorrhagic cystitis. Supportive treatment resulted in clearance of polyoma-BK from urine in 89.47% of the patients with polyoma-BK viruria.
Conclusion: None of the patients included in our study developed viral diseases due to Herpesviridae family members. Although the results of our study are limited by the small sample of patients analyzed, further evidence is brought to strengthen the importance, effectiveness and safety profile of pre-emptive antiviral therapy after allogeneic HSCT.

AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA – TARGU MURES BONE MARROW TRANSPLANT CLINIC EXPERIENCE.

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We present in this paper the experience of the Hematology and BMT Unit in Târgu Mureş in the single or double transplantation in multiple myeloma for period of 10 years. We emphasise the methodology of our clinic how to overcome the difficulties of mobilization in heavily pretreated patients, the timing of the first transplant and of the second one in case of a relapse and the methods of maintenance we indicate to prevent relapse.

THE OUTCOME OF AUTOLOGOUS TRANSPLANTATION IN A CASE OF T CELL NON HODGKIN'S LYMPHOMA, STAGE III, ASSOCIATED WITH REFRACTORY PSORIASIS.

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In this case prezentation we considered important to share our results obtained by the autologous transplantation of a patient with grade III T-cell non-Hodgkin lymphoma associated with a very extended severe psoriasis refractory to treatment. Psoriasis as an immune disease that does not have the indication of an autologous transplantation but we wish to present the good results we obtained by the autologous transplanation obtaining remission of the lymphoma and total regression of the psoriasis all this leading to the important improvment of the quality of life of the patient.

CLINICAL HAEMATOLOGY SECTION
ORAL PRESENTATION SESSION

GOLD NANOPARTICLE-BIOCONJUGATES AS CONTRAST AGENTS FOR CANCER CELL RECOGNITION AND DELIVERY AGENTS OF ANTI-LEUKEMIC TYROSIN-KINASE INHIBITORS FOR ACUTE MYELOID LEUKEMIA.

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Introduction.
Gold nanoparticles are extensively exploited in biomedical applications because of their easy preparation, ready bioconjugation and potential biocompatibility. Moreover, the reduced size of nanoparticles facilitates their delivery and incorporation into biological systems. Nevertheless, noble-metal nanoparticles have unique optical properties dominated by the excitation of so called localized surface plasmon resonances (LSPR) from the visible to the near-infrared (NIR) region of the electromagnetic spectrum which facilitates their detection in vivo using innovative, non-invasive techniques such as Surface-Enhanced Raman Scattering (SERS) spectroscopy. In the view of the above mentioned properties, one recent application of nanoparticles is their use for the development of new pharmacological molecular entities which can be delivered to targeted locations within the body in order to maximize therapeutic ratio and minimize systemic side effect.
Methods.
Having in mind the design of a specific, individualized therapeutic agent which relies on nanoparticle-structure properties, we chemically synthesized gold nanoparticles of various plasmonic responses (from Vis to NIR) and conjugated the particles either with fluorophores (eg. fluorescein isothiocyanate, cresyl violet perchlorate) for imaging applications or anti-leukemic drugs (quizartinib, midostaurin, sorafenib, lestauritinib) for therapeutic effect. Bioconjugated particles were characterized by transmission electron microscopy, UV-Vis-NIR absorption spectroscopy, dynamic light scattering, zeta potential, fluorescence and/or surface enhanced Raman scattering (SERS) and found to be biochemically stable and detectable inside cells. The functional tests included MTT assay, cell counting, cell cycle analysis and apoptosis assay.
Results.
Since a prerequisite for any therapeutic agent to be applicable in vivo is to be compatible with the healthy tissue nearby the targeted zone of malignancy, biological effects (proliferation and citotoxicity) of conjugated nanoparticles were investigated on OCI-AML3 acute myeloid leukemia cells and THP-1 human monocytic leukemia cells. Comparative tests between non-conjugated and conjugated nanoparticles revealed a direct dependence of cytotoxicity on particle concentration and also on their morphological and surface chemistry features. We have found that quizartinib, lestauritinib and sorafenib had an enhanced in vitro effect of conjugated with gold nanoparticles.
Conclusion.
The presented results evidence the potential of spectroscopic-active nano-conjugates to serve to combined purpose: as ultra-sensitive imaging tools for cancer cell identification and drug delivery vehicles for cancer nanotherapy.

IN VIVO ASSESSMENT OF THE POTENTIAL MEDULOTOXIC PROPERTIES OF GOLD NANOPARTICLES ON MALE CRL: CD1(ICR) MICE.

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Introduction
Nanotechnology is believed to bring unprecedented

advances in medicine by improving or developing new diagnostic and therapeutic methods. While the potential of some nanoparticles have been extensively studied in biomedical research, the vast majority of such studies are in the field of oncology. Gold nanoparticles (GNPs) have been reported to influence cell viability of CLL cell lines in vitro and have been suggested as a possible aiding therapeutic agent for CLL and other types of leukemia. However, toxicological in vivo studies on the full effects of these GNPs on healthy organisms have yielded insufficient and inconclusive data.

Aim

The aim of the present short-term toxicity study was to assess the medulotoxic properties of GNPs on male Crl:CD1(ICR) mice after 21 days of intravenous administration.

Materials and methods

To determine the possible medulotoxic effects of GNPs in an animal model, 20 male Crl:CD1(ICR) miceweighing between 28-30 grams were used. The animals were housed in polycarbonate type III open-top cages and had access to filtered tap water and pelleted feed ad libitum. The animals were kept in standard conditions: at a temperature of 24 ± 2 °C and a relative humidity of 55 ± 10%, 12:12-h light: dark cycle (lights on, 07:00 to 19:00). All experimental procedures were approved by the ethics committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy from Cluj-Napoca. The mice were randomly assigned into two groups. Group A received a daily intravenous retro-orbital injections of GNPs+TWEEN for 21 days by the method developed by Tal Yardeni et al., using a dose of 1000 µg/kg under general anesthesia with isoflurane, while group B served as a control group and received no injections while being anesthetized daily. After 21 days blood was harvested using the retro-orbital puncture method for hematological assessment and the sternum and femurs were collected. Even if it was not an objective of our study, the liver was also harvested due to its greenish, biliary stasis appearance in all individuals from the experimental group (group A). Hematological parameters were assessed using an Abacus Vet hematological analyzer while the gold concertation from the harvested organs was determined using ICP-MS. Histopathological analysis of the collected organs was also done using a standard hematoxylin-eosin staining.

Results

Results have shown a significant effect of the GNPs on hematological parameters. The total WBC count in the control group showed leukopenia, most probably due to the well-documented isoflurane-induced leukopenia, while the experimental group expressed borderline leukocytosis, indicating myeloproliferation. Liver enzymes (ALAT and ASAT) were also determined and were elevated in the experimental group as opposed to the control group and to the standard mouse values. ICP-MS yielded uniform data in all harvested samples, with all individuals having between 500 and 650 µgof pure gold in the femoral bone-marrow and 8350 – 8500 µg in their liver. The histopathological evaluation of the bone marrow yielded results that confirm that systemically administered GNPs have a definitive effect on its' structure and functionality. The liver showed signs of billiary stasis and macrovesicular steatosis.

Conclusions

The present studyis the first one documenting the morphological cellular changes that occur after a sub chronic systemic administration of GNPs in an animal model. GNPs have altered the cellular structure of the bone marrow, a phenomena which mirrored itself in the hematological values of the experimental animals. The dose of 1000 µg/kg was the NOAEL (No-observed-adverse-effect level) according to some authors when administered orally, but it proved to have a certain toxicity, especially of the liver when administered parenterally.

CLINICO-PATHOLOGICAL STUDY OF A CASE SERIES OF LENNERT'S LYMPHOMA.

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Introduction

Positive Nodal T-Cell Lymphomas/ Epstein-Barr virus (EBV) are highly aggressive, with an mean survival rate of 3.5 months. In this study, we present 8 cases from Ion Chiricuta Oncology Institute casuistry, Cluj-Napoca, with a similar phenotype, except EBV positivity.

Materials and methods

We performed 8 cases (2 women and 8 men) from Ion Chiricuta Oncology Institute casuistry, Cluj-Napoca samples collected between 1987 and 2014 through immunohistochemistry-parts of biopsy and clinical evolution of patients.

Anatomopathological samples were tested for CD3, CD4, CD8, PD1, TIA1, granzyme B, CD15, CD20, CD21, CD30 and Epstein-Barr virus latent membrane protein (EBV LMP-1) following the recommended protocols producers, assessing the percentage of small

and large lymphoid cells, epithelioid histiocytes, nucleated cells Reed-Sterngergbi, plasmocytes and samples vascularity.
All patients received the standard CHOP treatment (cyclophosphamide, doxorubicin, vincristine and prednisolone), previously determining the International Prognostic Index (IPI) and Eastern Cooperative Oncology Group (ECOG) effectiveness status. The patients were called to receive biannual inspections in the first two years and then annually.
Results
Anatomopathologicaly, most of the cases presented cytotoxic T lymphocyte phenotype inactive (TIA + Granzyme B -) with most of the lymphocytes CD4 + and CD8 + and all cases being EBV LMP-1 negative. Considering the evolution and clinical status the samples vascularity was consistent with the description of Lymphoma T angioimunoblastic. At seven patients from totaly of eight cases initially in stage III-IV was observed remission after treatment with CHOP mostly.
Conclusion
These results provide information that EBV infection is a variable for survival in this disease, future studies are mandatory for this purpose.

GOLD NANOPARTICLES CONJUGATED WITH RITUXIMAB FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA.

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Chronic lymphocytic leukemia (CLL) is a monoclonal disorder that is characterized by a continuous accumulation of malignant lymphocytes. Despite progress in targeted therapy options for CLL, relapse or progression to a Richter syndrome still appears. Thus, a more focused and targeted therapeutic option is required. This can be achieved by increasing the concentration of a cytostatic drug in the tumor while reducing its' systemic toxicity. In the continuous effort toward the development of more efficient therapeutic approaches for the treatment of CLL, in the current study we report the conjugation of rituximab antibody drug onto spherical gold nanoparticles. Their effective trans-membrane delivery inside CLL cells and their validation as real-potential therapeutics with increased efficacy, in comparison with drug alone.
The efficient formation of drug-nanocarriers was proved by spectroscopic characterization of the particles. The internalization of rituximab-nanocarriers was proved as a result of the strongly scattered light from gold nanoparticles and was correlated with the results obtained by TEM and dark field microscopy. The therapeutic effect of the newly-designed drugs was investigated by several methods including cell counting assay as well as the MTT assay and was found to be superior when compared with the drug alone, data confirmed by state-of-the-art analyses of internalization, cell biology (flow cytometry, apoptosis and autophagy assay) , genomics (RT-PCR for MS4A1) and proteomics (confocal microscopy and western blotting for CD20).
Key words: rituximab, gold nanoparticles, chronic lymphocytic leukemia

PHARMACOLOGICAL STUDY FOR THE SCREENING OF PRIMARY MYELOFIBROSIS.

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Introduction
Primary myelofibrosis is characterized by uncontrolled proliferation and activation of fibroblasts under the influence of different signals transmitted by the abnormal megakaryocytes but also by various elements of the bone marrow microenvironment. Following this activation, fibroblasts will produce an abundant extracellular matrix, which eventually will replace the bone marrow and interfere with its normal operation.
Materials and methods.
Currently, the treatment of this disease is limited and mostly nonspecific. Therefore, the development of new therapeutic strategies is very important. Several clinical

trials with promising results is based on the use of ruxolitinib, inhibitor of JAK2 tyrosine kinase, mutant myelofibrosis, in combination with an anti-fibrotic agent (# NCT01369498, NCT01981850 #).
In this study, we present the screening of 1240 substances, drugs currently used in various diseases to identify those who will inhibit the activation and differentiation of fibroblast into myofibroblasts phenotype, extracellular matrix producer. It was used mesenchymal stem cell line SR-497 submited to inactivation process by hanging drops, which can be isolated from chemical, biological and physical stimuli. After inactivation, cells were exposed to drugs investigated.
Results.
After the screening, we obtained three substances which inhibited the proliferation and differentiation of fibroblast: cyclosporine, mycophenolate mofetil and bisphosphonate risedronate.
Conclusions.
Further, these three substances will be investigated individually, both established cell lines and primary cell lines isolated from patients with primary myelofibrosis.

THE IMPACT OF MOLECULAR BIOLOGY AND CYTOGENETIC ABNORMALITIES ON SURVIVAL IN ACUTE MYELOID LEUKEMIA.

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Background: Chelation therapy is recommended for transfused patients that have an increased serum ferritin level (over 1000 microg/l). Deferasirox has shown efficacy and safety in maintaining or reducing body iron (assessed by liver iron concentration and serum ferritin). Patients' adherence to Deferasirox treatment was superior to others chelator drugs because it is administrared per oral. The goals of our study is to analyze the results of Deferasirox treatment of a group of adult patients diagnosed and treated in Hematology Departments of Nord-West Romanian hospitals (Cluj, Maramures, Satu-Mare and Salaj Counties).
Methods: We have done a retrospective, transversal study including all the patients with myelodysplastic syndromes (MDS), thalassemia and other anemias that received blood transfusion and chelator treatment. Data collected: profile of serum ferritine during deferasirox treatment, reasons for treatment discontinuating, evaluating adverse effects of Deferasirox. We created a data collection sheet that included: demographics, information about patients' disease, serum ferritine level at start of the treatment and during treatment, evaluation of commorbidities that could increase serum ferritine level, number of blood transfusion before and after starting the treatment, Deferasirox dose and data about dose modification, adverse effects of the treatment. We studied cardiac and liver hemochromatosis, too (if medical information available).
Results: We included in the study 35 patients treated with Deferasirox in the NW region of Romania. The diagnosis included MDS, thalassemia and other anemias. Ages: 55-98 years. MDS patients were treated with erythropoietin, low dose chemotherapy, epigenetic treatment, blood transfusions and bethatalasemic patients were transfused. The baseline value of serum ferritine was between 1075 and 6187 microg/l (median- 3631 and mean- 2321). Deferasirox dose that was administered to the patient was 20-30 mg/kg. There was a significant reduction in serum feritine from baseline for all the patients. We identified six cases of treatment discontinuation. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode) and Deferasirox was restarted after treating the adverse effect. In three cases, treatment was temporarily stopped because low ferritin level (under 500 microg/l). Packed red blood cell transfusions were administered after starting Deferasirox treatment (0-3 units/months, median- 1.5 units/months, mean 1.3 units/months). Two patients died during Deferasirox treatment because of main disease or its complications. We cannot present any conclusions about cardiac and liver hemochromatosis at the start of the treatment or during the treatment because of the leak of data in patients' files.
Conclusions: Analyzing our small group of 35 patient, serum feritine levels decresed after Deferasirox treatment, which proves the efficacy of the drug. Adverse reactions that determined a temporary stop of the treatment were mild/medium short time digestive reactions (diarrhea and digestive bleeding), so we can consider the chelator

EVALUATING THE EFFICACY OF CHELATION THERAPY. STUDY ON A GROUP OF PATIENTS FROM NORD-WEST OF ROMANIA.

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2- Baia-Mare Couty Hospital
3- Zalău Couty Hospital
4- Satu-Mare Couty Hospital

Background: Chelation therapy is recommended for transfused patients that have an increased serum ferritin level (over 1000 microg/l). Deferasirox has shown efficacy and safety in maintaining or reducing body iron (assessed by liver iron concentration and serum ferritin). Patients' adherence to Deferasirox treatment was superior to others chelator drugs because it is administrared per oral. The goals of our study is to analyze the results of Deferasirox treatment of a group of adult patients diagnosed and treated in Hematology Departments of Nord-West Romanian hospitals (Cluj, Maramures, Satu-Mare and Salaj Counties).
Methods: We have done a retrospective, transversal study including all the patients with myelodysplastic syndromes (MDS), thalassemia and other anemias that received blood transfusion and chelator treatment. Data collected: profile of serum ferritine during deferasirox treatment, reasons for treatment discontinuating, evaluating adverse effects of Deferasirox. We created a data collection sheet that included: demographics, information about patients' disease, serum ferritine level at start of the treatment and during treatment, evaluation of commorbidities that could increase serum ferritine level, number of blood transfusion before and after starting the treatment, Deferasirox dose and data about dose modification, adverse effects of the treatment. We studied cardiac and liver hemochromatosis, too (if medical information available).
Results: We included in the study 35 patients treated with Deferasirox in the NW region of Romania. The diagnosis included MDS, thalassemia and other anemias. Ages: 55-98 years. MDS patients were treated with erythropoietin, low dose chemotherapy, epigenetic treatment, blood transfusions and bethatalasemic patients were transfused. The baseline value of serum ferritine was between 1075 and 6187 microg/l (median- 3631 and mean- 2321). Deferasirox dose that was administered to the patient was 20-30 mg/kg. There was a significant reduction in serum feritine from baseline for all the patients. We identified six cases of treatment discontinuation. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode) and Deferasirox was restarted after treating the adverse effect. In three cases, treatment was temporarily stopped because low ferritin level (under 500 microg/l). Packed red blood cell transfusions were administered after starting Deferasirox treatment (0-3 units/months, median- 1.5 units/months, mean 1.3 units/months). Two patients died during Deferasirox treatment because of main disease or its complications. We cannot present any conclusions about cardiac and liver hemochromatosis at the start of the treatment or during the treatment because of the leak of data in patients' files.
Conclusions: Analyzing our small group of 35 patient, serum feritine levels decresed after Deferasirox treatment, which proves the efficacy of the drug. Adverse reactions that determined a temporary stop of the treatment were mild/medium short time digestive reactions (diarrhea and digestive bleeding), so we can consider the chelator

PET-CT IN ASSESSEMENT OF EXTRANODAL INVOLVEMENT IN NON HODGKIN LYMPHOMA.

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Introduction: Lymphomas are the most frequent hematological malignant disorders which implies lymph nodes. Extranodal limfomatous involvement is frequently seen, mainly in non-Hodgkin Malignant Lymphoma (NHL), in correlation with histology subtype and disease staging.
Material and Methods: We will try to present the usefulness of Fusion Imaging Tehnique PET-CT, which combines morphological and functional data, in primary disease staging, interim assessment, end of therapy evaluation and also in long-term follow-up in case of complete remission/suspected recurrence. We will present the main standard accepted diagnosis criteria (Chesson, Deauville, Lugano) which are applied in NHL subtypes presented. PET-CT scan was performed at 60 minutes following iv administration of 3,7-MBq/kg of 18F-FDG, with use of contrast agent when required and when allergic antecedents were missing, with a scan range from tentorium to proximal third of thighs. Scanning protocol was modified in correlation with suspected clinical lesion location (cerebral, cutaneous, cavum). Comparison with other

imaging techniques (mainly CT with iv contrast agent, MRI)were made when this type of scans was performed with maximum 45 days before PET-CT scan.
Results: Extranodal involvement is frequently seen in high-grade and low-grade NHL, in our cases being located mainly in spleen, bone marrow and lungs. Main limits of standard imaging tests, when PET-CT was proved to be superior , were in diffuse infiltrative lymphomatous disease (mainly spleen and bone marrow). Also, we had some cases with normal aspect of bone-CT scan and PET-CT is showing extensive extranodal disease. PET-CT limits are due to extranodal disease location in areas with physiologic F18-FDG uptake, associated infectious pathology and decreased avidity of some histology subtypes.
Conclussions: PET-CT scan is very useful, with use of standard diagnosis criteria, in extranodal involvement in NHL patients, mainly in aggressive forms. Extranodal suspected signs in some NHL types (including follicular, marginal zone, primary cutaneous anaplasic) must be assessed knowing the reduced avidity for 18 F-FDG for this histology subtypes.

THE TREATMENT WITH 5 AZACITIDINE IN MDS AND AML – PRACTICAL ASPECTS FROM THE CLINICAL EXPERIENCE OF COLENTINA CLINICAL HOSPITAL.

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The hypomethilating agents: azacitidine and decitabine have been approved by the FDA for use in patients with MDS. Although intensive therapy is preferred for patients with IPSS higher scores, hypomethilating agents are commonly used to treat patients with higher risk scores, which are not eligible for intensive therapy.
A meta-analysis of using hypomethilating agents for the treatment of MDS published in 2010, included data from 952 patients enrolled in clinical trials. Hypomethilating agents significantly improved overall survival (hazard ratio 0.72, 95% CI 0.60 to 0.85) and the combined endpoint of time to transformation to AML or death (hazard ratio 0.69, 95% CI 0.58 to 0.82). In a subgroup analysis of the drug, these benefits were observed for azacitidine, but not for decitabine. Azacitidine - azacytidine (5-azacytidine, 5-aza, Vidaza ™) is a pyrimidine nucleoside analogue of cytidine, which is believed to exert its beneficial effects / antineoplastic by causing DNA hypomethylation/ demethylation. This agent also has direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. Azacitidine was approved by the FDA for use in patients with the following forms of MDS: RA or RARS (if accompanied by neutropenia or thrombocytopenia or requiring transfusions), RAEB, RAEB-T, and CMML.
Materials and Methods: We present the evolution under treatment with hypomethilating agents in 15 patients, diagnosed with intermediate/high risk MDS and AML in our Department between 2009-2015. Cytogenetic examination was performed in all cases and an abnormal karyotype was obtained in 5 cases: a woman with LAM post MDS with del (5) (q32; qter), two patients with MDS and complex karyotype, which included the del (5) (q32; qter); a karyotype 45, XY, rob (13; 15) (q10; q10); a karyotype 46, XX, t (2; 6) (p16; q22.1), t (5.11) (p14; q23.1), del (13) (q10; 14.1) and one patient with MDS 12 monosomy. The selected schedules were: 5-Aza 75 mg/m2/d, for 7 days, repeted every 28 days
Results: The overall response to 5-aza was heterogeneous, with significant differences in the percentage of blasts, with a complete response in patients with monosomy 12.
Conclusions: The data indicate similar results to those in the literature. The hypomethilating agents are a less toxic alternative to classical cytotoxic/antimetabolites agents.
Aknowledgements: This presentation has been elaborated and written by Daniela Georgescu, MD and third year PhD student since 2011 at UMF Carol Davila under coordination of Prof. Dr. Anca Lupu, MD, PhD.
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GLOBAL BURDEN AND TREATMENT OUTCOMES OF INVASIVE PULMONARY ASPERGILLOSIS IN HEMATOLOGICAL PATIENTS – CASE SERIES.

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The specific term invasive pulmonary aspergillosis (IPA) is defined as invasion of the pulmonary parenchyma by the growing hyphae of Aspergillus Species, with evidence of vascular invasion by the fungus. IPA is seen primarily in patients with

haematological or solid organ malignancy and stem cell transplant recipients. We conducted an 18 months prospective survey on the IPA cases that were treated in the Hematology Department of Colentina Clinical Hospital. During the monitored period 5 patients were diagnosed with IPA and treated in our unit (4 with Voriconasole and 1 with Caspofungin). All of them were severely immnocompromised patients, with recent history of profound and prolonged neutropenic states. Pulmonary disease was present in all our patients, with evidence of disseminated infection in 40% of the cases. A high antifungal failure rate occurred and complete antifungal responses, with complete recovery of the patient, was noted in only one case. These results confirm that mortality from IPA in immunosuppressed patients remains high even if the newest, aggressive and sustained antifungal therapies were promptly initiated. We registered positive outcomes in less immuno-suppressed patients who did not required new chemotherapy courses after the IPA episode. The registered high fatality rates, which are in concordance with the recent international published data, highlight the importance of introducing new diagnostic tools, adjusted approaches and new antifungal therapies for the IPA in patients at risk.

THE MANAGEMENT OF ACUTE LEUKEMIA – THE EXPERIENCE OF COLENTINA HEMATOLOGY DEPARTMENT.

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Introduction: Patients with acute leukemia constitutes a major proportion of the cases hospitalized in the Hematology Department of Colentina Clinical Hospital. These patients are admitted in emergency either from internal medicine departments of Colentina Hospital, from emergency units of hospitals in Bucharest or from territorial hematology departments.
Materials and methods: It will be analyzed the group of patients with acute leukemia hospitalized in the Colentina Hematology Department in the last 2 years. We present demographics, distribution by type of acute leukemia considering the 2008 WHO classification of myeloid neoplasms and acute leukemia, types of therapy and response to therapy, prognostic factors, evolution, mortality data that will be compared with those in the literature.
There will also be presented particular cases as concerning presentation, complications, response to treatment, such as a case of acute lymphoblastic leukemia with acute liver failure at onset, a case of acute myeloid leukemia secondary to myelodysplastic syndrome with persistent partial response after 3 cycles of 5-azacytidine, a case of acute myelo-monocytic leukemia complicated by severe hypokalemia in post-chemotherapy aplasia phase.
Regarding the causes of death, it is found almost exclusively the role of sepsis, whose incidence and severity increases with the duration of neutropenia, the number of previous infectious episodes and prolonged broad spectrum antibiotics use. It can be found cases of sepsis evolving rapidly with death within hours despite preventive measures, including isolation, hygienic-dietary measures and use of antibiotics, antifungals and antivirals in prophylaxis scheme, quick delivery of empirical broad spectrum antibiotics and intensive care measures.
Conclusions: It requires better management of infectious complications, which are the leading cause of death in patients hospitalized with acute leukemia in Colentina Hematology Department. The main measures identified are increasing the level of training of medical staff involved in care of patients with severe neutropenia, judicious use of broad spectrum antibiotics, development of national protocols for treatment of febrile neutropenia in patients with acute leukemia, multidisciplinary hematology - infectious disease - intensive care management of septic patient with acute leukemia.

NEW TREATMENT OPTIONS FOR PATIENTS WITH MULTIPLE MYELOMA.

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Introduction: Multiple myeloma is a malignant haematological malignancy with poor prognosis. Over the past 15 years there has been a significant change in treatment strategy that led to the extension of the average survival rate up to double. In the era of so-called "New" drugs are the treatment options improved significantly and at the same time increased comfort care for patients (convenient outpatient regimen, fewer side effects on the gastrointestinal tract etc.). The basic "new" drugs include thalidomide, bortezomib and lenalidomide. Each of these drugs has its unique place in the treatment of patients with multiple myeloma, on the other hand, each of these drugs has its own specific side effects. The effort planned presentation is a summary of the general strategy of treatment, compared to 3 essential medicines of a new era, including the mechanism of their effects and their side effects. The last part is planned introduction of new treatment options

such as monoclonal antibodies etc.
Summary: General strategy for the treatment of patients with multiple myeloma, evaluating existing treatment with thalidomide, lenalidomide and bortezomib, compared to their adverse effects and an outline of possible future therapy (monoclonal antibodies ...)

ASSOCIATION OF MULTIPLE MYELOMA WITH LIGHT CHAIN AMYLOIDOSIS - VISCERAL DAMAGES, TREATMENT AND IMPACT ON SURVIVAL: A SINGLE CENTER EXPERIENCE.

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Amyloidosis light chain type (AL) is a disease through protein conformation disorder characterized by tissue accumulation of free light chains (or fragments thereof) that form amyloid fibrils. This disease can occur independent (primary amyloidosis) or in the context of another monoclonal gammopathies as multiple myeloma (MM), Waldenstrom's disease, non-Hodgkin's with secretion of monoclonal protein. In these conditions, the diagnosis will be of light chain amyloidosis associated with one of the diseases listed above.

This retrospective study shows the impact of association MM with AL regarding the clinical evolution with the type of visceral involvement, response to treatment and survival impact.
Material and Methods: 266 patients with MM admitted to our clinic between 2005 and 2014 were tested for AL; specific analysis for diagnosis and monitoring of multiple myeloma . Specific analysis of amyloidosis: Congo - Red examination in polarized light on samples from abdominal fat biopsy, renal biopsy, liver biopsy, rectal biopsy, lymph node biopsy, bone marrow biopsy; immunohistochemistry; electron microscopy; free light chain assay; proteinuria / 24 hours; EMG; Echocardiography; EKG; fibroscan; cholestasis tests;
Results and discussion: AL was identified in 44 patients (16.54%) from 266 patients with multiple myeloma. In this group of 44 patients with AL and MM: 66% had onset signs of of multiple myeloma related, but 34% patients had onset signs of amyloidosis (fatigue, weight loss, edema, peripheral neuropathy). 28 patients (63.63%) had at least 2 organ involved. 50% of patients had renal impairment manifested by chronic kidney disease and / or nephrotic syndrome. 16 (36.36%) patients had proteinuria; of these 14 (31.81%) nephrotic rank. Hepatic involvement was present in 11 patients (25%) being expressed clinically by hepatomegaly 7 (15.90%) patients; the remaining 4 patients experienced cholestasis, for these fibroscan examination was performed which revealed a liver infiltrated. 7 patients (15.9%) showed diastolic dysfunction delayed relaxation type; of these 6 patients had and restrictive cardiomyopathy. Regarding nervous system, 14 patients (31.81%) had clinically symptomatic peripheral neuropathy. Carpal tunnel syndrome was diagnosed in only 3 patients (6.81%). Autonomic neuropathy occurred in 34.18% of patients: 9 patients (20.45%) have experienced arterial hypotension, 5 patients (11.46%) disorders of bowel and 1 patient with urinary retention. Macroglossia was only found in 3 patients (6.81%).
Regarding the treatment of patients with MM and AL, it must be adjusted for each patient depending on the type of visceral lesion related by amyloidosis. Maximum attention should be given cortisone, bortezomib , and oral melphalan (not absorbed). Also very important is supportive therapy for organs injured by amyloid . Autologous bone marrow transplant is not contraindicated in patients with MM and AL, but it must be done more accurate assessment of heart function.
The impact on survival in association with amyloidosis, as a risk factor in patients with multiple myeloma, is dramatic. Mortality in these patients was 61.6% (22 patients died) and median survival period was reduced from 77 months in the control group (patients only with MM without AL), to 44 months in patients with MM and AL. Severity of the prognostic is based on the number of organs involved: in patients with more than 3 organ damage - median survival was reduced by 4 times, just 17 months compared with controls and mortality reached almost 70% of patients. For patients with MM and AL with cardiac involvement, survival dropped dramatically just 10 months, and 85% of them died.
Conclusions: The association of AL amyloidosis in patients with multiple myeloma is a real complication (16% of our patients with MM), much underdiagnosed in daily medical practice, with a major impact in the evolution and survival of multiple myeloma patients. The appropriate adaptations of treatment for each patient improves survival in these patients.
This work was supported by the grant CEEX 74/2006 from the Romanian Ministry of Research and Technology.

SECONDARY ACUTE MYELOBLASTIC LEUKEMIA – THE EXPERIENCE OF COLENTINA HEMATOLOGY DEPARTMENT.

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Introduction. Secondary AML (s-AML) encompasses AML evolving from myelodysplasia (AML-MDS) and treatment-related AML (t-AML) after exposure to chemotherapy, radiation, or environmental toxins. S-AML has traditionally been considered a devastating disease, affecting a vulnerable population of heavily pretreated, older adults. A limited understanding of disease pathogenesis/heterogeneity and lack of effective treatments have hampered overall improvements in patient outcomes. With the recent understanding that the secondary nature of sAML does not by itself incur a poor prognosis and incorporation of cytogenetics and molecular genetics into patient care and the advancement of treatment, including improved supportive care, novel chemotherapeutics agents, and nonmyeloablative conditioning regimens as part of allogeneic hematopoietic cell transplantation (HCT), modest gains in survival and quality of life are beginning to be seen among patients with s-AML. The development of t-AML has been reported following treatment of cancers ranging from hematological malignancies to solid tumors.
In this presentation we would like to present the experience of our Hematology department with diagnosing and treating secondary AML, an emerging disease, observed by our physicians, considering the increasing number of such patients who were admitted in our Department in the last year.
Materials and methods. We included in our retrospective study all the patients admitted into the Hematology Department of Colentina Clinical Hospital from January 2014 until July 2015 with the diagnosis of AML. From these patients we selected 2 groups: one comprised of de novo AML and one comprised of s-AML. For all patients we analised the relationship between hematologic parameters at diagnosis and response to treatment, the relationship between the type of AML (de novo or secondary) and the response to the treatment, the treatment used for AML in relation to the prior treatment for the first neoplasia, the outcome of the disease in relationship to the treatment used. Also, we analised the epidemiological aspect of these 2 groups. We compared the results of the 2 groups in order to see the particularities of each group.
Results. The results show that this entity, the s-AML, is an emerging disease, considering the increasing number of patients diagnosed with solid tumors which undergo intensive treatment (chemotherapy) with the purpose of curing them. The vast majority of the patients have been previously treated for breast cancer, althought one patient developed s-AML after the treatment for osteosarcoma at an early age (19 years old). Depending on the prognostic factors present at the onset we established the indication for allogeneic stem cell transplant. Furthermore, the chemotherapy regimens were chosen based on the prior treatment which was received for the first neoplasia (e.g. total cumulative dose of the anthracicline used), age, comorbidities, cardiac function, prognostic factors.
Conclusion. The management of patients with s-AML represents a challenge for the hematology specialist. The diagnostic procedure is very important, the molecular and cytogenetic exams being mandatory in order to establish from the onset the correct therapy regimen. The decision for treatment is difficult, and the indication for allogeneic stem cell transplant should be stated at the onset of the AML.

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CLINICAL HAEMATOLOGY SECTION
POSTERS

THE RESULT OF MOLECULAR ANALYSIS TO DETECT BCR-ABL HYBRID GENE IN LINGUAL EPITHELIUM CELLS IN SUBJECTS WITH PH CHROMOSOME POSITIVE / BCR-ABL POSITIVE CML.

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Background: The clinical and biological remarks on subjects with CML Ph + / BCR-ABL positive suggests that the intensity of hybrid bcr-abl gene expression in erythroblasts and megakarioblasts and its extension in other cells of hematopoietic and non hematopoietic systems like: lymphocytes, macrophage, fibroblasts, cells of vascular endothelium, epithelial cell have, on one hand, prognostic implications and on yhe other allow a deeper understanding of the disease
Aims: It is known even since 1961 the absence of Philadelphia chromosome in non hematopoietic cells like research cultured epidermal cells taken from subjects with Ph + CML (Tough IM , Court-Brown WM, et al - Lancet 1: 411, 1961),later Jacqueline Whang, Emil Frei et al (Blood, 1963,22,664), and the presence of Philadelphia chromosome in leukemia cells only, like myeloblasts , erythroblasts and megakariocites ; Researches are continued in the same direction by Martin PJ et al (Nature 287: 49.1980), HP Koeffler et al (Blood 55: 1063.1980), Segneurin D. et al (ExpHematol 15:822.1986) too. We consider it appropriate and useful to continue deepening in researching this theme so we tried to prove the presence/ absence of BCR-ABL hybrid gene in oral epithelial cells
Methods: In one male patient aged 31 years old, admitted in our Hematology Clinic on 11th of February 2015, with important splenomegaly (spleen diameters 32/16/14 cm) and a leukocyte number of 611000/mmc, peripheral blood samples were taken with explicit patient consent. In parallel, after prior mouth toilet with sterile saline water, epithelium cells from his mouth were taken with the help of the BRUSH CERVEX toothbrush. We have preserved this cells in saline sterile containers and containers SURE PATH (BD DIAGNOSTICS Tripathi); Samples were processed using equipment PCR- Real Time System, Applied Biosystems One-Step RNA isolation, reverse transcription stranded after clasic methods. DNA single-stranded was amplified by real-time PCR with specific primers of the p210 BCR-ABL p210 transcript and abl gene as control gene (after Gabert et al, Leukemia 2003);
Results: The analysis was performed in duplicate; In peripheral blood result was positive: Ratio major BCR-ABL / ABL: 100% IS; Due to the lack of genetic material extracted from oral epithelial cells (control abl gene was <10,000 copies) the BCRABL hybrid gene expression was negative in this cells
Summary/Conclusion: Due to the lack of genetic material extracted from oral epithelial cells (control abl gene was <10,000 copies) the BCR-ABL hybrid gene expression was negative in this cells. We will continue research on a larger number of cases with improved harvesting and extraction techniques and we will present the results to a later session. În parallel, we will extend this research, namely the magnitude of the BCR-ABL hybrid gene expression în individualized elements like erythroid, megakaryocytic and myeloid cells.
Keywords: BCR-ABL hybrid gene in nonhemato-poietics cells

EVALUATION OF THE SIGNIFICANCE OF TNF (TUMOUR NECROSIS FACTOR) ALFA VARIATION IN SUBJECTS WITH ESSENTIAL THROMBOCYTHAEMIA (ET) RECEIVING ANAGRELID/TROMBOREDUCTIN TREATMENT. PRELIMINARY DATA / PILOT STUDY.

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Background: We postulate that PDGF (Plateled Derived Growth Factor) could be the link between ET and mielofibrosys that could complicate the evolution of this disease.
Materials and methods: Starting from the observation that PDGF and TNF alfa have a similar influence on fibroblast proliferation rate and activity we have analysed TNF alfa concentration through out the evolution of ET under treatment with ANAGRELID /TROMBOREDUCTIN.
Seven patients newly diagnosticated with ET have been evaluated; the monitored parameters were: the thrombocyte number, ESR, CRP, fibrinogen level,

LDH, DD, iron level in parallel with the evaluation of serum TNF alfa concentration. Inclusion criteria for the evaluation of TNF alfa concentration have been normal levels of CRP, fibrinogen, DD, normal iron level and the absence of any recent infection. The level of TNF alfa has been determined at the moment when the diagnostic was made, 30 days after the initiation of therapy, 90 days after the initiation of therapy and later, at six months.

Results: All subjects had high levels of TNF alfa at the moment of diagnostic, with a mean value of 16.1 pg/ml (cut off<8.1 pg/ml). Evaluation at 30 days showed a significant raise in TNF alfa values, with a mean value of 41.5 pg/ml. At 90 days a regression of TNF levels was noted to a mean value of 13.4 pg/ml. There was no correlation between thrombocytes count, LDH values and TNF alfa variation.

Conclusions: The analysed data indicate that TNF alfa is a potential factor that could mediate mielofibrosis. We could not find any correlation between TNF alfa value and the thrombocytes count. In order to establish long term effects of therapy on the TNF alfa levels and secondary mielofibrosis, we need to extend the batch and the follow-up period.

RARE ASSOCIATION BETWEEN HEMOPHAGOCYTIC HISTIOCYTOSIS AND A LYMPHOPROLIFERATIVE SYNDROME.

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Hemophagocytic lymphohistiocytosis is a syndrome of pathologic immune activation characterized by clinical signs and symptoms of extreme inflammation. It is an aggressive, life threatening disease that frequently affects children and young adults, but can occur at any age. It is known in two forms: primitive, which is due to a genetic defect and secondary, which appears as a pathological manifestation that occurs secondary to other diseases (viral infection, autoimmune disease, malignant proliferation - T lymphomas, acute leukemias). The pathogenetic mechanism consists of an uncontrolled proliferation of macrophages, explained by the decreased functionality of NK lymphocytes and cytotoxic T lymphocytes (CD8 +) caused in their turn by mutations in the gene encoding perforin synthesis.

This paper presents the case of a 79 years old female who submitted to the hospital with febrile syndrome occurred 6 weeks prior to the submission. The following clinical and biological investigations excluded a possible infectious process, raising the suspicion of autoimmune hepatitis, and she was directed to an infectious disease clinic, where they were all removed: meningitis, endocarditis, a viral infection, autoimmune hepatitis (all this time the patient was febrile under extended spectrum antibiotics with negative urine cultures and hemocultures). A positive Quantiferon test indicated a possible tuberculosis, suspicion that was ruled out in a pneumology hospital.

Meanwhile we noticed a decrease in haematological constants (that were near normal initially) to the occurrence of cytopenias. In the haematology clinic, following the lack of response to broad-spectrum antibiotics, and the thorough investigations conducted, which excluded other pathology, the suspicion of hemophagocytic histiocytosis occurred, later confirmed by the presence of 5 criteria out of 8, sufficient for the diagnosis according to guidelines HLH 2004. We initiated specialized treatment, resulting in remission of febrile syndrome, improvement of general health and haematological constants. Thereafter, osteomedulara biopsy result was suggestive for follicular lymphoma.

In conclusion, prompt initiation of chemotherapy is essential for survival, but an early diagnosis was a challenge because of the rarity of the disease, variable presentation and the time required to perform diagnostic testing.

PAROXISTIC NOCTURNAL HEMOGLOBINURIA MANAGEMENT AND EVOLUTION IN AN ELDERLY PATIENT.

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Background: Paroxistic nocturnal hemoglobinuria (PNH) is a clonal acquired stem cell disease, characterized by intravascular complement-mediated lysis of red blood cell, white blood cell and platelet. PNH is a rare disorder that can affect any age, but most frequently occurring in early adulthood, with a median age at the time of diagnosis of 32 years.

Materials and method: We present the case of an 81 years old patient admitted in the Hematology Clinic of Craiova in October 2014 accusing fatigue, dizziness and moderate dyspnea. The medical history, physical examination and laboratory investigations revealed an underweight patient, with dry and pale skin and mucous, dark colored urine (hemoglobinuria), hepatomegaly with the lower edge of the liver at 3 centimeters below the lower edge of the ribs, grade I splenomegaly,

anemia (Hb- 9,6 g/dl), thrombocytopenia (42000/mmc), TBIL- 3.96 mg/dl, DBIL- 0.41 mg/dl, LDH- 586 mg/dl, negative indirect Coombs test and positive HAM test. Peripheral blood smear detected poikilocytosis (dacryocyte, codocyte, schistocyte), platelet- 50.000/mmc. Bone marrow examination shows: erythrocytic series 75% with binucleated macroeritroblasts, erythroblasts with intracytoplasmic bridges. Bone marrow biopsy revealed hypercellular bone with erythroblastic hyperplasia, many erythroblastic groups and dyserythropoiesis. Thorax and abdomen computed tomography showed hepatomegaly (left liver lobe= 7.3 cm, right liver lobe= 15 cm) and splenomegaly (12.78 cm).

Results: Based on clinical and laboratory investigations we have established the diagnosis of paroxistic nocturnal hemoglobinuria. Consecutively the treatment consisted of supportive measures and corticotherapy (Dexamethasone 16mg/ day iv divided q 12hr for 4 days) every 21 days. During follow-up the patient showed multiple remissions and acute episodes, and it developed the following complications: candida esophagitis, insulin-dependent type II diabetes mellitus, left basal pleurisy and acute bacterial pneumonia.

Conclusions: The particularity of our case resides in its advanced age and co-morbidities, thus the treatment posing numerous problems. Another particularity lies in the aggressive course of this disease, with the patient presenting multiple relapses in a limited timeframe.

MANAGEMENT OF A REFRACTORY CASE OF IMMUNE THROMBOCYTOPENIC PURPURA.

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Introduction. The immune thrombocytopenic purpura (ITP) is defined as a secondary pathological condition of a peripheral thrombocyte hyperdestruction occurring through an immunologic mechanism exceeding the ability of normal compensatory thrombocyte formation and in a clinical translation through a purpura syndrome and at hematology level, through thrombocytopenia with megakariocytosis.

Clinic case. We submit here the case of a patient of 52 years old with the diagnosis of ITP identified in January 2012 and having Morb Pott antecedents since 1993 ; she underwent surgery and treated with tuberculostatics. At the time of the diagnosis determination, the patient showed epistaxis , petechiae ecchymosis at the level of the lower and upper limbs, headache, acute thrombocytopenia and mild microcytic hypochrome anemia. The patient underwent investigations to exclude a secondary thrombocytopenia. There have been done several investigations detecting an infection with Helicobacter Pylori and positive HBs antigen and for these reasons she followed a medical treatment. Initially, she followed pulse therapy with Solu-Medrol, but no therapeutic response was obtained. A new line of corticosteroid therapy -Dexamethasone was tested, but no response either. As the corticosteroid therapy provided no response, there was started the treatment with Revolade obtaining a favorable response, on short term. The patient temporarily responded at the increase of the Revolade dosage associated to Dexamethasone. In July 2012, she came back because of the disease relapse and she was administrated immunoglobulins, followed by splenectomy with good response, but, 3 weeks since the surgery the severe thrombocytopenia was present again. Between September 2012 and July 2013 she was periodically hospitalized for substitutive treatment, showing severe persistent thrombocytopenia. The treatment with Vinblastin was tested but no response was obtained. In October 2013 there occurred a hemorrhagiparous syndrome predominantly at the lower limb level accompanied by hemorrhagic bullae at the level of the oral mucous membrane and the treatment with Mabthera was started, but no therapeutic response was obtained. Between January and November 2014 she was periodically hospitalized for substitutive treatment, and in December 2014 the treatment with Romiplostim with weekly dosages started and no adverse effects were noticed. After one month of treatment the dosage increase was required, and the level of thrombocytes was maintained within the normal range.

Conclusions. The evolution is characterized by a refractory feature, the lack of response at the corticosteroid therapy, rapid relapses after therapy and splenectomy and requiring multiple therapeutic lines, but with favorable sustained response to treatment with Romiplostim.

THERAPEUTIC RESULTS IN AGGRESSIVE NON-HODGKIN LYMPHOMAS PATHOLOGY IN THE ANTI CD20+ MONOCLONAL ANTIBODY ERA.

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INTRODUCTION
The Non- Hodgkin malign lymphoma (NHML) represents a monoclonal malign cellular proliferation which starts from the lymphoid tissue and impacts the B and T lymphocytes. There is a broad range of Non-Hodgkin lymphoma (NHL) subtypes with high malignity , the most frequently encountered being the diffuse large B-cell.
PURPOSE
The study proposes to evaluate the response to the CHOP (Ciclofosfamida, Doxorubicina, Vincristin, Prednison) regimen in association with a monoclonal antibody (Rituximab) in case of patients diagnosed with aggressive NHL.
METHODS
Between January 2010 and February 2015 I conducted a retrospective analytical study on 80 patients diagnosed with high-grade NHL in the Hematology Department of Timisoara. The diagnosis was established based on the full blood count corroborated with the biochemical tests (LDH), VSH, marrow aspirate, histopathological and *immunohistochemical tests*, cytogenetic tests and radiology investigations (Rx, CT).
RESULTS
Of the total 80 patients, 56,25% are younger than 60 years old, 43,75% are older than 60 years old including 46,25% women and 53,75% men.
The patients were staged based in the Ann Arbor criteria as follows: 12,5% were in stage I, 25% in stage II, 21,25% in stage III and 41,25% in stage IV.
The calculation of the IPI score reveals a reduced risk for 37,5% of the patients while 62,5% show a higher risk.
Of the 80 patients, 50% followed a treatment in accordance with the CHOP regimen and for 50% of them it was associated with Rituximab.
For the patients who undertook chemotherapy in accordance with the CHOP regimen , 35% were in complete remission, 30% were in partial remission and for 35% of them the lack of response to the treatment or relapse were detected. Of the 40 patients who undertook chemotherapy in association with monoclonal antibody in accordance with the R-CHOP scheme, 60% presented a complete response, , 30% partial response and 10% relapsed or showed no response to the treatment.
CONCLUSION
The treatment in accordance with the CHOP regimen represents the therapeutic standard for high malignity NHL with B lymphocyte expression on both young and old patients , but the addition of Rituximab resulted in a major benefit obtaining a significantly higher complete response rate.

COMPLICATIONS OF CHEMOTHERAPY WITH VELCADE AND DEXAMETHASONE IN PATIENTS DIAGNOSED WITH MULTIPLE MYELOMA.

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Introduction
The multiple myeloma (MM) is a disease caused by the malign proliferation of the plasmocyte cells and is characterized by bone lesions , disturbance of the immunoglobulins (Ig), medullar failure and renal failure.
Purpose
The study proposes the evaluation of the complications incurred by chemotherapy with Velcade in association with Dexamethasone at the patients diagnosed with MM.
Methods
Between January 2009 and December 2014 I conducted a retrospective analytical study on 90 patients registered at the Hematology Department of Timisoara. The MM diagnosis was established by revealing the presence of the monoclonal Ig in the serum , excretion of light chains in the urine (kappa and lambda) over 10% medullar plasmocytes ,lythic bone lesions. All patients were subject to complete clinical examination, full blood count , biochemical tests, radiography (skeleton). CT or RMN investigations were performed based on the clinician recommendation.
Results
The average age of the patients was 60 years and the group consisted of 59% men and 41% women. Of the 90 patients, 50% showed IgG, 30% IgA, 2% IgD, 1% IgM and 17% free chains. B2-microglobulina indicated values less than 3,5 mg/L for 10.5% of the patients , between 3,5 and 5,5 mg/L for 35,5% of the patients and over 5,5 mg/L for 54% of the patients.
The staging was based on the Salmon – Durie criteria as follows: 63% of the patients are included in the stage III ,27,5% in stage II and 9,5% in stage I.
The analysis of the complications developed secondary to the chemotherapy with Velcade in association with Dexamethasone reveals that 53,5% of the patients present cytopenia, 39,1% present gastro-intestinal disturbances, 27,05% neurological disturbances, , 5,6% metabolism and nutrition disturbance and 3,23% present cutaneous lesions.
Conclusions

This study reveals that the most frequent complications encountered at patients with MM diagnosis and who were subject to chemotherapy with Velcade in association with Dexamethasone are cytopenia , gastro-intestinal and neurological disturbances.

BCL-2 EXPRESSION AS A FACTOR OF NEGATIVE PROGNOSIS IN HODGKIN LYMPHOMA.

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Introduction

Although significant progress has been made in the field of BH treatment, in many cases the prognosis related to this disease is unfavorable. This is the reason why a series of factors may positively influence the evolution and prognosis of the disease, considering that their detection in an earlier stage since the start up itself of the disease allow the application of a suitable therapy.The molecular immunology studies revealed that for classic Hodgkin lymphoma the HRS cells (Hodgkin Reed Sternberg) derive from the germinal center of the B cells including the immunoglobulins gene rearrangement, but without the surface expression of the B cell receiver. BCL-2 was identified as the first gene involved in the HRS cell apoptosis. The BCL-2 expression by HRS cells can prevent the apoptosis caused by the absence of functional B cell receivers which explain the tumorigenesis. Also the BCL-2 expression can explain the resistance to the treatment inducing the HRS cell apoptosis.

Purpose

The study proposes the determination of the BCL-2 expression at patients suffering of Hodgkin lymphoma , the correlation between its expression and the biochemical and immunology modifications as well as the evaluation of survival.

Methods

Between April 2008 and April 2013, I conducted a retrospective analytical study on 151 patients diagnosed with Hodgkin lymphoma at the Hematology Department Timisoara . The main method to establish the diagnosis was the biopsy followed by the hystopathological and immunoshytochemical investigation of the sampled tissue.

Results

The average age of the patients included in the study was 49.69±17.46 with a minimum age of 18 years and a maximum age of 89 years , of which 37.7% were women and 62.3% men. The monitoring period since the diagnosis determination was 13.92±6.24 months. For 33.7% of the patients the remission was complete , while for 45.7%, of them the remission was partial and the 17.2% indicated a progressive disease , 0.6% relapsed and 2.5% died.

BCL-2 was identified at 82 patients(54,3%), the intensity being different and depending on the histological level of the disease as follows: BCL-2 expression has been found at 19,8% of those with mixed cellularity, at 39,1% of the patients diagnosed with nodular sclerosis and at 5.3% of those with lymphocyte depletion.

The survival duration of the patients with BCL-2 expression is shorter that of those who do not express the gene, as its expression, from statistic point of view, is significantly positively correlated with the increased values of other immunological markets:CD15, CD20, CD30 and biochemical ones: fibrinogen.

Conclusion

Further to the study completion, it was noticed a lower survival rate for the patients with BCL-2 expression associated to the intense expression of the CD15, CD20, CD30 markers as well as with the increased values of the fibrinogen. The VSH, LDH biochemical markers and the ceruloplasmin are inversely corelated with BCL 2 presence , and the survival rate does not modify significantly when such values are higher than the normal ones.

THE RESULTS OF TREATMENT WITH IMATINIB MESYLATE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA. RETROSCPECTIVE STUDY.

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Background. Imatinib Mesylate, a Tyrosine Kinase inhibitor, is one of the drug of choice for chronic myeloid leukemia (CML) in chronic phase (CP). During therapy, a few patients develop myelosuppression. Adverse side-effects of the drug could be: edema, nausea, vomiting, diarrhea, cramps and cutaneous reactions. Adverse hematologic side-effects could include anemia, neutropenia, and thrombocytopenia.

Aims. The aim of this study was to evaluate the results of treatment and the safety of imatinib as first line therapy in patients with newly diagnosed CML-CP.

Methods. Between January 2008 and January 2015, 92 patients with Ph+CML-CP were included in the study. They were diagnosed in Department of Hematology, County Hospital Timisoara, Romania. Eligibility criteria included age 18 years and older, ECOG performance status of 0 to 2, adequate hepatic and renal functions, no prior imatinib therapy, non-pregnant patients. CML-CP was defined as less than 10% blasts and less than 20% basophils in the peripheral blood and bone marrow and a platelet count between $100x10^9$/ L and < $1000x10^9$/L. Therapy was initiated with imatinib 400 mg orally daily and patients were monitored for any adverse effects.

Results. Out of 92 cases with CML-CP included in the study, male: female ratio was 0.8:1.3 with median age 45 (ranged from 18-70). After starting Imatinib a CHR was achieved at 3 month by 89.5% patients. The CyR achieved was major in 65%(with 58% CCR) no CyR in 18 patients. The molecular response was complete in 29% and major in 32% patients. The doses were increased in 23 patients and improved response was achived in 14 patients. Six patients were switched to Dasatinib and for to Nilotinib. The median follow-up was 62 month(range 22-80) and under Imatinib was 49 months. The twelfth patients died, seven of blastic transformation. The study showed that the commonest hematological side effects were grade 2 anemia (13%), followed by leucopenia 11%, and thrombocytopenia 7%. The most common non-hematological adverse effects were superficial edema and weight gain 30%, followed by musculoskeletal pain 29%, then gastro-intestinal (vomiting, diarrhea) 9%.

Conclusions. Data indicated that imatinib mesylate remains effective for the most of the patients is a well tolerated drug, and all adverse effects could be managed for patients with CML-CP. The most common hematological side effect was anemia, while the non-hematological side effect was fluid retention.

CHRONIC IMMUNE THROMBOCYTOPENIA-RETROSPECTIVE STUDY ABOUT METHODS AND RESULTS OF TREATMENT.

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Background. The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely.

Aims. To evaluate the treatment and modality of treatment of ITP patients in Departament of Hematology, County Hospital, Timisoara during 15 years (I 2000-XII 2014).

Methods. A retrospective study for 330 ITP patients was performed. Patients demographics, medical history, current treatments and side effects , were abstracted from the patient's medical charts for the 12 months prior to their most recent visit.

Results. The mean age was 47 years, with 61% women and 39% men. Median time from the diagnosis of ITP to the start of the observational period was 22 months. Prior to the observational period, 32% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 76% of all patients were treated. The most frequent reasons given for treatment were platelet count (72%), followed by bleeding symptoms (55%). Corticosteroids represented 60% of treatments, followed by IVIg (20%), azathioprine (10%), rituximab (5%) and 5% thrombopoiesis stimulating agents(TPO-receptor agonists). Splenectomies (32% of patients) and platelet transfusions (35% of patients) were performed during the observational period.Splenectomy went without complications.There was no intraoperative death.Postoperative complications were observed in 11% of patients.Postoperative hospitalization media was 5.7 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 costicosteroids therapy did not affect postoperative remission rate. For monitoring the platelet levels, 82% of patients visited their hematologist 1 to 10 times during the observation. Main reasons for a visit were a low platelet count (42% of visits) and bleeding (34% of visits). Overall, 42% of patients required hospitalization. Mean duration of hospitalization was 13,2 days.

Conclusions. The retrospective study of 330 patients provides therapeutic outcomes resulting from treatment methods from our department. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids represent the most used treatment from our department.
Splenectomy is a seif technique with satisfactory remission rate in patients with ITP that do not respond to medical treatment

HIPERFERRITINEMIA IN MYELODISPLASTIC SYNDROME(MSD) PATIENTS. CORELATION WITH EVOLUTION AND SURVIVAL.

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Background. Most myelodysplastic (MDS) patients have anemia and many of them require red blood cells (RBC) transfusions leading to iron overload. Hematological improvement during iron chelation therapy was first pointed out more than twenty years ago. This phenomenon seems to be more frequent after introduction of Deferasirox. The most simple test assessing iron overload is serum ferritin concentration .
Aims. Assessment of hyperferritinemia incidence in MDS patients at the moment of MDS diagnosis, and correlation between ferritin level and evolution an survival in patients diagnosed with MDS.
Methods. The retrospective data collection from a single center experience (Department of Hematology County Hospital, Timisoara, Romania) between January 2005 and December 2014 included 131 patients (73men and 58 women) with MDS. All the patients had complete blood count and serum ferritin level, and complete follow-up data.
Results. Ferritin level above 1000 ng/mL was found in 45 patients (31%) (Group 1) and ferritin level ≤1000 ng/mL in 86 patients (69%) (Group 2). Most patients with significant hiperferritinemia,
were RBC transfusion dependent (78% of patients). Among patients with ferritin level ≤1000 ng/mL, 36% were RBC transfusion dependent. Serum hemoglobin concentration was lower in Group 1 patients in comparison with Group 2 patients (7,3 g/dL vs 9,6 g/dL, p< 0,001). The most frequent MDS subtype in Group 1, were patients with refractory anemia (RA) (31%), compared with patients with ferritin ≤1000 ng/mL - 14% (p<0,04). According to IPSS score, there were no differences between studied groups. Median follow up was 14 months. There was an improved overall survival (OS) in RBC transfusion independent patients compared to RBC transfusion dependent patients, but mean OS was not significantly statistically different in studied groups. No correlation was found between ferritin level and time to acute myeloid leukemia(AML) transformation.
Conclusions. Hiperferritinemia >1000 ng/mL does not influence survival and time to AML transformation in MDS patients. The most frequent MDS subtype in patients with ferritin level >1000 ng/mL was MDS RA. Among patients with ferritin level >1000 ng/mL 81% were RBC dependent.

ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS. RETROSPECTIVE STUDY OF 92 CASES.

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Background. The management of old patients with acute myeloid leukemia remains controversial, specially in those cases affecting very old patients (aged >=70) in which the dilemma therapeutic abstention versus treatment (with low or high intensity schemes) is a major subject.
Aims. We present the experience in our centre with this group of patients in the period 1990-2011. **Methods.** During the period of study 92 cases were diagnosed (relapses, FAB M3 cases and patients initially treated with 5-AZA were excluded). Patients were divided into 3 groups according to the treatment: no treatment (supportive treatment), low intensity treatment (low doses Ara-C: 10 mg/m^2/12h s. c. days 1-21) and high intensity treatment (adapted ICE: Idarubicin 10 mg/m^2 days 1 and 3; Ara-C 100 mg/m^2/12h days 1-3; Etoposide 100 mg/m^2 days 1-3).
Results. The mean age of patients was 73.2 years (60-85); sex distribution was 50 males and 42 females; mean Karnofsky index was 70; 52 patients received treatment and 40 only did not; overall survival was 6,1 months (median 2.3) significant differences were observed in the mean overall survival between the treated and no-treated groups (8.2 vs 2,1 months respectively; p=0,016). In the low intensity group (27 patients) an overall response of 31% (5 CR, 6 PR, 9 NR and 7 not evaluable) was observed while in the high intensity one (25 patients) overall response was 52% (10 CR, 4 PR, 5 NR and 6 not evaluable); no statisticaldifferences were observed between both groups considering all subgroups of response (p=0,15).Considering overall survival, no statistical differences were observed between the low and high intensity groups 7,1 vs 13,4 months (p=0,21) respectively.
Conclusions. Overall survival in the treated group is higher than in the non-treated one, differences reached statistical significance. Comparing both arms of treatment no statistical differences were observed in the quality of response, though a higher proportion of complete responses were observed in the high intensity

group . No statistical differences have been observed in the overall survival between both groups of treatment.

THE IMPORTANCE OF CLOSE MOLECULAR MONITORING IN A CASE OF CHRONIC MYELOID LEUKEMIA WITH NEGATIVE EVOLUTION IN THE ERA OF TYROSIN KINASIS INHIBITORS.

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Introduction: Chronic Myeloid Leukemia (CML) is a proliferation of the polynuclear granulocytes associated with the apparition of the Philadelphia chromosome (Ph). From the clinical point of view CML is characterized by splenomegaly, paleness, headaches, sweating and recurrent infections. The treatment for CML are inhibitors of the tyrosin kinasis. The prognostic for CML is favorable because of the appearance of the tyrosin kinasis inhibitors
Case presentation: We present the case of a 60 years old patient, diagnosed in April 2008 with CML chronic phase Ph1+ which was given citoreductory treatment with Hydroxiurea until June 2008 when he starts treatment with Glivec. The patient presents complete cytogenetic remission at 6 months and major molecular remission at 1 year from beginning of treatment but in March 2010 he loses the Ph1+ cytogenetic response and presents the pathological clone +8 (14%). He starts treatment with Dasatinib with favorable evolution and complete cytogenetic and molecular remission at 6 months after the start of treatment. At the monitoring from June 2015 it is suspected a molecular relapse and is considered necessary cytogenetic monitoring in view of further treatment.
Conclusions: The treatment with tyrosin kinasis inhibitors is intended to obtain complete cytogenetic and molecular response. Our patient obtained this response only for short periods of time but did not maintain this response, therefore we recommend a close cytogenetic and molecular monitoring of patients with CML in view of optimal treatment.

MANAGEMENT AND TREATMENT OF HIGH GRADE NONHODGKİN LYMPHOMA - CLINICAL CASE –

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Introduction: Diffuse large B cell NonHodgkin lymphoma is the most common form of non-Hodgkin lymphoma, representing approximately 30% -40% of total lymphomas.
It is an aggressive lymphoma, fast growing if left untreated but with a high chemiosensibility which can cause long-term remission. Primary mediastinal large B-cell lymphoma is a unique clinico-pathological subtype. Typically affects young women who have a mediastinal mass with rapid growth causing respiratory symptoms often pairing with superior cava vein syndrome and / or deep vein thrombosis. Standard treatment is chemotherapy R-CHOP with or without local radiotherapy in case of residual tumor mass.
Case Presentation: We present the case of a 38 years old young female which presented 11 months ago asthenia, fatigue, dyspnea and dysphonia. Neck and chest contrast substance computerized tomography and ultrasound Doppler were performed showing a mediastinal solid mass of 5.5 / 4.5 cm, incomplete filling defect in the internal and external jugular vein, left subclavian vein without flow containing parietal thrombi. Histopathological and immunohistochemical exam of the mediastinal tumor mass were made resulting a B cell NonHodgkin lymphoma with mediastinal primary site. Blood count (within normal limits), 4 regions computerised tomography and bone marrow aspirate staged the patient IIA.
R-CHOP chemotherapy was decided and initiated and anticoagulation therapy for subclavian vein thrombosis was administrated. VIII chemotherapy applications were performs, well tolerated , with complete remission. (evaluated by computerised and positron emission tomography)
Conclusions: The patients required anticoagulant dose adjustments and had secondary neutropenia throughout the entire chemotherapy. Given the young age and high degree malignancy of the disease, the patient will be addressed to the Centre for Bone Marrow Transplantation for hematopoietic stem cell harvesting and will be imagistic evaluated to determine further course of treatment.

DIFFERENT THERAPEUTHIC METHODS OF A CASE OF CHRONIC MYEOLID LEUKEMIA WITH PROLONGUED EVOLUTION.

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Introduction. Chronic myeloid leukemia is a clonal disease of the stem pluripotent hematopoetic cell, characterised by the presence of the Philadelphia chromosome and/or the presence of the BCR/ABL rearrangement with a pathogenetic proven role.
Clinical case. We present the case of a 58 year old female patient diagnosed in August 1998 with Chronic Myeloid Leukemia, chronic phase, Philadelphia chromosome positive, initially trated with Hydroxiuree and afterwards with Interferon with a complet hematological response but without cytogenetic response. In February 2008 she starts treatment with Glivec 400mg/day with the achievement of a minor molecular remission, but in June 2009 the BloodCellCount suggests the passing of the disease in the accelerate phase and it is decided to increase the dose of Glivec at 600mg/day without achieving a complete cytogenetic response. Also the patient presents multiple side-effects of the treatment with Glivec: nausea, vomit, rectal bleeding, therefore in 2011 it is initiated treatment with Dasatinib 100mg/day, with the periodic adjustment of the dosys because the patient again presents multiple side-effects without a therapeuthic response. From November 2014 the treatment with Dasatinib is stopped and the patient started treatment with Tasigna 600mg/day, obtaining major cytogenetic remission and the decreasing in value of BCR/ABL for the first time at 3 months after the start of the treatment.
Conclusions. The patient with long term evolution, multiple therapeuthic lines, the last three being done with tyrosin kinasis inhibitors, with the presence of numerous side-effects at Dasatinib and Glivec and the accelerate phase of the disease at treatment with Glivec, but with favorable response in the end, after the start of the treatment with Tasigna and without any side-effects. It is important to periodically monitor a patient with Chronic Myeloid Leukemia molecular, but also by cytogenetic means, in order to be able to establish an optimal treatment.

CARDIOVASCULAR COMPLICATIONS SECONDARY TO CHEMOTHERAPY IN AGGRESSIVE NONHODGKIN LIMPHOMAS.

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INTRODUCTION
The non-Hodgkin lymphoma is a malignant cell proliferation. The starting point is affecting lymphoid tissue B cells and T In aggressive NHL, most often encountered chemotherapy, according to protocol, is multi-agent CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) in combination with monoclonal antibodies (rituximab).
PURPOSE
The purpose of the study is tracking secondary cardiovascular complications that arise polichimio-therapy type CHOP and R-CHOP respectively.
METHODS
We conducted a retrospective study on a group of 47 patients from Timisoara Hematology Clinic, with non-Hodgkin lymphoma highly malignitate, during 01.2010-02.2015. The diagnosis was based on clinical features, histopathological, immunohistochemical exams , bone marrow aspirate, cytogenetics and radiological investigations. (radiography, CT scan).
RESULTS
Of the 47 patients, age incidence is highest between 40-60 years 48.94%, followed by 31.91% between 60-80 years and 19.15% between 20-40% are women ani.57.45 , 42.55% men; 44.68% alive, 55.32% died. Of the 47 patients, cardiovascular complications that arise after treatment, 29.79% had hypertension, ischemic heart disease 19.15%, 6.38% heart failure, 4.25% thrombosis, arrhythmias type atrial fibrillation, right bundle branch block. and 2% valvular type mitral regurgitation and aortic
CONCLUSION
Cardiovascular complications that occurred after treatment according to the protocol CHOP and R-CHOP that are in the forefront hypertension, ischemic cardiomyopathy and heart failure.

EXTREME THROMBOCYTOSIS AND BASOPHILIA IN A CASE OF CHRONIC MYELOID LEUKEMIA.

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Chronic myeloid leukemia (CML) is a hematopoietic malignancy originating from transformation of a pluripotent haematopoietic cell.
CML was the first malignant disease found to be consistently associated with a specific cytogenetic abnormality, the Philadelphia chromosome, resulting in the formation of the BCR-ABL fusion oncogene.
The most common feature of CML is an elevated WBC count usually >25.00x10^3/µl and frequently >100,00x103/µl, the WBC differential usually shows

granulocytes in all stages of maturation from blasts to mature morphologically normal granulocytes.
The platelet count is elevated in 30 – 50% of patients and is higher than 1000,00x10^3/µl in a small percentage of patients with CML. Excessive thrombocytosis like that are seen in essential thrombocythemia (ET) are uncommon (described as Ph1 positive ET in the past).
Basophils are constantly elevated but only 10 – 15% of patients have ≥7% basophils in the peripheral blood. In contrast to mastocytosis hyperhistaminemia is uncommon
Elevated basophil count is a treasure of accelerated phase but excesiv basophilia is a very rare condition at diagnosis and is suggestive for basophilic leukemia. Extreme basophilia at presentation impose a differential diagnosis with rare chronic or acute basophilic leukemia.
We present the case of 30 years old female who was reffered to our department for anaemis syndrome. Clinical: pallor, no organomegalies. Blood count cell showed: anaemia, Hb= 10 g/dl, leucocytosis 66,00 x 103/µl, extreme thrombocytosis 3600,00 x 103/µl and on peripheral blood surprising an elevated procent of 66% of mature basophils, the lack of imature granulocytes. JAK2V617F mutation is not detected. BCR-ABL transcripts: major molecular response, cytogenetic analysis: 100% presence of Ph chromosome. With the diagnosis of MCL can start a treatment with Hydroxiuree, than dasatinib 100 mg/day. The result is a spectacular one, a normal blood count cell after one month.
Presentation with extreme thrombocytosis and basophilia in MCL is exceptional rare representing a diagnosis provocation: MCL, ET or basophilic leukemia.

JAK INHIBITOR THERAPY (RUXOLITINIB) IN IDIOPATHIC OSTEOMYELOFIBROSIS - NEW HOPE ? THE EXPERIENCE OF THE HEMATOLOGY CLINIC- IRO IASI

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Idiopathic osteomyelofibrosis (IMF) is a hematopoietic clonal stem cell disease characterized by: anemia, splenomegaly and systemic manifestations. The relationship between alteration of the JAK - STAT signal transduction and the signs and symptoms of IMF is clearly established. Classic therapies administered to date in patients with IMF had modest results. The appearance of JAK inhibitor therapy is considered a new therapeutic hope for patients with this condition.
The purpose of our study is to evaluate the efficacy and tolerance of JAK inhibitor therapy in patients diagnosed with primitive and secondary osteomyelofibrosis.
Material and Methods: We evaluated a group of 17 patients diagnosed with idiopathic osteomyelofibrosis who initiated treatment with Ruxolitinib in the period August 2014 - May 2015 in IRO Hematology Iasi. In all patients we analyzed clinical and biological parameters at 3 stages: in the moment of the diagnosis, at initiation of the therapy and after 3 months of treatment.
Results: The average age of our patients at the initiation of therapy with Ruxolitinib was 61years old, the majority being diagnosed with primitive osteoyelofibrosis (76%) and only 4 patients with secondary osteomyelofibrosis. Therapeutic response assessment was performed by measuring spleen size at diagnosis and after 3 months of treatment; in some patients, also when reaching 6 months of therapy. We found spleen decreasing with at least 2cm in 85% patients comparing to the initial evaluating dimensions. In 2 patients we observed progressive evolution of disease under treatment trough spleen increasing. In 70 % of patients was noted hemoglobin decreasing by at least 2 g / dl comparing to the initial value, and in 50 % patients increased transfusion requirements. In a number of 3 patients severe infectious events in context of GRD III -IV neutropenia WHO occurred, in 1 case being necessary to stop therapy. In 1 patient we noted the combination of renal failure and hepatic cytolysis, so it required the adjustment of the dose of Ruxolitinib. 4 patients (23%) presented thrombocytopenia GRD III WHO, which also demanded a dose adjustment.
Conclusions: Treatment with Ruxolitinib was effective in most patients diagnosed with osteomyelofibrosis from our group. There was noted decreasing spleen size, but also the presence of adverse effects that needed dose adjustment and closely monitoring treatment intolerance.

PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE ASSESSMENT BY FLOW CYTOMETRY AND MOLECULAR BIOLOGY IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA DIAGNOSED IN THE HEMATOLOGY CLINIC, IRO IASI.

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The evolution of patients with acute lymphoblastic leukemia and establishing a correct therapeutic approach increasingly depends on the ability to identify and track the minimal residual disease (BMR) outstanding both after induction chemotherapy and during consolidation. The methods of detection ranges/varies, from the identification of a leukemia associated phenotype by flow cytometry or of fusion genes by molecular biology techniques, to immunoglobulin or TCR gene rearrangement.
The purpose of this paper is to identify the role of minimal residual disease detection in making therapeutic decisions post-induction and in following patients during therapy.
Material and Methods: We evaluated a group of 35 patients diagnosed with acute lymphoblastic leukemia in 2012-2015. Most patients (85.7%) were diagnosed with ALL B and only 14.3% of T-ALL. Minimal residual disease was evaluated in all patients post-induction and every three months over the cures of consolidation.
Results: The evaluated prognostic factors at diagnosis showed that most patients were under 30000 / mmc WBC at diagnosis (63%), phenotypically there were included in the high risk class 17.1% of patients with ALL B and % with ALL T, a total of 22.9% of patients were classified as being in the increased risk class and 20% in very high risk class. The fusion genes evaluation at diagnosis identified their presence in 27% of the patients - 11.4% BCR-ABL p190 positive 8.6% MLL-AF4 positive. Post-induction rating minimal residual disease by flow cytometry showed its presence in 30% of patients. Also, in the assessment of minimal residual disease by molecular biology, the gene fusion identification was positive in 5 patients (14.3%). Based on these assessments and on the risk classes patients received the indication of an allogenic transplant at the first complete remission – a number of 3 patients in the high-risk class with persistent minimal residual disease post-induction. The patients of our study were evaluated during therapy and had a positive BMR (50%) which has directed therapy to reinduction chemotherapy followed by allogenic transplant at 5% of patients, who achieved a complete second remission.
Conclusions: Identification of minimal residual disease at any time during therapy through both flow cytometry and molecular biology allows orientation to the best therapeutic approach and is a factor with amajor prognostic impact in patients with ALL.

THE EXPERIENCE OF A SINGLE CENTER OF HEMATOLOGY IN THE TREATMENT WITH HYPOMETHILATING AGENTS.

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Introduction: The clinical studies demonstrated that treatment with hypomethilating agents (5-azacytidine and decitabine) in intermediate/high risk MDS resulted in complete cytogenetic responses even in cases with a complex karyotype. On the other hand, for patients with AML who do not qualify for aggressive chemotherapy and allogeneic medullary transplantation, treatment with hypomethilating agents leads to transfusional independence and increase in quality of life.
Materials and methods: We present the evolution under treatment with hypomethilating agents in 12 patients, diagnosed with intermediate/high risk MDS and AML in our Department between 2009-2015. There were 8 men and 4 women, with ages between 56-84 years, 7 of them diagnosed with intermediate/high risk MDS, and 5 diagnosed with AML, unfit to chemotherapy. 9 patients (6 patients with MDS and 3 patients with AML) received treatment with 5-azacytidine and 3 patients, one man with AML post MDS and 2 women with AML de novo, received Decitabine.Cytogenetic exam was performed in all cases and a abnormal karyotype was obtained in 2 cases, both with MDS, one patient with a complex karyotype, including del (5)(q32;qter) and one with 12 monosomy. The selected schedules were: 5-Aza 75 mg/m2/d, for 7 days, repeted every 28 days and Decitabine 20 mg/m2/d, for 5 days, repeated every 28 days.
Results: All patients had a good tolerance to therapy, without significant adverse events. The overall response to 5-Aza was heterogenous, with no significant differences regarding blast percentage, with one complete response in the case with 12 monosomy. Unfortunately in the 3 patients with AML treated with Decitabine, there was a delay in the time of treatment initiation due to administrative and financial isues, and they died due to disease progression. There were no side effects.
Conclusions: The presented data indicate similar results to that in the literature. The most important effect of treatment was on the quality of life by the reduction in the transfusional demand. The hypomethilating agents are a less toxic alternative to classical cytotoxic/antimetabolites agents.
Aknowledgements: This presentation has been elaborated and written by Daniela Georgescu, MD and

third year PhD student since 2011 at UMF Carol Davila under coordination of Prof. Dr. Anca Lupu, MD, PhD.
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STUDY ON MEMBRANE FLUIDITY IN CHRONIC MYELOID LEUKEMIA AND MYELOPROLIFERATIVE NEOPLASMS AND MEDICATION INFLUENCES.

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Background Patients with chronic myeloproliferative neoplasms (MPN) have a variety of structural and functional abnormalities of platelets The patients with JAK2-positive MPN have a higher incidence of venous thrombosis compared with noncarrier. Platelet function is influenced by changes in membrane fluidity (MF) which has an important role in the expression of platelet receptors and in modulating the activity of protein membrane. The aim of our study was to determine whether presence of JAK 2 mutation influences platelet MF and if changes of MF may be correlated with the treatment of these patients **Materials and Methods** We present a retrospective study on 36 cases MPN (20 JAK2-positive MPN) and 14 CML admitted in Colentina Clinical Hospital Bucharest. The determination of platelet membrane fluidity was performed by fluorescence anisotropy measurements using as marker 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA DPH). We analyzed the fluorescence anisotropy of platelet membrane and correlate the result of with different kind of treatment. **Results and Discussion** Patients with MPN and JAK2 mutation present have a high level of fluorescence anisotropy (equivalent to high fluidiy of platelet membrane) than the JAK-negative group. Median value for JAK2 positive MPN group 148.5 95% CI for median value (141-152.8)) vs JAK2 negative MPN group 132 (126.4-137.5) p = 0.0009. There are no differences between CML and MPN group. Our results confirm that fluorescence anisotropy is influenced by medication taken. We observe that MPN patient who have taken Hydroxiureea alone had a high level fluorescence anisotropy than patient who have taken association Hydroxiureea and Anagrelid ; median value and 95% CI for median value 151 (137.1-158.6) vs 136 (126-137.5) p=0.03. At the begining Anagrelid was used as inhibitor of platelet aggregation and after that it was observed its effect in decreased count of platelet. Its effect is inhibitor of phosphodiesterase, blocking megakaryopoiesis and represses proplatelet formation. In recent clinical studies it was not observed any differences in prevention of thrombotic complications MPN patients in treatment with Hydroxiurea and Anagrelid. Patient who have treatment with tyrosin kinase inhibitor (TKI) - Sprycel or Glivec, had a low level of fluorescence anisotropy, median value and 95% CI for Hydroxiureea group 151 (137.1-158.6) vs TKI group values 138 (124.4-147.8) p=0.04. No differences of fluorescence anisotropy was observed between group of MPN patients who received JAK inhibitor (Jakavi) and group MPN with Hydroxiureea treatment.or between TKI inhibitor group and Jakavi group. **Conclusion** Presence of JAK 2 mutation in MPN patient is associated with low fluidity of platelet membrane. In literature this group of MPN patient had frequently thrombotic complication. We have to observe in the future if this group with high level of fluorescence anisotropy had a high risk of thrombosis. Association of Anagrelid or TKI inhibitor is associated with lower level of fluorescence anisotropy value. Both medications Anagrelide and TKI inhibitors influence protein with high role in signaling transduction. It must observe if the modification of MF influences proplatelet formation and Anagrelid effect in decreased level of platelet count.

CHRONIC MYELOID LEUKEMIA – TREATMENT RESPONSE AND INVOLVED FACTORS.

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Chronic myeloid leukemia (CML) is probably the most studied malignancy and served as a pacemaker in developing new concepts and strategies in Oncology. More than 90 % of patients with CML Philadelphia-chromosome (Ph +) have in translocation of the Abl gene from chromosome 9 on chromosome 22 , t [(9; 22) (q34 ; q11)] . At the molecular level this translates into the emergence of a new hybrid gene , fusion (BCR-ABL) that encodes for an oncoprotein (p210 , rarely p190 or P230) , with tyrosine kinase activity
According to the latest European LeukemiaNet guidelines (ELN) published in the journal Blood journal (August 2013), the response to treatment with tyrosine kinase inhibitors (TKI) is the most important

prognostic factor in the disease.
Given the new recommendations we made a database with about 90 patients hospitalized in Hematology Clinical Hospital Colentina and CML- diagnosed with chronic, accelerated and blastic phase, in the last 5 years. Both retrospectively and prospectively, we have completed individual files in which we monitored several variables that may influence the response to treatment: clinical parameters and especially paraclinical (sex, age, comorbidities, splenomegaly, stage of disease onset, number of cells at the onset, prognostic scores (score EUTOS, Sokal), the initial dose of TKI, the cytogenetic and molecular exam every six months. Very important are the new elements emerged during TKI therapy (clonal cytogenetic abnormalities when changing therapy, mutations in the kinase domain of BCR -ABL1).
Depending on the results, newly diagnosed patients were included from the beginning in two groups: low risk and high risk of progression disease , aiming to correlate data with the literature .
Patients in the study group were hospitalized in Colentina and Coltea Clinical Hospitals, in the period 2009-2014 diagnosed with CML in all phases.So far I I have identified 88 patients from both hospitals
I have compiled a data sheet for every patient with the leading individual hematologic known prognostic factors in treatment response. I filled up 43 sheets with tracking parameters. Partial results have identified as favorable prognostic factors : female sex (61 % of those with CMR to 12 months), the early molecular response (64% of these patients had CMR to 2 years) , low risk-Sokal score: 86 % of these patients had CCR 6 months. The unfavorable factors: clonal cytogenetic abnormality- one case with trisomy 8 had lost RMM and one case of trisomy Ph that progressed to acute leukemia. The BCR-ABL domain mutations were important and significant: T315I -1 case- went to allotransplant early in the course of the disease, 1 case with V299L mutation - loss of MMR.
Regarding the response with second generation TKI administered after Imatinib: 2 of the 5 patients receiving Dasatinib achieved MMR or RMC , none of the 2 on Nilotinib.

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DIAGNOSTIC PROBLEMS OF THROMBOTIC TRROMBOCYTOPENIC PURPURA IN A PATIENT WITHOUTH THE CHARACTERISTIC CLINICAL PRESENTATION- Case report.

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Thrombotic Thrombocytopenic Purpura (TTP) is a disease characterized by a congenital or acquired deficiency of the protein von Willebrand factor cleavage called ADAMTS13, which is manifested by the ”pentad”: microangiopathic hemolytic anemia, thrombocytopenia, neurological manifestations, renal failure and fever.
Below we present a case that raised issues of differential diagnosis of TTP before measuring ADAMTS13 activity and dosing the anti-ADAMTS13 antibodies.
Our patient, female, aged 33 years old, without significant pathological personal history came in the hematological service accusing asthenia, fatigue, bruising to the legs and chest in the absence of trauma. On admission with easily influenced general state, skin and scleral jaundice. Paraclinically normochromic normocytic anemia (Hb = 7.5g / dL), marked reticulocytosis (281.9 ‰) with schistocytes on the the blood picture, severe thrombocytopenia (12,000 / mm^3). In the biochemical evidence LDH= 3047 U / L and significant mixed hyperbilirubinemia with predominantly indirect bilirubin.
In the absence of clinical features specific to TTP (neurological signs, fever and renal failure),a differential diagnosis with paroxysmal nocturnal hemoglobinuria (test HAM negative), a neoplastic cause(markers negative + nonsugestive imaging), with collagen diseases (immunological profile nonspecific), valvular heart disease (echocardiography in normal relations) with infectious causes (negative markers), disseminated intravascular coagulation and Evans syndrome (negative immunological tests) was made. ADAMTS13 activity assay revealed a downturn of this protein, the presence of anti-ADAMTS13 antibodies and ADAMTS13 antigen decline confirmed the diagnosis of TTP. Further therapy by plasmapheresis was initiated with the normalization of the hematological profile.
PTT clinical expression may be less suggestive, with the consequence of delaying specific treatment for a disease whose mortality rate is about 90% without treatment.

ANTICOAGULANT RELATED SPURIOUS THROMBOCYTOPENIA: CASE REPORT AND BRIEF REVIEW.

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EDTA-related spurious thrombocytopenia is a well known phenomenon in the routine haematology laboratory, unfortunately underrecognized in our medical community.The management of suspected falsely low thrombocyte count is repeating the measurement on blood prelevated on a citrate tube (coagulation tube), while the more elaborate, but resource-consuming labour-intensive method is the confirmation of platelet count on cytological examination of blood smears. We here report a case where aggregation and consecutive spurious thrombocytopenia occurred not ony on EDTA, but also on heparin and citrate anticoagulants.

THE DIFFERENTIAL DIAGNOSIS DIFFICULTY WITH INHERITED FACTOR VII DEFICIENCY.

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Inherited factor VII deficiency is a rare coagulation disorder and its prevalence is estimated to be less than 1 case in 500,000 people in the general population. Immune mediated acquired factor VII deficiency, is also uncommon and has been associated with drugs such as cephalosporins, penicillins and oral anticoagulants; it has also been reported to occur spontaneously and alongside certain conditions, such as myeloma and aplastic anemia.
We present a case of an inherited factor VII deficiency with a difficult diagnostic process.
50-year-old Caucasian female with a history of benign uterine leiomyoma presented with menorrhagia and was found to be anemic with hemoglobin of 5.9 g/dl, PT 82 seconds and INR 7 despite not taking any anticoagulants and having never suffered any peri or postoperative bleeding despite multiple surgical interventions. Toxicological analysis was performed and revealed no evidence of warfarin or coumarin-type drugs. The INR was reversed to 1 with fresh frozen plasma both in vitro and in vivo. We therefore considered that this was an inherited mechanism rather than autoimmune. The measured factor VII level was 1 %.
Inherited factor VII deficiency is a rare coagulation disorder but should be considered with patients presenting with acute coagulation disorders even with no previously reported history of a bleeding disorder.

A CASE OF ACUTE LYMPHOBLASTIC LEUKEMIA ASSOCIATED WITH ACUTE PANCREATITIS AT ONSET.

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Introduction: Acute lymphoblastic leukemia is a (LAL) clonal, malignant disease characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood or other organs and by the supression of normal haematopoiesis, which leads to medular failure, clinical manifested by anemia, hamorrhage , infections . LAL represents 75 to 80% of acute leukemias among children and only 20% of all leukemias among adults.
Acute pancreatitis can be one of the associated complications in acute leukemias, frequently associated with Asparaginaze administration.
Acute pancreatitis is one of the most common disease of the gastrointestinal tract, beeing a severe disease characterised by sudden inflammation of the pancreas . Acute pancreatitis is a common complication which occurs in patients sufferig from vesicular biliary lithiasis or chronical alcoholism. Other possible causes incriminated : drug reaction , iatrogenic (post ERCP),lipidic metabolism disorders, idiopatic.
Case report . A 23 year old patient, without any previous patologic or hereditary antecedents, diagnosed in april 2015 with acute lymphoblastic leukemia with B lineage, to whom chemotherapy was not initiated, develops in 48 hours of hospital admission acute pancreatitis.The diagnosis was supported by clinical simptomatology, biological status (amylase 995 UI/L, lipase 1196 ui/L), imagistic results.
After gastrointestinal and infectious disease consults,but also after further more investigations we excluted as possible causes : vesicular biliary lithiasis, sistemic infectious diseases (viral, bacterial, fungal). remainig as possible etiological factors: drug related (the pacient was receving Dexamethazone treatment) or secundary to the haematology disease.
The evolution after suportive, symptomatic treatment was favorable with remission of clinical symptoms and normalization of pancretic enzymes , without beeing

necessary the termination of Dexamethazone , only reduction of the dose. This advocates for the secundary infiltration do to the acute leukemia as the main etiologic factor. After the administration of Asparaginase the pacient did not developed any pancreatic affectation.
The patient after received chemotherapy according to UKALL XII/ECOG 2993 protocol leading to complete remission of the disease.
Case particularity: acute lymphoblastic leukemia in a young patient, without associated comorbidities complicated by acute pancreatitis at onset , probably by secondary determination of the haematology disease.

MULTIPLE MYELOMA IN YOUNG PATIENTS - CLINICAL SPECIFICATIONS, EVOLUTION AND TREATMENT OPTIONS.

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Introduction: Multiple myeloma (MM) is a neoplasic plasma cell proliferation that usually occurs in the elderly patient (aged 60 to 70 years), the MM diagnosis in young patients accounts for 2% of all myelomas.
Materials and Methods: We present the evolution of the newly diagnosticated young patients (maximum age at diagnosis 55 years) with MM in the Hematology Department of the Colentina Clinical Hospital Bucharest.Our patients data base records 20 new diagnosed cases of MM during the surveyed period, 1 January 2014 up to 1 June 2015. Sub-categorizing our patient, based on age distribution, we report that 8 cases were encountered in patients aged under 55 years, our youngest patient being a 40 years old woman. Based on the Durie Salmon classification of myelomas 2 cases were classified in the IIA stage, 3 in the IIIA stage and other 3 in the IIIB stage. For 2 cases, the citogenetic examination evidentiated the t(4,14) variant and attributed the high risc mSMART sub-classification to the cases. In one patient we recorded a hipervascosity state, that was well tolerated by the patient.
Results: The young patients diagnosed with MM evolved favorable under chemotherapic treatment - protocol type VCD (Velcade, Ciclofosfamide, Dexamethasone). In four cases we recorded the complete response to treatment and the patients are scheduled to undergo autologous hematopoetic stem cell transplant, the rest of the patients being still under chemotherapy.
Conclusion: Gathering data from MM cases diagnosted among young patients - aged under 55, we want to emphasize on the fact that we registered a relatively high incidence of cases during a relatively short period of time (18 months), in this particular age group.

ACUTE MYELOID LEUKEMIA AFTER OSTEOSARCOMA IN A 19 YEAR OLD PATIENT: CASE REPORT.

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Abstract. Introduction. As patients with osteosarcoma become long-term survivors, increasing attention has turned to the burden of late effects. Recent studies showed an increase in the incidence of secondary malignant neoplasms in patients with osteosarcoma compared with the general population. The risk of developing leukemia was reported to be in an increasing rate in last decade Case report. In this report we present the case of a 19 year old patient admitted in the Hematology Department of Colentina Clinical Hospital in February 2015 for fever (38°C), extreme fatigue, intense pallor. The patient reported that he had been diagnosed with osteogenic osteosarcoma of the right femur 2 years ago which was operated and treated with chemotherapy (COSS EURAMOS protocol) and radiotherapy, achieving complete remission. The full blood count performed at admission revealed severe anemia, thrombocytopenia and leukocytosis and the blood smear revealed the presence of myeloblasts in proportion of 97%. The bone marrow aspirate showed a percentage of 95% blasts with the morphological characteristics of myeloid lineage, with the dislocation of normal hematopoiesis. The flow cytometry performed on the bone marrow aspirate stated the diagnosis of Acute Myeloid Leukemia M1 FAB subtype (WHO 2008). The cytogenetics revealed an abnormal karyotype with 91 chromosomes (XXY). The molecular biology did not find mutations in the FLT3ITD, FLT3D835 and NPM1 A genes. The patient started the first course of induction therapy with Cytarabine 5 days and Anthracicline 2 days (the calculated total dose of anthracicline used for treating the osteosarcoma permitted us to further administer anthracicline this time as well) and the bone marrow aspirate performed at day 21 revealed a percentage of blasts of 4%, this being interpreted as complete remission. Next the patient underwent a first consolidation course of chemotherapy with high dose Cytarabine followed by a severe post chemotherapy aplasia. The bone marrow aspirate performed after this course revealed a percentage of 3% blasts, showing that the patient is in sustained complete remission.

Considering the prior treatment for the osteosarcoma consisting of radiotherapy and chemotherapy we considered this to be a secondary Acute Myeloid Leukemia, this representing a major negative prognostic factor, along with the cytogenetic abnormalities found. These factors strongly indicate that the first complete remission should be consolidated by allogeneic stem cell transplant. To be noted that the patient has a sibling which is not HLA compatible, but efforts are being done to find a suitable unrelated donor.
Conclusion. The particularities of this case are the association of the two malignancies in a young patient, the presence of the abnormal karyotype (hyperploid), and also the good response of the patient to the induction therapy, achieving complete remission after the first induction course, and of course, sustaining that response.

CLINICAL CASE OF APLASTIC CRISIS ASSOCIATED WITH EXTRAMEDULLARY HEMATOPOIESIS IN AN ADULT WITH HEREDITARY SPHEROCYTOSIS AND PARVOVIRUS B19 INFECTION.

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Hereditary spherocytosis is an inherited hemolytic anemia due to red cell membrane defects, characterised by chronic hemolysis with different severity degrees, splenomegaly and macrospherocytosis on the blood film.
Among the possible complications in these patients are aplastic crisis and extramedullary hematopoiesis.
Aplastic crisis arise as a result of an acute parvovirus B19 infection, characterized by fever, maculopapular rash ("slapped cheeks"), vomiting, diarrhea, anemia with reduced reticulocyte number, lower bilirubin and medullary aplasia. The acute parvovirus B19 infection appears mostly in children (rarely in adults) and is self-limited.
Extramedullary hematopoiesis is localized in the liver, spleen, lymph nodes and mediastinum, as a compensatory response to insufficient bone marrow cell production.
In this poster we present the case of a 42 years old man with hereditary spherocytosis diagnosed during childhood (without transfusion demand), which presented with accentuation of anemia (Hb 6,1 g/dl, Ret 3,8%), fever, chills, bone and muscle pain.
The evolution was with a decrease in Hb value to 4,2 g/dl, of reticulocyte number (Ret 0,8%) and bilirubin (BT 3,8 g/dl).
ADN Parvovirus B19 was 100.000.000 copies/ml
The CT exam showed extramedullary hematopoiesis areas situated paravertebraly in the inferior thorax and hepatosplenomegaly.
The infectious episode was self-limited and improved with substitution treatment.

CORRELATIONS BETWEEN BCR-ABL LEVEL AND HEMATOLOGICAL ONSET OF CHRONIC MYELOID LEUKEMIA AT DIAGNOSIS.

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INTRODUCTION

A rapid research development of chronic myeloid leukemia (CML) were made for a fully understanding of the mechanisms underlying these diseases.
Inhibition of the BCR-ABL tyrosine kinase activity is the most efficient way for silencing this oncoprotein. The development of tyrosine kinase inhibitors (TKI) for chronic phase CML patients was revolutionized the treatment of this disease and profoundly transformed its evolution from a fatal disease to a chronic condition. The amount of leukemic cells that remain in the patient's body during treatment with TKI can be monitored accurately by using 3 ways: examination of peripheral blood (hematologic response), cytogenetic examination of metaphase analysis in bone marrow (cytogenetic response) and quantification of BCR-ABL transcripts (molecular response).

MATERIALS AND METHODS

Diagnosis and monitoring of CML patients in chronic phase was performed on peripheral blood samples, processed to obtain cell lysates and TRIzol preservation. Detection of BCR-ABL transcripts was achieved by Multiplex PCR of cDNA. Real Time Quantification of BCR-ABL transcript level was achieved by the Hybridization Probes detection method on the LightCycler 1.5 Instrument.

RESULTS

The study included 222 patients in 2014-2015. In patients positive for BCR-ABL fusion gene, the most frequently identified transcripts were b3a2 - 28% and b2a2 detected in 11% of cases. Rare cases of atypical transcript b3a3 were identified in 2% of cases, and the presence of both transcripts b3a2 + b2a2 was detected in 2% of cases at presentation.

In this study we have investigated the correlation between the BCR-ABL transcript type and hematological parameters at diagnosis: hemoglobin, hematocrit, leukocyte and platelet counts. Regarding the hemoglobin and hematocrit levels, there was not a significant difference between the main two types of transcripts. A significant difference in BCR-ABL expression at diagnosis was observed regarding leukocyte levels, where in the case of transcript b2a2, we have found the average of 107,383 (103/µl), compared with patients with b3a2 transcript - 76,832 (103/µl).

Regarding platelet levels, a significant difference was identified: patients with transcript b2a2 have an median average of 740 (103/µl) and patients with b3a2 transcript have a lower average of 473 (103/µl) platelet count at diagnosis.

This observation suggests that BCR-ABL with b2a2 transcript has a lower expression, is less agresive and thus the time from symptomatic onset to the diagnosis is prolonged. Patients with b2a2 transcript would have milder symptoms and faster response to treatment.

In patients with b3a2 transcript, symptoms can be more aggressive and thus, the time from symptomatic onset to diagnosis is shorter.

The average of BCR-ABL level (BCR-ABL1 IS%) at diagnosis was found to be slightly different. In patients with b2a2 transcript, the average of BCR-ABL was 22.6% and 35.2% for patients with b3a2 transcript.

The next aspect of the work is related to the importance of achieving major molecular response (MMR) as soon as the start of imatinib mesylate. Getting an MMR at 12 months of treatment initiation was associated with an optimal response to imatinib mesylate therapy.

CONCLUSIONS

For patients enrolled in this trial , the transcript b3a2 type was more frequently identified than those with b2a2 and there were identified patients with atypical transcripts and patients with both types b3a2+b2a2. High levels of leukocytosis and thrombocytosis at diagnosis for patients with b2a2 transcript suggests a lower gene expression of BCR-ABL and thus a longer time from first signs of illness until diagnosis. The molecular response to TKI therapy was faster than those with b3a2 type.

In patients with b3a2 transcript, symptoms can be more aggressive and thus, the time from symptomatic onset to diagnosis is shorter and molecular response to treatment with TK inhibitor is delayed.

IDENTIFICATION OF MOLECULAR MARKERS FOR DIAGNOSIS OF ACUTE LEUKEMIA IN ADULD AND PEDIATRIC PATIENTS.

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INTRODUCTION

In acute leukemia (AL), was identified a growing number of molecular genetic changes, highlighting biological heterogeneity of this disease. Moreover, the characterization of specific molecular abnormalities provides the basis for targeted therapies, such as using of all-trans retinoic acid (ATRA) and arsenic trioxide in the treatment of acute promyelocytic leukemia or using of tyrosine kinase inhibitors in the treatment of acute myeloid leukemia with FLT3 mutations. A detailed evaluation of molecular markers at diagnosis is crucial for risk stratification groups of patients with LA. This allows the earlier identification of patients at high risk of relapse eligible for allogeneic stem cell transplantation. Finally, molecular markers are important for detecting minimal residual disease after initial therapy and during long-term follow-up. These allow a more tailored treatment approach for each patient and are the premises of personalized medicine.

MATERIALS AND METHODS

The most common fusion genes identified in acute myeloid leukemia (AML) are: MLL - AF4 , BCR - ABL1 , AML1 - ETO, PML- RARα , CBFb - MYH11 and MLLAF9 . In acute lymphoblastic leukemia (ALL), the most common gene changes are: MLL - AF4 , BCR - ABL1, TEL - AML1, E2A - PBX1 and SILTAL1 . Detection of these genetic markers in AL was perform by using Multiplex PCR method on complementary DNA . Identifying these fusion genes

allowed us to stratify the patients into risk groups. Molecular monitoring of minimal residual disease by quantitative detection (RQ-PCR) and Nested PCR allow of early prediction of relapse.

RESULTS

272 patients with suspected acute leukemia at prezentation were investigated in 2014-2015, 179 (66%) - adults and 93 (34%) - children. In adults patients, 73% were diagnosed with AML and 27% with ALL. For the diagnosis of AML fusion genes were identified as following: FLT3-ITD - 45%, PML-RARα - 29%, CBFβ-MYH11 - 13%, AML1-ETO - 13%, and MLL-AF9 - 0%. For the diagnosis of ALL were identified: BCR-ABL - 70%, MLL-AF4 - 17%, E2A-PBX - 7%, TEL-AML - 6% and SIL-TAL - 0%.

In pediatric patients, 17% were diagnosed with AML and 83% with ALL. For the diagnosis of AML were identified: PML-RARα - 62%, MLL-AF9 - 25%, AML1-ETO - 13%, CBFβ-MYH11 – 0% and FLT3-ITD - 0%.

For the diagnosis of ALL have been identified: TEL-AML1 - 74%, BCR-ABL - 13%, E2A-PBX - 9%, MLL-AF4 - 4% and SIL-TAL - 0%. Observing these two groups, there is a clear majority frequency of cases of AML in adults and ALL in pediatric patients. According to publications in the literature, ITD mutation-FTL3 (Internal Tandem Duplication in FLT3) is one of the most common mutations found in adult AML. Detection of FLT3-ITD is associated with resistance to chemotherapy, early relapse, and the progression of the disease. The data identified in this study with 45% prevalence of FLT3-ITD in adult AML, may partially explain why adult AML has a poorer clinical outcome than pediatric AML. The association of FLT3-ITD tandem duplications in PML-RARα translocation is frequently described in the literature. In the study group we have analyzed, three patients with FLT3-ITD showed PML-RARα translocation also.

CONCLUSIONS

For acute leukemia, the most common in adults is acute myeloid leukemia and the most common in pediatric patients is acute lymphoblastic leukemia.

Detection of minimal residual disease in acute leukemia is a useful and effective method for identifying patients at high risk of relapse. Early detection and treatment can improve clinical outcome molecular relapse in acute promyelocytic leukemia with PML- RARα positive. FLT3 gene with tandem duplication is a negative prognostic indicator for AML.

Fron all analyzed markers in adults we did not find any patient with TEL-AML fusion in ALL and no MLL-AF9 in AML. In children no FLT3-ITD and CBFβ-MYH11 cases were identified.

SECTION TRANFUSION MEDICINE
EDUCATIONAL SESSION

THE VALUE OF A HIGH QUALITY IMMUNOHAEMATOLOGIC DIAGNOSIS TO ENSURE BLOOD SAFETY.

Al. Dobrota
BTC Constanta

Objective: Faithfull to its assumed mission to contribute to the education and training of professionals working in the transfusion field, The Romanian Blood Transfusion Society is organizing the 3rd educational session in transfusion medicine, focused on the complex and challengeable field of immunohaematology.
This session is addressed to all those interested in acquiring, developing or updating knowledge and skills to solve "problem" cases in immunohaematology, to ensure adequate transfusion treatment whenever necessary .
Method: The program includes both the presentation of basic concepts in immunohaematology, updated information on blood groups classification, impact of erythrocytic antigen polymorphism on the capacity to ensure compatible transfusion treatment and practical matters of interest: issues concerning the algorithms used in Romania for testing and diagnosis of various categories of groups (blood donors, patients with transfusion indication and pregnant women, newborns). The speakers will illustrate with real cases from practice the multiple facets of the immuno-hematological testing activity addressed, be specific for a blood transfusion center or a hospital blood bank. The presentations and discussions will offer participants the opportunity to identify practical solutions to ensure safe transfusion therapy for immunized patients .The potential consequences when the pretransfusion testing protocol is not followed in multitransfused patients and implications on the capacity to select compatible blood components are exemplified by presenting a case of severe adverse reaction. Cases of hemolytic disease of the newborn with irregular antibodies of other specificity than anti-D will bring an add-value to the practical information provided along the session.
Conclusions: The subjects diversity ensures a wider scene for discussion and experience sharing among participants, hopefully stimulating the interest to increase knowledge and practical experience in a particular field of laboratory medicine / transfusion medicine. The educational session is expected to have a positive outcome, given the nationwide lack of any educational program to cover specialists' training needs. The educational session should strengthen blood transfusion centers capacity to support clinicians by providing a specialized response in solving special patients' transfusion needs. Ad-hoc formation of an informal network of experts, supporting the „problem" cases solving would be expected in the absence of a national reference immunohematology laboratory.

TRANSFUSION THERAPEUTIC SOLUTIONS IN MULTIPLE MYELOMA

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

Introduction
Transfusion is always saving in a very simple context: when given the right patient at the right time, for the right diagnosis, the right product, but find the right adjective for many nouns, something not always so simple.
Case Presentation Material
Patient 67 years without a pathological history, hospitalized on 21/09/2013 in Hospital MS, Department of Hematology, the symptoms more pronounced in the last two weeks: malaise, asthenia and fatigue intense, marked pallor, without bleeding syndrome, and without organomegaly. Complementary examinations show: important inflammatory syndrome, severe pancytopenia, hyperproteinemia, hypersideremia. On peripheral blood smears are seen with severe leucopenia difficult because some plasma and several blasts. Managed marrow puncture extracting a minimal amount of material to be examined, which reveals an important plasmocitosis.
Biological values of the patients: Hb -5.1 g / dl, WBC - 1800 / mmc, platelets - 40,000 / mmc, ESR -140 mm / 1h, bilirubin -1.3 mg / dl, Fe - 239 mg / dl, urea -37 mg / dl, creatinine - 0.7 mg / dl, ALT- 45 microns / L, AST-36U / l, GGT-98U / l, total protein -8,7mg / dl, and blood smears show: erythrocytes in wrapping machines with the following formula WBC: Bl 3%, 52% S, E 2% Lf 36%, 2% Mo, Plasmocite5% EBL 4/100 items.
After the first consultation and biological evaluation of

hospitalization, to diagnosis, treatment administration hematologist decide symptomatic with packed red blood cells to treat anemia. The red cells in order Transfusion Unit of the hospital. When performing blood typing are difficulty in defining group, both in point of transfusion and hospital laboratory where blood group is found discordant at Beth Vincent and Simonin, presenting both samples panaglutination.
Blood-grouping and verifying the compatibility required immunohematology Laboratory of CTS, which receives pretransfusional patient samples. Indeed, both Beth Vincent and the agglutination finds Simonin also positive allo witness, the witness AB positive, positive self-control. For verifying the Beth Vincent, washed red cells 3 times with saline and after washing, the result block is A + C + c + E - e-, K-, and for the test Simonin patient serum diluted to 1/4 and the result group A + is obtained. It makes you patient research irregular antibodies, also with 1/4 diluted serum, resulting absent irregular antibodies. Initial test compatibility with patient serum, result incompatibility bags tested, but with 1/4 diluted serum is found compatible red blood cells. It manages four CER group A + C + c + E – K-, which corrects anemia. Later on radiographs, bone lesions characteristic indicating the spine, it is diagnosed multiple myeloma. For now discharged improved patient following to be readmitted in Colentina Hospital.
Conclusion
Sometimes blood grouping difficulties may delay the administration of blood products. Even if establishments or hospital laboratory, fail to determine the patient's blood group, immunohematology Laboratory Transfusion Center has available and can give appropriate transfusion solution.

NEWBORN HEMOLYTIC DISEASE CAUSED BY ANTI-e ANTIBODY.

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Blood Bank Ploiesti

Introduction
Immunohematology investigation of pregnant women, in routine medical activity, often treated with enough lightly, turns out to be insufficient to predict the occurrence of hemolytic disease of the newborn. Sometimes neonatologist is facing a newborn with serious and prolonged jaundice, born of a mother with Rh positive, which is quite surprising for it, there is need for emergency diagnosis. Direct Coombs test is positive newborn. Irregular antibodies can be emphasized in the serum of children, but also in maternal serum. We are dealing with a hemolytic disease of the newborn.
Material
Case Presentation: In O.G. Hospital, a pregnant women at the time, born a healthy child, 2500 g, Apgar 8, which in the next 24-48 hours develop jaundice, with bilirubin 14 mg / dl, with signs of moderate anemia, Hb = 9 mg / dl. Mother is the third birth, uninvestigated biologically and clinically during pregnancy, coming from a socially disadvantaged environment.
Mother's biological constants are normal in routine investigations carried out in maternity include maternal blood group, which is O+. The child's blood group at birth, performed cord blood, the mother is the same. However prolonged jaundice makes the neonatologist ask conducting a direct Coombs test. Direct Coombs test, positive IgG is quite surprising neonatologist.
CTS resumes immunohematology investigations related to the case: blood group and Rh Kell phenotype of the mother: O +, C + c + E + e- K-, with irregular antibodies mother's research and finding an anti-e, in titer of 1/64; Group and child phenotype O +, C + c+ E + e + K-, the child research irregular antibodies, with the presence of anti-e titer of 1/8. It performs father's blood type, showing: O +, C + c + E + e+ K-. To correct anemia blood transfusion deciding compatible with maternal serum O +, C + c+ E + e-K-, and without e antigen, along with other therapeutic measures balances newborn.
Results:
The comparison results groupage erythrocyte mother, child and father in various systems already allow to eliminate a number of possibilities and retain various assumptions. Antigen responsible must be present in the fetus and the father, and absent mother. The choice depends on the specific blood transfusion of maternal antibodies. It should not take charge of the immunization antigen. Transfusion of blood must be made compatible with maternal serum.
Conclusions:
It is very useful: Investigation pregnant since the third month: type and Rh, group, Kell phenotype Rh, Rh and whatever research irregular antibodies to red blood cells to a panel joint. Moreover, it is useful to use red blood cells and father, expressing the antigen that may cause immunization. ABO serum of incompatibility between the mother and father's red blood cells, but not always allow for the diagnostic use of these red blood cells.

HEMOLYTIC DISEASE OF NEWBORN DUE TO ALLOANTIBODIES IN KIDD SYSTEM.

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Haemolytic disease of the fetus and newborn (HDFN) is caused by maternal alloimmunization against red cell antigens. Although the Rh antibody was and still is the most common cause of severe hemolytic disease of the newborn, other alloimmune antibodies belonging to Kell (K and k), Duffy (Fya), Kidd (Jka and Jkb), and MNSs (M, N, S, and s) systems do cause severe hemolytic disease of the newborn. In severe cases, HDFN may lead to fetal anaemia with a risk for fethal death and to severe forms of neonatal hyperbilirubinemia with a risk kernicterus.

We present the case of a multigravida (5G, 4P), blood group A Rh(D) neg ccddeekk,immunized during previous pregnancies in Rh (D) system. In history, one of the infants presented hemolytic disease which required two exchange transfusion. Investigations conducted during last pregnancy revealed only the presence of anti-D, titer increased (1/1024). At birth, the blood group of the fetus is A Rh (D) negative, but TCD (IgG) positive. Laboratory investigations revealed the presence in mother's postpartum and fetus serum, aloantibodies anti-JKa, the mother's phenotype (Jka-Jkb+) and the newborn phenotype (Jka + Jkb-). The selection of compatible blood for exchange transfusion was difficult as there wasn't available units of leucodepleted blood Jka (-) in the blood bank.
The case reveals the importance of detecting maternal alloimmunization early in pregnancy to facilitate the identification of high-risk cases to timely start antenatal and postnatal tratment.

TRANSFUSION MEDICINE SECTION
ORAL PRESENTATIONS

SEARCHING FOR AN UNRELATED COMPATIBLE STEM CELL DONOR FOR PATIENTS ON BONE MARROW TRANSPLANT LIST.

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Allo-transplantation of hematopoietic stem cells from a compatible unrelated donor is one of the options for the treatment of patients with hematological that do not have a matched donor in the family. Graft success is influenced by many factors, the most important being the degree of HLA, type of the disease and disease stage, age etc. During the last 24 months, at RNDVCSH has been registered a continuous increase of search activities in order to find the best donor for each patient on the waiting list. Thus, only in our laboratory, we have recorded in 2015, an increase of 66% regarding the number of patients that are looking for an unrelated donor and an increase of over 80% in the number of extensive and /or verification testing. Although the selection rate for TE / TV of potential compatible donors has remained constant over the last 24 months (34-30% national donors versus 66-70% donor international donors), during 2015 we have noticed a significant increase, from 19 % to 30% of national donors who, after testing high resolution, have been find at least 90% compatible and have been accepted for donation by Romanian Bone Marrow Transplant Centers. Regarding the category of donors found 100% compatible, the percentage was definitely favorable to the national donors: 66% national donors versus 49% international donors validated. Despite all these encouraging results, it should be mentioned that for approx. 40% of patients tested in our lab it has not been yet identified an acceptable donor. It is about patients with rare phenotypes / HLA alleles. It is widely recognized and accepted that the highest chance of identifying a compatible unrelated donor is in the geographical area / population that the patient belongs. Definitely the continued increasing of the number of national donor registered and tested will help to identify more and more national donors for our patients.

UPDATES IN IMMUNOLOGICAL EVALUATION STRATEGY OF CARDIAC RECEPTOR. NATIONAL HLA LABORATORY EXPERIENCE

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Heart transplantation as a therapeutic option remains surgical procedure for patients with heart failure in terminal stage or who suffer from severe coronary artery disease. Special efforts have been made in the direction of immunological and viral evaluation HLA as complex cardiac patient on the waiting list. From published the data, the influence of HLA compatibility between the recipient and the donor in heart transplantation was controversial, and the prospective HLA matching seldom achieved. There are studies that show that a matching of HLA A, B, DR did not have too much effect in patient with heart transplant concerning the general survival. There are other studies that demonstrate that the patient's survival post-transplantation could be correlated with the degree of HLA compatibility. The study conducted in our Institute of Hematology and Transfusion is based on immunological and viral assessment protocol for patients candidate to heart transplantation. The evaluation are done in pre and post transplant, in agreement with the Institute of cardiovascular diseases CC. ILIESCU. HLA typing and infectious disease tests done for candidates are presented in the study. Discuss the role and influence of determining anti-HLA Antibodies, especially of specificities class I and II in the serum of the recipients in the pre and post transplant. It is a study that is started and will continue with immunological evaluation of a larger number of heart transplant candidate patients.

ESTIMATION OF RESIDUAL RISK OF TRANSFUSION TRANSMITTED HIV-1 AND HCV DURING 2011-2014.

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BACKGROUND: The residual risk(RR) of transfusion transmitted infections(TTI) is expressed as the probability of having a potentially infectious blood unit/component validated by the current screening technologies and its estimation is a vital tool for evaluating the safety of blood supply. The RR of TTIs is mainly generated by window period (WP) infections in repeat blood donors(RBD) and depends on the characteristic length of WP for the screening tests and the incidence. Mathematical models have been developed based on these parameters, including the interval between the negative and the positive donation. The accuracy of such models is validated by measuring the observed against the predicted outcome. We had previously estimated the row potential RR based only on the observed frequencies of incident cases among RBDs. Changes in screening and confirmation technologies since 2011, inprovement in data collection, increased availability of repository samples for retrospective testing and the prevalence and incidence changes in first-time blood donors (FTBD)and RBDs after 2010, conducted to reevaluation of the RR for, that we report here.
METHODS: An incidence/WP model incorporating the median interval between donations for RBDs seroconverting within 2 years (Seed et all,Internal Medicine Journal, 2005; 35:592-598) has been used. WP values published for the current screening tests are 15 and 20 days for HIV and HCV respectively. Prevalence and incidence are expressed for 100,000 donations. Data from serological screening of 1.655.732 donations (382.283 FTBD and 1.273.449 RBD) were analysed and, for HIV and HCV positive RDB the median interval between donations was determined. Where available, repository samples from previous negative donations were tested for HIV-1 p24 antigen, HIV-1 and HCV RNA respectively, to determine the infectious status.
RESULTS: The HIV-1 prevalence and incidence were 43 in FTBD and 4.3 in RBD respectively. 51/58 positive RBDs seroconverted in a median interval of 152 days the resulting theoretical RR for HIV-1 being 1/253025. For HCV a prevalence of 467 and an incidence of 2.4 were observed. 25/31 cases seroconverted within 2 years with a median interval of 95 days resulting in a theoretical RR of 1/241955. RNA testing was performed on 57%(29/51) and 20%(6/25) repository seronegative samples from seroconverting RBDs for HIV-1and HCV respectively. 3 viremic blood donations were identified for each virus corresponding to an observed frequency of infectious donations of 1/424483.
CONCLUSIONS: For HCV the estimated RR is similar to those reported in Western EU and USA prior to the introduction of NAT screening, but is well above those reported for HIV. The observed frequency of seronegative viremic donations is probably underestimated due to unavailability of all repository samples. The computed RR points to the probability of collecting at least 1 infectious blood units per year for each virus, and was restricted to incident cases among RBD, but the risk from seroconversion among FTBD should be taken into account. The local prevalences for TTIs and the detection of viremic WP-donations indicate that further reduction of the residual risk would occur only through introducing the NAT testing of all donations together with improving standards for donor selection.

EVOLUTIONS OF PREVALENCES AND INCIDENCES OF HTLV-I IN BLOOD DONORS; IMPACT ON DONATION SCREENING STRATEGIES.

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INTRODUCERE: First retrovirus directly associated with a human malignant haemopathy(ATL), HTLV-I is endemic in some parts of the world, and specifically in South-West Japan, Caribbeans,South America and parts of Africa, where the carrier rate is high. Screening the blood donors for HTLV in Europe and North America was introduced primarily due to immigration from endemic areas and susequently some areas of moderate /high endemicity unknown before were identified. According to the new criteria issued by ECDC (2012 and 2015) an area with „a prevalence of 1% in the general population or a prevalence of over 1/10.000 first-time blood donors(FTBD)”is considered a „high prevalence” area with „strong evidence of HTLV-I infection” and testing of blood and tissue donors is recommended. In Romania screening of blood donations for HTLV was introduced in 1999 and additionally pacients with suggestive malignant haemopathies, polytransfused pacients, some of their contacts, as well as potential stem cell and bone marrow donors were investigated. Our last communication on the results of HTLV screening up to 2010 reported a prevalence of 4.8/10.000 in FTBD and together with the unusually closely related circulating virus isolates are

consistent with the existence of an endemic area and a specific HTLV-1 clade. Updates on prevalence and incidence of HTLV-I are reported here together with their impact on donation screening strategies. .
METHODS: The current screening is performed with sandwich enzyme immunoassay(EIA) or CLIA exclusively based on recombinant antigens. The confirmation of EIA/CLIA reactives is done with a Line-Immuno-Assay (LIA), observing the H.E.R.N. recomandations. Results of HTLV screening in 2011-2014 were analysed as compared to previous data.
REZULTS: During 2011-2014, 1655732 donations have been screened and 108 FTBD were confirmed positive for HTLV-I, with a resulting prevalence of 2.83/10.000 as compared to 4.8/10.000 registered previously. Two additional incident cases were identified in 2011 and 2014. The corresponding incidence for the considered period is 0.2/100.000 donations. HTLV-I positive donors were confirmed in 25 districts from all parts of the country. Local prevalences over 5/10000 were still registered in the South-East and the 2 incident cases originate fron the same area. Bucharest together with other 3 districts in this area concentrate 72% of all cases while accounting for only 36% of all blood donations.
CONCLUSIONS: The general prevalence for HTLV-I among FTBD have decreased as compared to that previously reported but is still significant and stongly supports the existence of an endemic area centered on the South-East of Romania, at least among blood donors. Nevertheless, over time,isolated cases have been confirmed all over the country. Due to lack of incident cases among RBD, in some blood services in EU the possibility of discontinuing the anti-HTLV screening of all donations had been considered and swiching to donor testing had been proposed. The incident cases registered over the last four years prevent us from considering such a strategy for blood screening, at least for the next years.

IMPACT OF NONSPECIFIC REACTIVITIES IN SCREENING TESTS UPON BLOOD COLLECTION.

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BACKGROUND: Screening of blood donations relies primarily on enzyme immunoassays (EIA). Continuous refining of EIAs improved their sensitivity and specificity. As the senzitivity of the last generations of EIAs has reached almost the last limit minimizing the negative window and increasing the capacity to detect a large range of mutans and viral varians, a specificty of 100% and will probably never be achieved with current technologies. Nonspecific reactivities are frequently donor related or assay related and represent a challenge for blood services. Voluntary nonremunerated blood donors are recruited from low-risk populations and the majority of reactive results obtained in screenig turn out to be nonspecific upon confirmatory testing, determine loss of blood products and defferal of donors who require counseling, additional testing and sometimes medical evaluation. We report here the prevalence of non specific reactivities and its impact on blood collection.
METHODES**:** 1.655.732 blood donations collected during 2011-2014 were screened for HIV, HBV, HCV and HTLV by Blood Transfusion Centers(BTC) and repeat reactive samples sent to the Central Reference Laboratory for confirmation. A sequential algorithm is used for confirming anti-viral antibody reactivity which includes retesting in the original EIA followed by an alternative EIA and imunoblot for samples reactive in 2 EIAs or with high sample-to-cut-off ratios. An isolated rectivity in one EIA and an indeterminate or negative imunoblot result requires retesting on a follow-up sample after a time to exclude the potential serological window. HBsAg is confirmed by specific neutralization with anti-HBsAg and additional testing for anti-HBc, HBeAg/anti-HBe, Anti-HBs.
REZULTS: 15825 blood units were discarded due reactive screening tests and sent for confirmation. 12044 (76%) were actualy confirmed as positives for the suspected virus.Between 2%(HBV) and 22%(HTLV) of samples were not confirmed in the initial screening test. The yield of reactive samples by suspected virus and the rate of confirmation (HIV:801/22%; HBV:10865/91%; HCV:3608/50%; HTLV:329/34%) are concordant with the local prevalences and higher for specific antibody EIAs. The apparent specificity resulted from large scale screening ranges from 99.89% for HCV to 99.99% for HTLV with a frequency of nonpositive cases from 1/926 for HCV to 1/7630 for HTLV. Some nonspecific reactivities were only tranzient (from 2% for HBV up to 8% for HIV) and retesting the donor after several months gave negative results, but up to 74% are persistent. Donors with tranzient nonspecific reactivity or due to tests replaced by more performant ones may be eligible for future reentry.

CONCLUSIONS: The rate of nonspecific reactivity resulted from current screening of blood donations over the last four years suggests nonsignificant loss to blood collection(<1%). The donors with persistent nonspecific reactivities, though noninfected, are permanently deferred to minimize loss of resources. The challenge for the BTCs is to inform these donors of their results and to minimize donor anxiety, since it is very difficult to explain to donors that although not infected their blood is not suitable for transfusion. Additionally, misinterpretation by donors of such results, can lead to medicolegal implications.

OCCULT HEPATITIS B INFECTION AND RESIDUAL RISK.

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Blood Bank Ploiesti

Introduction
Hepatitis B is a worldwide public health problem. It is estimated that at global level there are over 2 billion people infected in life.
Material
A particular form of HBV infection identified by molecular biology in recent years, occult HBV infection is. IOB is defined by the presence of HBV DNA in liver tissue, sometimes in person's serum, HBs Ag absent. IOB prevalence is unknown, despite numerous studies showing the disadvantage of including a relatively small number of people, the lack of standardization of laboratory tests, which almost all are retrospective. Regarding the apparently healthy population, the prevalence was investigated IOB blood donors and more than in the general population.
IOB blood donor's prevalence is much lower in developed countries, unlike the one significantly higher in developing countries. In the general population it was found in 16% of cases studied. IOB were advanced in the pathogenesis of these assumptions: host immunity, viral interference, and epigenetic factors. Diagnostics for detecting HBV DNA at very low serum using technology requires sensitive and specific PCR or NAT. By using real-time PCR can be detected most occult infections (<10 copies / ml).
Over 92 million blood donations are collected globally every year. A single blood donation in products labile, can reach three people and stable products from a single donation can reach hundreds of patients. Although testing is increasingly drawn towards increased transfusion safety is unanimously recognized the existence of a residual risk of HIV, HCV, HBV, WNV, CMV, etc. Screening donated blood for infectious diseases is a key measure of safety, worldwide, to protect patients, which prompted the introduction of NAT in many countries since 1997, according to the epidemiological situation and the financial potential of the country.
IOB transmitted infection can be transmitted by transfusion in liver transplantation, hemodialysis and bone marrow transplantation. Although post-transfusion hepatitis risk is now very low, risk of infection through transfusion of blood products is still very high (1: 100,000) compared to HCV (1: 700,000), and HIV (1: 2,000,000). Post-transplant liver from HBV infection risk donor is IOB 25-94%, but is lower than after bone marrow transplantation. IOB prevalence is higher in hemodialysis patients (14-19%)
Conclusion
IOB is a complex biological entity, mainly related to indefinite persistent HBV DNA in hepatocytes and strong suppression of viral replication. IOB unable essential contribution eradication of HBV infection. In Romania IOB prevalence remains unknown, it is underdiagnosed entity. IOB can be transmitted by transfusion, perinatal and liver transplantation. Testing for anti-HBc, anti-HBs, anti-HBe blood used in transfusions as an option. Blood testing by real time PCR notable remains an option for Romania.

WEST NILE VIRUS INFECTION, A NEW CHALLENGE IN ENSURING BLOOD TRANSFUSION SAFETY.

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Blood Bank PLOIESTI

Introduction
The epidemiological situation in the European Union created by the increasing number of cases of infection with WNV between 2008-2010 led to the need for a guide of recommendations applicable to ensure transfusion safety measures in the affected areas, depending on the assessed risk. After the 1996 WNV epidemic, MS has implemented in the territory at risk (counties bordering the Danube), the vector activity period (May-October) surveillance system with WNV infection.
Material
There have been confirmed cases of human infection

annually. Specific measures have been taken transfusion system since 2008. Applying ensuring blood transfusion safety measures allowed without impairment of transfusion therapy management in counties affected areas. West Nile virus of the family Flaviviridae, found in both tropical regions and in the temperate. It mainly infects birds, but infects and humans, horses, dogs, cats, bats, chipmunks, skunks and domestic rabbits. The main route of human infection is through the bite of an infected mosquito. Transmission is by infected mosquitoes. In very few cases, there was a virus transmitted through blood product transfusion, organ transplants, breastfeeding and even trans placental.
Interest practitioners with West Nile virus increased when it was discovered in the US in New York, in 1999, when 66 cases were confirmed that resulted in seven deaths. About 80- 90% of infected people show no symptoms. The incubation period is 3 - 14 days after contact with the virus.
Infection with West Nile Virus has three clinical forms.
Meningitis or encephalitis 1. About 1 in 150 people infected with West Nile virus develop severe symptoms of the disease: high fever; headache; neck stiffness; stupor; disorientation; coma; tremors and convulsions; muscle weakness; vision loss; numbness and paralysis;
Symptoms may persist over several weeks, and neurological effects may be permanent. Recovery is a long period of convalescence.
2. Febrile syndrome About 20% of infected people show symptoms of moderate severity: fever; headache; nausea, vomiting, anorexia, diarrhea; muscle and joint pain; rarely swollen lymph nodes;
The symptoms for a period of 7-10 days, although weakness may persist for up to several weeks and lymphadenopathy to 2 months.
3. Asymptomatic infection: approximately 80% (4 of 5 people) infected show no symptoms. West Nile virus infection diagnosis is made by molecular biology and clinical examination. Testing is performed only in patients who have severe symptoms because there is no treatment for West Nile virus infection. Acknowledge by PCR testing, or analysis of CSF. There is no specific treatment for the infection W NV. In the case of the medium severity manifestation of symptoms, improves the patient's condition after a few days without treatment. In case of severe, hospitalization is required and treat the patient with symptomatic medication for supportive.
Conclusions: Testing of donated blood for WNV by PCR is useful for Transfusion Safety.

SEROLOGICAL SCREENING FOR BORRELIA BURGOFERI IN BLOOD DONORS FROM ARAD COUNTY.

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Borrelia burgdorferi (BB) is the agent of Lyme disease, which is the most common vector-borne disease in the temperate zones of the northern hemisphere. The tick Ixodes ricinus is the most responsible vector for Borrelia burgdorferi, the pathogen transmitted to humans and animals by the tick during feeding.
Given the distribution of vector Ixodes ricinus, the main carrier of the Borrelia burgdorferi spirochete in Romania, and in particular, in Arad, in this work we aimed to identify the prevalence of IgG and IgM antibodies to Borrelia from healthy blood donors in Arad county, in order to continue the investigation regarding the presence of this pathogen in the general population and the rest of the country.
The study was initiated on blood donors residing in Arad. 187 donors were chosen both from the urban and rural area, according to age groups, and in approximately equal proportions of men and women, 105 men (56%) and 82 women (44%) – a greater number of male donors since, according to the literature, the risk of contacting potentially infected ticks is higher in men than in women.
Following the analysis performed, the results of both tests indicate a rate of over 85% negative results and compared with one other, there is a higher percentage of positive IgM than IgG both in the urban and rural areas. The number of positive IgM donors is higher in three age categories, respectively 20-30, 31-40 and 41-50 IgG, as compared to IgG positive donors.

CTS-UTS IMPORTANCE OF COLLABORATION IN SOLVING CASES OF TRANSFUSION.

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In the transfusion we must always have in mind that there is a risk and if not managed properly it will have a negative effect on the achievement of safety objectives transfusion we must take into account the residual risk and the consequences it might have on the smooth

running of the act transfusion
Risk management is coordinated activities to direct and control the activity in a compartment in terms of risk
Attitude towards risk is important and especially its anticipation
The ability to detect the consequences can The influence and risk assessment.
The interface between the prescriber, the doctor in charge of the UTS and the CTS is fundamental to risk management in transfusion activity.
Applications of blood in the hospital precinct required the use of a standardized method safer with minimal risk to transfusion safety.
It is important:
continuously identify nonconformities that emerge, their registration, their classification,
taking immediate corrective measures, their periodical analysis,
undertaking preventive measures resulting from this analysis that contributes to a significant risk reduction, such as the changing circuit UTS and increase space for this activity
Collaboration CTS-UTS
The biggest problems we had with patients suffering from haematological malignancies or otherwise associated with AHA, direct antiglobulin test positive they have an increased risk of hemolysis after blood transfusion. This is due to the presence of unidentified erythrocyte alloantibodies, including haemolytic autoantibodies with strong potential. This mechanism makes selection of a unit of blood transfusion is extremely difficult, complex and risky for the patient. Screening and identification of irregular antibodies was performed by specialized personnel from the CTS's.
Also, cases have posed problems with deficit of immunoglobulins, especially in the elderly, where the need has been working with CTS to be transfused.
Big problems we had patients that have necessitated politransfuzaţi extended phenotype, identification of irregular antibodies to be transfused.
In 2014 I worked especially if you were sick politransfuzaţi number 633 , of which 419 men and 214 women
We performed tests : AI, extended phenotype and special procedures
We advised prescribers regarding the amount of blood , and the type of PSL appropriate for the case .
If there had not been this collaboration premanentă these cases could not be resolved, and patients could not benefit from transfusion.
Although UTS has acquired a standardized equipment to perform transfusions transfusion compatibility issues could not be achieved without special tests and intervention personnel from the CTS and thus require specially trained personnel for these tests which are necessarily required in these cases .

CLINICAL AND BIOLOGICAL EFFECTS OF BLOOD DONATION BY APHERESYS

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Introduction: The present study analyses the manner in which repeated blood donations by apheresys can affect the health of the donors.
Purpose: By knowing the clinical and biological alterations which occur post repeated blood donations by apheresys, the selection of the blood donors can be influenced.
Materials and method: The study group consists of 100 donors with repeated plasma and platelet donations by apheresys on Haemonetics blood cell separators.
Results: For the study group, minor reactions to citrate were observed on 54% of the donors, lipothymia on 20% and cardiac sympthoms on 17%. No platelet counts below that of 100 000/μL were recorded post donating. The mean leucocyte count for the ten donations was of 7 205/μ and it did not vary significantly (p=0,55). The minimal lymfocyte count decreased below 1000/μL for 6 successive donations. The mean lymfocyte count was of 2047 μL for the 10 successive donations. The minimum value of the total protein concentration dropped below 6g/dl after 10 donations. The value of total plasmatic calcium decreased under 8,4 mg /dl on 2,5% of the donors after 5 repeated donations. Ionic calcium decreased under 4 mg/dl on 50% of the donors after 5 repeated donations by apheresys.
Conclusions: The clinical and biological immediate and long term changes which occur on blood components by apheresys donors are transitory and do not endanger the health or the life of the donors.

LOW VARIANTS Rh STUDY AMONG BLOOD DONORS IN BTC BUCHAREST.

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OBJECTIVE:
The aim of this study was to monitor and to compare the different RhD variants and their frequency during 6 monts in CTSMB and their transfusion implications.

MATERIAL/METHOD:
Equipments: fully automated system Qwalys (Diagast), semiautomatic Ortho BoiVue System, semiautomatic Dia Med System.
The negative test was carried out in comparison with ID DiaMed Anti Ig G cassette + Anti D weak serum, technique based on the Coombs test and DiaClon ABO/D test.
The A number of 24.436 samples were taken and processed for Rh, tested routinely with different monoclonal AntiD (Ig M and IgM + Ig) All negative or weak reactions (1+ - 3+) were confirmed with polyclonal anti D ser and DiaClon ABO/D test.
Samples were monitored by using informatic system and archives data study.
RESULTS:
- 135 samples (0,55 %) were D weak and D partial from different category, tested by DiaClon ABO/D test.
- 27,8 % DVI variant and 72,2% Du
- different variant fenotyping are identifying in RhD negative samples :
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 %
- the correlation ABO/ Rh weak: 50,37%A//
31,12%O // 14,07%B // 4,44%AB
- in 2 cases we detected antiD antibodies
CONCLUSION:
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 weak confirmed by molecular biology.
No correlation can be made between blood group and occurrence of RhD variants weak
It is necessary to investigate the anti –D alloimunization rate of the variants to provide a better immune transfusion service

HEMOLYSIS - QUALITY CONTROL PARAMETER FOR BLOOD PRODUCTS ERYTHROCYTE.

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Background:
Hemolysis test is an important parameter of quality control for whole blood and red cell concentrates.
It performs storage at the end of the above products.
Testing is done through the HemoCue Plasma / Low Hb.
The results demonstrate the connection between hemolysis and observing the storage of blood products.
Materials, methods, results:
To test for hemolysis use two devices - a haematological automatic analyzer – Nikohn and a HemoCue microcuvettes analyzer Plasma / Low Hb.
At first analyzer measurements are made in total blood sample or red cells. The sample is harvested respecting the conditions of sampling, the blood component bag tested.
It will work: hemoleucogram retaining our study of hemoglobin and hematocrit value, on haematological automatic analyzer – Nikohn.
The HemoCue Analyzer, determining free Hb from a sample of plasma. The sample is harvested in the same bag the blood component (in the same conditions) is centrifuged (15 minutes at 1500 rpm), and the determination of the free hemoglobin will be obtained in g / dl.
Hemolysis is calculated using the formula: plasma Hb x (100 - Ht) / Hb
Control will be accepted value ≤ 0.8% of hemolysis red blood cells.
Conclusions:
This control parameter, hemolysis, depends of the intensity red cell metabolism during storage. In particular metabolism is influenced by the temperature of storage of the blood component.
Red cell concentrates, whole blood must be kept at a controlled temperature between + 2 ° C and + 6 ° C. The storage time depends on the solution of anticoagulant / preservative used.
For example, the shelf life in CPDA-1 is 35 days and anticoagulant system / additive storage time may be extended to 42 days.

HEMATOPHOBIA – CRYSTALLIZATION OF FEAR TRIGGERED BY A STIMULUS : BLOOD.

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The term “phobia” comes from the Greek word “phobia” – meaning fear, and indicates an anxiety disorder lacking in a precise objective or cause, provoking intense reactions- both psychological and physical. The person experiencing a phobia is self conscious of the irrational aspect of the fear, and anxiety felt, but can not control it, the consciousness being devoid of any rational cognition.
Hematophobia is an abnormal condition characterized by a morbid fear of blood. According to the WHO, it ranks third among the most common phobias after phobia of birds and vacuum.
The possible causes that can lead to the development of hematophobia are: the classical conditioning (associating an unpleasant stimulus to the situation),

transmission through phobic and anxious parental models creating emotional maladjustment reactions- "Take care not to stink yourself, there will be blood" (hematophobia tends to run in the familie).
Symptoms are as follows: slow heart rate and low blood pressure, which can lead to unconsciousness, sweating, persistent and intense fear irrational nature triggered by the presence or anticipation of confrontation with specific stimulus, fear generating situation is avoided by topic.
There is no laboratory test to diagnose this disorder. The psychologist will ask the client to describe the symptoms they experience, in which situations and how often they occur. The main objective of the treatment program is to train the client to be able to confront phobic stimulus, to see blood, to be able to donate blood or endure any other medical interventions involving venipuncture.
Psychotherapy can be achieved through behavioral therapy or cognitive therapy, which may be accompanied by anxiolytic medication. In behavioral therapy, the subject is taught to contract their muscle when confronted with phobic stimulus. This muscle tension will lead to an increase in blood pressure and cardiac syncope is prevented.
Since this is a specific phobia, therapy lasts not more than ten sessions, lasting about sixty minutes per session.

COMMUNICATION STRATEGIES WITH THE BLOOD DONORS FOR COLLECTION EFFICIENCY AND INCREASED SAFETY TRANSFUSION.

DG. Hanganu, M. Catana, D. Gheorghe, M. Coman
BTC Ploiesti

Introduction
Communication is an indispensable element to achieve the objectives interpersonal, organizational. The two entities interchangeable communication information, messages, meanings.
Material and method
Its objectives transfusion cannot be ensured without good communication. The meeting between potential donors / donors and medical staff represents an opportunity for communication in achieving the donation of medical and increase transfusion safety.
Communication is done consistently, the doctor collected by nurses in the area of public access centers recommended by the whole team.
The ability of staff to achieve effective communication with potential donors, donor attendants, non-donor population interacts with the center, the public may influence subsequent behavior and image of the institution.
Effective communication between staff and public centers, comply with the principles:
1) Full and open communication - encouraging health professionals share donors, potential donors, accurate, complete and objective;
2) communication full dignity and respect - respectful attitude towards the public, respecting their values, beliefs, knowledge in medical decision making;
3) participatory communication - donors, potential donors are encouraged to tell their fears, opinions, and participate informed medical act and the decisions that are involved;
4) collaborative communication - health professionals, donors, potential donors and collaborating leadership in achieving a quality medical service.
Effective communication facilitates the acceptance of the medical team potential donation by donors, improves quality of care, increasing compliance and wellbeing of donors. A good compliance donor depends on communication full professionalism, respect, commitment, empathy, confidentiality.
The attitude of the staff in the reception and selection of donors, attitude during medical procedures, giving donors the feeling that they are welcome.
Respect in the relationship between the donor and the medical staff during medical procedures is very important. Donors appreciate the recognition of their ability to understand medical information (want to be recognized as intelligent consumers of information), and knowledge of some aspects of their personal life, besides those related to health. Lack of medical education of many, prevents them understand certain strict medical information, the duty staff to translate this information into a language accessible to any level of education.
Direct communication is an important factor in ensuring blood transfusion safety, seriousness and sincerity of both sides. Hiding honest communication prevents unwanted pathological conditions, prevents negative emotional states affecting potential donors (emotions, fear of needles, blood, white robes, etc) that have unfavorable consequences donation.
Indirect communication, open to the public and can be addressed through: promotion, media paintings, communication panels for people who want to find out about donation and do not interact directly with health professionals in Central television, posters core business, etc. .
Conclusions:
Communicating send a clear, consistent and informative donors. Communication within the organization channeling entire medical team in meeting objectives. Vertical communication from management to the medical team, ensure understanding and acceptance to

organizational objectives, ensuring communication backwards adapting the strategy to achieve the objectives, horizontal communication within the team organization's success.

INCIDENCE AND CLINICAL ASPECTS OF PREGNANCY THROMBOCYTOPENIA – CASES OF THE NATIONAL TRANSFUSION HAEMATOLOGY INSTITUTE

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Introduction
Recent advances in the management of thalassemia have significantly improved life expentancy and quality of life of pacients with thalassemia with a consequent increase in their reproductive potential and desire to have children. Endocrine complication due to haemosiderosis are present in a significant number of thalassemia pacients and often becam bariers in their desire for parenthood.
Method
We investigated all of our adult pacients for endocrinological disease and causes of infertility. All patients have been tested in Endocrinological Clinic of Elias Hospital for LH, FSH, estradiol in women and testosteron in men, free T4, TSH,ACTH and cortisol stimulation in order to investigate hypotalamo-hypophisa axis and prolactin levels in women with amenorrhea. Transvaginal ultrasound has been made in order to estimated number and dimensionsof ovarian follicle. Iron overloud was dterminated by serum ferritin levels every 3 mounth and for few patients by MRI T2* or ferriscan.
Results
Although hypogonadism was frequently found in thalassaemia patients, there are patients with children: three of male(age 24-36 years) with 2 children each and 2 female, one age 35 with tweens and the other age 20 with 2 children from different pregnancies.
Conclusions
Fertility problems can be overcome and pregnancy may be possible and safe for patients eith thalassemia major in terms of transfusion and chelation optimal treatment and supervision of multidisciplinary teams of specialists.

INCIDENCE AND CLINICAL ASPECTS OF PREGNANCY THROMBOCYTOPENIA – CASES OF THE NATIONAL TRANSFUSION HAEMATOLOGY INSTITUTE

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Purpose of the study: We intended to establish the yearly incidence of pregnancy thrombocytopenia in the National Transfusion Haematology Institute. We also studied clynical ad therapeutical aspects related to the delivery movement in these special pregnant ladies.
Material and method: We have studied a number of 196 pregnant ladies, which were surveyed between 2013 and 2015 in the National Transfusion Haematology Institute. In these patients we have studied: complete blood cell caunt with blood film examination, in order to appreciate (or, eventually rectify) the platelet number; complete clotting tests; antiplatelet autoantibodies, anti-phospholipidic antibodies; occasionally – osmotic fragility test, screening test for thrombophylia and genetic mutations for thrombophylia. The patients were surveyed each 6 to 8 weeks.
Results: Out of the 196 pregnant patients we studied, 29 patients (representing approximate 15 per cent) had thrombocytopenia, with a number of platelets between 49.000 and 141.000 per μl. (when examining the blood film, we had values between 60.000 and 152.000 per μl.); we mention that 5 patients had, when examining the blood film borderline – normal values – "false thrombocytopenia"). We also mention that were of the patients surveyed during this period (2013 - 2015) had thrombocytopenia purpura (ITP); we have not notice in any of our pregnant ladies very low platelet values and we admit platelet sequestration in the placenta as the main mechanism of thrombocytopenia. Five of the patients associated pregnancy anaemia (iron and folic acid deficiency); four of them were diagnosed with hereditary spherocytosis and three – with thrombo-philia.
As for therapy – the thrombocytopenia pregnant ladies received vitamin C supplements – 500 mg. daily; we avoided larges doses, in order not to affect foetus metabolism; they also received folic acid, iron supplements and multivitamins.
In cases in which obstetrical conditions allowed physiological delivery, antiplatelet antiantibodies were tested and were absent. Both in cases that and needed Caesarean section and in cases with normal delivery, we adviced and administered packed platelets.
Conclusions: Pregnancy thrombocytopenia is a relatively frequent medical condition, that needs haematological survey during pregnancy and also packed platelets supplements at the delivery moment.

AUTOIMMUNE HEMOLYTIC ANEMIA – PEDIATRIC CLINICAL CASES.

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acquired condition of accentuated hemolysis due to the presence of the antibodies directed against the body's own red blood cells antigens. Autoimmune hemolytic anemia can be classified according to the optimum thermal activity of the anti-red blood cells antigens auto-antibodies in "hot" "cold" "mixed" type AHAI. Depending on the etiology, AHAI are classified in idiopathic, secondary to certain diseases or induced by certain medications. Medicines (penicillin, ampicillin, rifampicillin, tetracycline, cephalosporin etc.) can induce antibodies that connect to the surface of the red blood cells and can determine hemolysis by a series of mechanisms. The hemolysis produced by the action of the antibodies can be intravascular or extravascular and its consequence is severe anemia endangering the patient's life.
Materials and methods: In the last years we had a series of clinical cases of AHAI in children of various ages. We present a case of a three years old child admitted within the Pediatrics Clinical Hospital of Brasov with the initial diagnosis of severe hemolytic anemia, hemoglobin 4 mg/dl occurred during the treatment with broad spectrum antibiotics prescribed by the doctor for a respiratory disease.
The Blood Transfusions Center of Brasov received samples from the patient for the direct Coombs test; then we made some pre-transfusion tests to select the compatible blood.
We used the ID-DIAMED MICRO TYPING SYSTEM gel column agglutination technique: irregular antibodies screening and direct antiglobulinic test (TCD) made with poly-and mono-specific antiglobulic serums.
Results: the Coombs test revealed the presence on the surface of the red blood cell of $C_3c = C_3d$ complement fixating auto-antibodies. No irregular antibodies were highlighted in the serum.
The patient responded very well to the corticotherapy (SOLU-MEDROL) and to the blood transfusion (4 *50 ml CER)
Diagnosis when discharged: medication-induced autoimmune hemolytic anemia, good general condition, no signs of hemolysis, hemoglobin 8 mg/dl.
Conclusions:
Necessary immunohematological evaluation in case of hemolytic anemia
The positive role of a correct immunohematological diagnosis in orienting the clinical diagnosis and for the application of the appropriate therapy, including the transfusions with compatible blood components.
Importance of team work in solving a clinical case.

TRANSFUSION THERAPY IN AUTOIMMUNE HEMOLYTIC ANEMIAS – REVIEW OF THE CASES.

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Introduction. Autoimmune hemolytic anemia (AIHA) is characterized by the presence of auto-antibodies directed against antigens presented on the surface of red blood cells (RBC) which lead to their accelerated destruction. AIHAs are divided into warm antibody AIHAs, cold antibody AIHAs and mixed AIHAs. A major role in the diagnosis of AIHA is held by the Coombs test (direct antiglobulin test), which detects immunoglobulins or complement fragments bound to the RBCs.
Materials and methods. Retrospective study on a period of 2 years, in which we present our experience in the laboratory diagnosis of AIHA, solving the blood-type determination issues, validation of the pretransfusion tests, and finding compatible RBC units for patients diagnosed with AIHA.
We used hemotest serums and the Ortho BioVue micromethod, techniques and reagents which are based on column hemagglutination (Ortho BioVue cases with glass microsphere in an antiglobulin serum medium) in liquid phase at temperatures of 4C, 22C, and 37C, using ficin, papain enzymes.
Results. Twenty-three patients were diagnosed with AIHA, 18/23 (78.3%) patients with warm antibodies, 2/23 (8.7%) patients with cold antibodies, and 3/23 (13%) patients with mixed antibodies. Transfusion therapy was needed in only 7/23 (30.4%) patients. Two out of 23 (8.7%) patients had negative irregular antibodies tests, 21/23 (91.3%) patients had positive irregular antibodies tests, which issued further testing to determine the alloantibodies.
Conclusions. The presence of autoantibodies on the surface of RBCs, but also free in the patient's serum made determining the blood-type, the irregular antibodies, and finding a compatible RBC concentrate difficult.

NURSES SECTION

THE MANAGEMENT OF HAEMOPHILIA - A CHALLENGE IN CLINICAL PRACTICE?

Livia Doria Neacsu
Coltea Clinical Hospital – Hematology Departament

Prophylaxis is administration by intravenous injection of the concentrate of coagulation factors in order to prevent the occurrence of bleeding events.
Prophylactic administration of clotting factor concentrates is advisable to make prior to engaging in higher risk of injury.
Education and training for the patients and parents is an essential component for increasing compliance to prophylactic treatment
Peripheral venous access is associated with a reduced risk of developing infections and gives patients greater freedom, the method of administration recommended in patients with hemophilia.
In the event of a hemorrhagic event, first aid (RICE) is important - but treatment with clotting factor concentrate is absolutely essential. Coagulation factor should be administered as soon as possible to stop the bleeding.
Regular exercises and other measures to stimulate normal psychomotor development should be encouraged to provide muscle tone, develop coordination and balance and improve physical condition.
It is not recommended intramuscular injections and taking aspirin or anti-inflammatory drugs in patients with hemophilia nesteroidienne.

ELIGIBILITY CRITERIA FOR BLOOD DONORS IN PREDONATION LABORATORY

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Self-sufficiency principles arising from voluntary and unpaid donations were recommended and encouraged by the European Council [Article 2 of the Recommendation R (95)].1.4.
The main purpose of selecting blood donors is to determine whether the person is healthy, to protect both the health of donors and recipients.
Laboratory examination before donation:
1. Determining Rh blood group OAB by Beth-Vincent method (with known serum: anti A+B, anti-B, Anti-A);
2. The level of hemoglobin:
   15,5 g/dl. – 16,0 g/dl. for women
   13,5 g/dl. – 18,0 g/dl. for men

* Abnormal values – high or low hemoglobin should be investigated in detail, and decreased hemoglobin concentration less than 2.0 g/dl. between two successive donations.
* At the recommendation of the phisycian consultant, further evaluations may also include: measurements of blood glucose, complete blood count (CBC) from capillary blood.
In Predonation laboratory, a special attention is given to all donors, providing advice on education issues of donation, especially in terms of its impact on the body donor blood donation.

CAELYX THERAPY IN RELAPSED MULTIPLE MYELOMA PATIENTS.

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Colentina Clinical Hospital

Administration liposomal doxorubicin with bortezomib together polietlinglicate patients with multiple mileom.

Bortezomib (Velcade) alone or in combination with doxorubicin liposomal pegylated (Caelyx) is indicated for the treatment of adult patients with progressive multiple myeloma who have received at least one prior treatment and whose who underwent a transplant of hematopoietic stem cells or not indicative of such a transplant.

When the recommended combination therapy with pegylated liposomal doxorubicin (Caelyx), Velcade (3.5 mg powder for solution for injection) is administered by intravenous or subcutaneous injection of the recommended dose of 1.3 mg / m2 body surface area twice weekly for two weeks, on day 1, 4, 8 and 11, as part of a treatment cycle lasting 21 days. This 3-week period is considered a treatment cycle. The interval between consecutive doses of VELCADE must be at least 72 hours. Pegylated liposomal doxorubicin (Caelyx) is administered at a dose of 30 mg / m2 on day 4 of the treatment cycle with Velcade by intravenous infusion over 1 hour after injection VELCADE administered.

In a sterile syringe introduce an appropriate amount of Caelyx. Must be strictly aseptic technique because Caelyx contains no preservative or bacteriostatic agent. Before administration, the appropriate dose of Caelyx must be diluted in 5% glucose solution for infusion (50mg / ml). For doses <90 mg (valid for patients with multiple mileom), Caelyx is diluted in 250 ml. The solution can be infused for 60 or 90 minutes. The use of any diluent other than 5% glucose solution for infusion, or the presence of any bacteriostatic agent such as

benzyl alcohol may cause precipitation of Caelyx. It is recommended that the Caelyx infusion line is connected, through a peripheral catheter at an intravenous infusion of 5% glucose.
The infusion may be given through a peripheral vein. Do not use with in-line filters.

They can be administered up to 8 cycles of the therapy, as long as patients do not tolerate the treatment of the disease progression . Patients who achieved a complete response continue treatment for at least 2 cycles after the first evidence of complete response, even if it means more than 8 treatment cycles.
Also, can continue as long as the treatment is tolerated and continues to respond to this paraprotein patients whose values continue to fall after 8 cycles.

CENTRAL VENOUS CATHETER CARE FOR THE NEUTROPENIC PATIENT.

Stoica Gabriela, Modoran Lenuta, Adina Chivu, Tolan Adriana, Avram Sofia, Giurescu Denisa, Cornea Florentin, Moldoveanu Mihaela, Negrila Cristina, Mihai Beatrice, Nisip Stelica, Goran Mihaela, Manole Mihail, Constantin Dorel, Nita Florica, Sofron Laurentia
Clinical Hospital Colentina – Bucharest / Hematology Departament .

Central venous catheters (CVC), also known as central lines, central venous line or central venous access catheter, is a medical device placed into a large vein, the internal jugular vein, subclavian vein, axillary vein, or femoral vein. It is used to administer fluids and medication, in our particular case chemotherapy, obtain blood tests, and measure central venous pressure
We worked on developing an evidence-based survey on CVC care for patients with severe neuropenia that are treated for hematological malignancies in the Hematology department of Colentina Clinical Hospital – Bucharest. Our prospective study addresses catheter type, insertion site, and placement as well as prophylaxis and management of both catheter-related infection and thrombosis. The data we processed are gathered from a lot of 250 patients that were submitted to our clinic during the last 24 months and presented with or required a CVC. More than 80% of the catheters were used at the maximum-prescribed time interval and were safely removed in our clinic, under the strict supervision of our intensive care personnel. The most frequent challenge that we faced working with CVC in our clinic was the thrombosis of the venous device, with the possibility to re-obtain function after intensive care intervention in less than 30% of the cases. Catheter infection was the second main problem related with CVC handeling; we obtained positive cultures in 25% of the suspected cases of infection.The data that we managed to centralize highlight the already well know facts that appropriate catheter handling, sufficient operator experience, careful technique, and proper catheter maintenance with removal as soon as possible are associated with optimal outcome.

CURRENT CONCEPTS OF CARE OF THE ACUTE LEUKEMIA PATIENT IN POSTCHEMOTHERAPY APLASIA.

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Acute leukaemia is a malignant disorder characterized by proliferation of immature hematopoietic cells and cellular maturation of these precursors and by inhibiting normal haematopoiesis.

Depending of the affected cell line the acute leukemias are classified into two broad categories: myelogenous and lymphoblastic. The treatment in accordance with international protocols is represented by chemotherapy, in order to induce complete remission. As a side effect of chemotherapy, patients develop post chemotherapy aplasia, characterized by severe neutropenia, severe anemia, severe thrombocytopenia. The main complications of bone marrow failure syndrome are: major risk of infection, bleeding, affected general status with marked asthenia, fatigue, dyspnoea, tachycardia, fever.
The role of the medical assistant both before and especially after chemotherapy treatment is extremely important, it must prepare solutions for infusion in an aseptic, controlled manner, it must monitor the administration of treatment (risk of extravasation of chemotherpy) and patient's haemodynamic parameters.
Because of the severe neutropenia it is demanded that the patient is isolated in sterile rooms, has limited visitation, the medical personnel is using adequate sanitation materials (special sterile equipment by wearing gloves, gowns, disposable shoes and disposable protective masks) during the collection of biological samples, and during the administration of treatment. An important role in prevention of infections of the patient is represented by rigorous hygiene, and education in pursuit of its warning signs (fever, chills, bleeding, appearance of bleeding).
In patients with acute leukemia it is preferred that the treatment is administered threw a central venous catheter, which involves strict rules, aseptic handling

specifications, and requires adequate training of medical personnel. Complications of this CVC implantation include infection and the thrombotic risk.
Conclusion: The acute leukemia patient by the hematologic disease particularity, and the associated complications requires careful handling from a multidisciplinary team, experienced in managing these cases in order to ensure and improve the quality of life and decrease the risk of morbidity and mortality.

THE CHEMOTHERAPY TREATMENT IN THE HEMATOLOGY DEPARTMENT OF COLENTINA CLINICAL HOSPITAL.

Manole Mihail, Stoica Gabriela, Modoran Lenuta, Adina Chivu, Tolan Adriana, Avram Sofia, Giurescu Denisa, Cornea Florentin, Moldoveanu Mihaela, Negrila Cristina, Mihai Beatrice, Nisip Stelica, Goran Mihaela, Constantin Dorel, Nita Florica, Sofron Laurentia
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Chemotherapy is a category of cancer treatment that uses chemical substances, especially one or more anti-cancer drugs (chemotherapeutic agents) that are given as part of a standardized chemotherapy regimen. Chemotherapy may be given with a curative intent, or it may aim to prolong life or to reduce symptoms (palliative chemotherapy). Traditional chemotherapeutic agents are cytotoxic, that is to say they act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side-effects of chemotherapy: myelosuppression, mucositis, and alopecia.
Most chemotherapy is delivered intravenously, although a number of agents can be administered orally. There are many intravenous methods of drug delivery, known as vascular access devices. These include the winged infusion device, peripheral cannula, midline catheter, peripherally inserted central catheter (PICC), central venous catheter and implantable port. The devices have different applications regarding duration of chemotherapy treatment, method of delivery and types of chemotherapeutic agent.
The chemotherapy must be sterile, so the patient does not get infected by the chemotherapy as it is administered directly into his bloodstream. The chemotherapy must be made accurate, which means that it is made exactly as the doctor prescribes it. It must be the right drug and the right dose. The fluid that contains the drug must be right. Also, the chemotherapy must be labeled accurately to include the necessary information. The chemotherapy must be made in a timely manner that also guarantees sterility and accuracy. Timely means the least amount of time that still produces an accurate and sterile product.
In this presentation we would like to make an overview of how chemotherapy should be prepared in a Hematology Department, and we would like to show how we do it in our ward.
In the Hematology Department of Colentina Clinical Hospital we respect these 3 most important rules of chemotherapy preparation: first - the infusion is prepared exactly as the doctor prescribes it (after he calculates the body surface area and he makes sure that the blood tests permit the administration of the treatment without risks), second - the preparation is made at the precise time so every infusion bag stays sterile while in use, and third - the infusion bags are prepared in a sterile environment (Biological Safey Cabinet) by a trained medical assistant who is wearing protective equipment.
Of course, one other thing that we take into consideration is the management of the venous line that we administer the chemotherapy, weather is a peripheral cannula or a central venous catheter.

SPECIFIC FEATURES OF BLOOD TRANSFUSION IN IMMUNOSUPPRESSED PATIENTS.

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Introduction: Patients hospitalized in hematology-oncology departments are immunosuppressed both by the nature of their disease, in general as it is a haematological malignancy, and as a consequence of their treatment. They usually require frequent blood transfusions, with special issues in selection of blood products, blood administration, transfusion surveillance and potential complications.
Matherials and methods: There will be presented aspects of ABO groupage difficulty in some patients with lymphoproliferative diseases due to absence / poor expression of agglutinins in the serum of these patients, clinical, prevention and treatment of post-transfusion graft versus host disease in patients treated with purine analogues, after transplantation of hematopoietic stem

cells or some patients with lymphomas, aspects concerning the risk of transmission of bacterial infections or other infections, particularly cytomegalovirus, via blood products transfused, with high potential for severe adverse events in immunosuppressed patients, the risk of anaphylaxis reactions in patients with selective IgA deficiency.
There will also be presented the indications for transfusion of phenotyped in Rh / Kell systems blood products, leucocyte-depleted and irradiated blood products, and the role of nurses in providing transfusion safety and rigorous monitoring of immunosuppressed transfused patients.

Conclusions: Blood transfusion in immunocompromised patients require special precautions in selecting blood products and ensuring blood transfusion safety and nurse involved in care of these patients must know and apply actively measures to prevent potential severe transfusion complications in this population.