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3rd International Symposium and Advanced Postgraduate Course in CHRONIC GRAFT-VERSUS-HOST DISEASE: CLINICAL PRACTICE AND RESEARCH

Abstract Book

Zagreb, Croatia
September 20-21, 2016

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Nina Grković

Authors:

Basak Grzegorz
Bilić Ernest
Bilić Ervina
Bojanić Ines
Duarte Rafael
Duraković Nadira
Dušek Davorka
Dwilewicz-Trojaczek Jadwiga
Gooley Ted
Greinix Hildegard
Halter Jörg
Ilić Ivana
Jędrzejczak Wiesław Wiktor
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Klepac Pulanić Tajana
Knobler Robert
Krnić Nevena
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Lee Stephanie
Ljubas Kelečić Dina
Mravak-Stipetić Marinka
Nemet Damir
Olivieri Attilio
Pavletic Steven Z.
Perić Zinaida
Petriček Igor
Pulanić Dražen
Pusic Iskra
Schoemans Helene
Schultz Kirk
Serventi-Seiwerth Ranka
Vrhovac Radovan
Vukić Tamara
Wolff Daniel

Address:
Bilten Krohema - The Journal of the Croatian Cooperative Group for Hematological Diseases CROHEM,
Buzinski prilaz 1, 10 010 Zagreb, Croatia

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Welcome words

Dear friends and colleagues,

It is our great pleasure to welcome you to the 3rd International Symposium and Advanced Postgraduate Course in Chronic Graft-versus-Host Disease in Zagreb, Croatia, September 20-21, 2016.

Approximately 50% of patients after allogeneic hematopoietic stem cell transplantation (alloHSCT) will develop chronic Graft-versus-Host Disease (cGvHD), which can last for many years, causing severe medical, social and quality of life problems. However, more than ever before, the tools are at our hands to address cGvHD conclusively and improve outcomes of patients after alloHSCT. Such progress in a such relatively rare and complex disease can be achieved only by assertive interdisciplinary and multicenter international collaboration.

Chronic GvHD is now much better characterized using the National Institutes of Health classification, developed in 2005, prospectively validated and further refined in 2014. However, not a single agent has yet been approved by regulatory agencies for cGvHD prevention or treatment. The current standard front-line steroids therapy has a 50% failure rate with significant toxicity, and there are no standard salvage therapy options. The focus now is on further in-depth study of cGvHD biology, developing and validating new biomarkers, and pursuing clinical trials of emerging new agents. To break off from the 30-year-old suboptimal treatment paradigms, the goal should be that each cGvHD patient is either treated in a clinical trial or documented within a registry capturing essential clinical data.

This advanced postgraduate course brings together prominent experts in the field of cGvHD from Europe, United States of America, and Canada. For example, the lecturers are experts from the German-Austrian-Swiss cGvHD Consortium, the cGvHD subcommittee of the Complications and Quality of Life Working Party of the European Society of Blood and Marrow Transplantation, the National Cancer Institute National Institutes of Health (Bethesda, USA), the Fred Hutchinson Cancer Research Center (Seattle, USA), and many others. The goal is to systematically cover the basic principles of diagnosis, organ-specific and systemic therapy, and supportive care of cGvHD.

At this time, special focus is given to aspects of clinical trials development and design, with the goal to empower the participants to take part in and develop such studies in their own institutions.

This event is dedicated to physicians who are engaged in management and advancing care of alloHSCT patients. The goal of the meeting is also to provide a venue for networking and establishing contacts for our future collaboration in addressing cGvHD, and to create a critical mass of investigators and colleagues who will carry this field in the near future.

We hope that this 3rd GvHD symposium in Zagreb will continue to grow as a regular advanced continuing education experience in this rare but important and devastating disease.

With kind regards and a warm welcome to Zagreb,

Prof. Steven Z. Pavletic, Bethesda, MD, USA,
Assist. Prof. Dražen Pulanić, Zagreb, Croatia,
and
Prof. Damir Nemet, Zagreb, Croatia

Symposium and Course Information:

3rd International Symposium and Advanced Postgraduate Course in Chronic Graft-versus-Host Disease: Clinical Practice and Research, Zagreb, Croatia

Organizers:

University Hospital Center Zagreb and School of Medicine, University of Zagreb, Zagreb, Croatia
National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
Croatian Cooperative Group for Hematological Diseases (CROHEM), Zagreb, Croatia

Course directors:

Steven Z. Pavletic (Bethesda, USA),
Stephanie Lee (Seattle, USA),
Hildegard Greinix (Graz, Austria),
Dražen Pulanić (Zagreb, Croatia),
Damir Nemet (Zagreb, Croatia)

International Faculty and Lecturers:

Basak Grzegorz W. (Warsaw, Poland)
Duarte Rafael F. (Madrid, Spain)
Gooley Ted A. (Seattle, USA)
Greinix Hildegard (Graz, Austria)
Halter Jörg (Basel, Switzerland)
Knobler Robert (Vienna, Austria)
Lawitschka Anita (Vienna, Austria)
Lee Stephanie (Seattle, USA)
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Schoemans Helene (Leuven, Belgium)
Schultz Kirk R. (Vancouver, Canada)
Wolff Daniel (Regensburg, Germany)

Local Scientific Committee and Speakers:

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Desnica Lana (hematology)
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Pulanić Dražen (hematology)
Serventi-Seiwerth Ranka (hematology)
Vrhovac Radovan (hematology)
Vukić Tamara (physical and rehabilitation medicine)
Zadro Renata (medical biochemistry and laboratory medicine)

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Croatian Academy of Sciences and Arts, Illyrian Hall, Opatička 18, 10000 Zagreb, Croatia

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September 20-21, 2016

Official language:

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Badges:

All participants are required to wear their badges throughout the symposium

Certificate of attendance:

Certificate of attendance will be distributed the last day of the symposium (September 21st, 2016)

Further information available at:

https://eventyco.com/e/12179

Program overview

Tuesday, September 20th

7,30-08,30 Registration of participants

1. Basic principles (chairs M. Mohty, D. Nemet)

8,30-8,40 D. Nemet (Croatia) and S.Z. Pavletic (USA): Opening and introduction to the symposium
8,40-9,00 R.F. Duarte (Spain): Incidence, trends and risk factors
9,00-9,30 H. Greinix (Austria): Pathophysiology/Biology of cGVHD

2. Clinical diagnosis and management (chairs S. Lee, D. Pulanić)

9,30-10,00 R. Serventi-Seiwerth (Croatia): Diagnosis and staging of cGVHD
10,00-10,30 I. Ilić (Croatia): Histopathology of cGVHD
10,30-11,00 M. Mohty (France): Prevention of cGVHD

11,00-11,20 Coffee break

11,20 -11,40 R. Vrhovac (Croatia): Front line systemic therapy
11,40-12,00 D. Wolff (Germany): Salvage systemic therapy
12,00-12,20 S.Z. Pavletic (USA): Emerging drugs for cGVHD

12,20-12,40 Panel Discussion, Moderator: H. Greinix

12,40-13,40 Lunch and poster viewing

3.a Organ specific manifestations and treatments – brief overview and case reports
(chairs S.Z. Pavletic, J. Halter) – 1st part

13,40-14,00 R. Knobler (Austria): Cutaneous manifestations
14,00-14,20 M. Mravak-Stipetić (Croatia): Oral cGVHD
14,20-14,40 I. Petriček (Croatia): Ocular cGVHD
14,40-15,00 T. Klepac Pulanić (Croatia): Genital cGVHD

15,00-15,40 Panel Discussion and Case Studies, Moderator: D. Wolff

3.b Organ specific manifestations and treatments (chairs G.W. Basak, R. Vrhovac) – 2nd part

15,40-16,00 J. Halter (Switzerland): Lung cGVHD
16,00-16,20 E. Bilić (Croatia): Neurological manifestations in cGVHD
16,20-16,40 T. Vukić (Croatia): Joint/fascia manifestations and rehabilitation medicine role in cGVHD

16,40-17,00 Panel Discussion, Moderator: A. Olivieri

17,00-17,20 Coffee break

3.c Other manifestations and treatments (chairs R. Knobler, R. Serventi-Seiwerth) – 3rd part

17,20-17,40 D. Dušek (Croatia): Infections in cGVHD
17,40-18,00 D. Pulanić (Croatia): Platelets and coagulation in cGVHD
18,00-18,20 Z. Perić (Croatia): Psychosocial issues and HRQOL in cGVHD
18,20-18,40 D. Ljubas-Kelečić (Croatia): Nutrition in cGVHD

18,40-19,00 Panel Discussion, Moderator: H. Schoemans

Wednesday, September 21th

4.a Clinical trials, biomarkers and drug development – 1st part (chairs R.F. Duarte, R. Zadro)

8,30-8,50	K.R. Schultz (Canada): Translating from mouse to man
8,50-9,10	K.R. Schultz (Canada): Biomarkers in cGVHD
9,10-9,30	G. Lauc (Croatia): IgG glycosylation in cGVHD
9,30-9,50	I. Bojanić (Croatia): ECP mechansims and efects in cGVHD
9,50-10,10	Panel Discussion, Moderator: D. Batinić
10,10-10,30	Coffee break

4.b Clinical trials and drug development – 2nd part (chairs T.A. Gooley, L. Desnica)

10,30-11,00	S. Lee (USA): Issues in the design of cGVHD clinical studies
11,00-11,30	A. Olivieri (Italy): Evaluating therapeutic response in cGVHD by NIH criteria
11,30-11,50	H. Schoemans (Belgium): Modern media technology in cGVHD evaluation
11,50-12,40	T.A. Gooley (USA): Study design - observational cohort studies, early and late therapy
12,40-13,40	Lunch and poster viewing
13,40-14,30	Logistical and regulatory challenges in conducting studies in cGVHD: Panel Discussion and practical examples, Moderators: S. Lee and T.A. Gooley

5. General principles and putting it all together (chairs H. Greinix, D. Nemet)

14,30-14,50	D. Nemet (Croatia): Establishment of multidisciplinary team for cGVHD
14,50-15,10	A. Lawitschka (Austria): Pediatric aspects of cGVHD
15,10-15,30	E. Bilić (Croatia): Late effects in children
15,30-15,50	N. Duraković (Croatia): Late effects and cGVHD – and are they connected?
15,50-16,10	G.W. Basak (Poland): GvL effect of allogeneic transplantation
16,10-17,00	Panel Discussion: Models and barriers to cGVHD care delivery, Moderator: R.F. Duarte
Introduction:	I. Pusic (USA) – models of care delivery in the US (10 min)
Introduction:	H. Schoemans (Belgium) – models of care delivery in Europe (10 min)
Participants:	S. Lee, H. Greinix, D. Wolff, S.Z. Pavletic, D. Pulanić
17,00	Closing of the symposium: S.Z. Pavletic, D. Nemet, S. Lee, H. Greinix, D. Pulanić
20,00	Farewell dinner and networking reception

Incidence, trends and risk factors

Rafael F. Duarte
Department of Clinical Hematology, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain

With over thirty thousand allogeneic hematopoietic cell transplants (HCT) performed worldwide every year, and a continuous increase in allogeneic HCT numbers for more than three decades with no signs of saturation, the incidence and prevalence of chronic Graft-versus-Host Disease (cGvHD) in allogeneic HCT recipients will continue to increase in the coming years. In addition to being the main cause of non-relapse mortality late after allogeneic HCT, cGvHD is the main determinant of late morbidity, impaired functional status and reduced quality of life in allogeneic HCT survivors.

Broadly speaking, approximately half of allogeneic HCT recipients develop cGvHD. The incidence, however, may vary widely from 20% to over 80% depending on patient and transplant risk factors. In recent years, the NIH criteria for diagnosis of cGvHD have allowed us to distinguish the pleiotropic manifestations that characterize cGVHD from those of acute GvHD regardless of timing after HCT. Classic cGvHD can be now distinguished from overlap subtype of cGvHD and from late acute GvHD. Of note, the diagnosis of cGvHD according to the NIH criteria has an impact on its reported incidence, which would be naturally lower than with previous diagnostic criteria that included as well late acute forms of the disease. Such differences in definitions and diagnostic criteria have also had an impact on the identification of risk factors in previous studies.

A prior history of acute GvHD is perhaps the most important antecedent to the development of cGvHD. However, acute and cGvHD are not a continuation of a single syndrome, have differences in pathogenesis and clinical presentation, and beyond some common risk factors, there are predictors with a particular importance for cGvHD. It is worth noting that associations between risk factors and cGvHD risk and outcomes vary among studies, as they are mostly retrospective in nature and heterogeneous regarding patient and transplant characteristics. Overall, it is well established that the increasing use of mobilized peripheral blood grafts as cell source, compared to bone marrow, increases the risk of cGvHD and the duration of immunosuppressive therapy that it requires, without apparently influencing acute GvHD. In addition, older patient age and female to male recipient/donor sex mismatch both seem to pose a greater impact on the risk of cGvHD than on acute GvHD. Progressive onset of cGvHD from prior acute GVHD and thrombocytopenia are consistently associated with increased non-relapse mortality and poorer outcome in patients with cGvHD. A large CIBMTR registry analysis developed a 10-variable risk score that could clearly stratify non-relapse mortality and overall survival in patients with cGvHD diagnosed prior to the NIH criteria. Albeit externally validated, their applicability to NIH-diagnosed cGvHD needs further consideration. Multiple additional risk factors play a role in the development and severity of cGvHD, such as the use of donor lymphocyte infusions, HLA-mismatched donors, donor type, the use of TBI, Karnofsky performance status and serum bilirubin level. Further research into cGvHD biology and pathophysiology, on the development and validation of new biomarkers and prospective analysis of the natural history of the disease will give us further understanding of the trends of incidence and the risk factors involved in this most important complication of allogeneic HCT.

Pathophysiology/Biology of chronic Graft-versus-Host Disease

Hildegard T. Greinix
Division of Hematology, Medical University of Graz
Graz, Austria

Allogeneic hematopoietic cell transplantation (HCT) is an established curative treatment for selected patients with hematologic and oncologic diseases. Graft-versus-Host Disease (GvHD) has remained a serious complication of allogeneic HCT occurring in an acute and chronic form. Chronic GvHD (cGvHD) is a major cause of mortality after HCT and deleteriously affects the quality of life in surviving patients otherwise cured of their underlying disease. During the last decade the incidence of cGvHD has increased due to older age of transplant recipients, use of peripheral blood stem cells instead of bone marrow as stem cell source, use of mismatched and unrelated donors, and treatment with donor lymphocyte infusions for recurrent malignancy after HCT. Manifestations of cGvHD resemble those seen in autoimmune diseases and autoantibodies have been frequently observed.

Recent preclinical and clinical studies provide an improved insight into the pathophysiology of cGvHD that had been poorly understood for decades. Thymic damage caused by the conditioning regimen, as well as prior acute GvHD, leads to decreased negative selection of alloreactive T cells, immune deviations resulting in release of inflammatory and fibrogenic cytokines such as interleukin 2 (IL-2), interleukin-10 and transforming growth factor ß, activation of macrophages and fibroblasts, and eventually in tissue fibrosis. A relative deficiency of regulatory T (Treg) cells as a consequence of abnormalities in Treg homeostasis in patients with lymphopenia and cGvHD has been observed. Low-dose IL-2 administration resulted in enhancement of Treg thymic neogenesis, restoration of Treg homeostasis and clinical improvement of patients with cGvHD.

Besides donor-derived $CD4^+$ and $CD8^+$ T cells, other cell populations such as B cells are also of major importance in the biology of the GvHD reaction. Donor B cell responses to recipient HY antigens have been associated with the development of cGvHD in the setting of gender-mismatched HCT. Besides these alloantibodies, autoantibodies against platelet-derived growth factor (PDGF) receptor are of importance in sclerodermatous cGvHD, since they are known to induce tyrosine phosphorylation, accumulation of reactive oxygen species (ROS) and stimulate type I collagen gene expression through the Ha-Ras-ERK1/2-ROS signaling pathway. All these processes are implicated in inflammation and tissue fibrosis. A distortion of B cell homeostasis with increased proportions of $CD19^+CD21^{low}$ B cells and reduced numbers of $CD19^+CD27^+IgD^+$ non-class switched and $CD19^+CD27^+IgD^-$ class-switched memory B cells has been observed in patients with cGvHD. Besides abnormalities in B cell subpopulations, elevated levels of B cell activation factor (BAFF) leading to increased BAFF/B cell ratios characterize patients with cGvHD. Lack of BAFF consumption by BAFF-R expressing peripheral B cell subpopulations due to B lymphopenia could lead to expansion and survival of autoreactive B cell clones newly emigrated from the bone marrow. Excess of BAFF also led to increased metabolic activity and survival of B cells in patients with cGvHD.

Thus, deficiencies of Treg cells and dysregulation of B cell homeostasis can result in emergence of autoreactive B cell subpopulations and the production of autoantibodies contributing to cGvHD. In view of the dismal prognosis of some patients with cGvHD, their prolonged need for and lack of response to immunosuppressive therapies novel research findings are urgently needed to improve prophylaxis and treatment of cGvHD.

Main educational points/Learning goals:

- Current concepts of T cell and B cell involvement in the pathophysiology of cGvHD;
- Role of regulatory T cells in cGvHD;
- Dysregulation of B cell homeostasis in cGvHD;
- Pathogenesis of tissue fibrosis as hallmark of cGvHD.

Diagnosis and staging of chronic Graft-versus-Host Disease

Ranka Serventi Seiwerth
Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb, Zagreb, Croatia

Chronic Graft-versus-Host Disease (cGvHD) is a multisystem alloimmune and autoimmune disorder that occurs in 10 - 80 % (50%) of stem cell transplantation long-term survivors. Changing conditioning regimens from standard myeloablative to reduced intensity regimens and decreasing early transplant related mortality resulted in increased proportion of patients who develop cGvHD. Reliable incidence of cGvHD was compromised by the lack of standardized diagnostic criteria, polymorphic chronic clinical course that can mimic alternative diagnoses and still poor understanding of the disease, combined with diversity of observer experience, limited expert follow-up as well as different statistical methods applied. Lack of standardized criteria and definitions of diagnosis of cGVHD were the major obstacles for treatment progress.

In the last decade, transplant physicians have made huge efforts to overcome these problems.

In 2005, the NIH Consensus Conference on cGvHD developed criteria for diagnosis and staging of cGvHD that define minimal clinical diagnostic criteria for cGvHD diagnosis, as well as clinical distinction between acute and chronic GvHD.

Manifestations of cGvHD may be restricted to a single organ or tissue, but more often 2 or 3 organs are affected. Skin, mouth and eyes are most commonly affected in about 50% of patients. Gastrointestinal tube, liver, lungs, and female genital organs are also frequent sites of the disease. **NIH Consensus Criteria** define criteria for each site as ***diagnostic*** (sufficient to establish diagnosis of cGvHD); ***distinctive*** (seen in cGvHD, but alone insufficient to establish diagnosis of cGvHD) and ***other features*** or ***unclassified entities***. Revised 2014 NIH criteria recognize **common features** as signs and symptoms found in both acute and chronic GvHD. The milestone for the diagnosis is always physical examination and history accompanied with laboratory findings and imaging or functional testing for localizations, or even endoscopy procedures followed by biopsy and histopathology. Chronic GvHD ***severity*** is graded for each affected organ **from 0 to 3**. NIH 2014 Consensus proposed some changes in diagnosis and severity staging.

Global NIH* score** can be ***mild, moderate or severe due to number of affected sites and severity of symptoms in affected organs.

Scoring organ symptoms and global cGvHD severity score are important for individual therapy planning (local/ancillary therapy and supportive care or systemic immunosuppressive therapy) for the best balance between devastating and debilitating effects of cGvHD and Graft-versus-Tumor effect till immunotolerance is achieved.

Symptoms of cGVHD usually occur between 3 months and 2 years after stem cell transplantation and are present in about 75% of patients within the first year. Earlier definition of cGvHD as disease occurring later then day + 100 after stem cell transplantation or donor lymphocyte infusion (DLI) is recognized as imprecise and unreliable since acute GvHD can occur late after transplant ***(late onset acute GvHD)***. There are no time limits for the onset of ***classic cGvHD.*** In some patients symptoms of acute and chronic GvHD are present at the same time ***(overlap syndrome***). Overlap syndrome, a distinct category of cGvHD, has been precisely defined in 2014 revised NIH criteria.

The pattern of onset of cGvHD also differs: it can progress from acute GvHD ***(progressive cGvHD*)**, appear after symptoms of acute GvHD were resolved (***quiescent cGvHD***) or it can appear as the first manifestation of GvHD (***de novo cGvHD***). Patients with progressive cGvHD have worse prognosis than other two groups, since these are usually patients who progress while still on corticosteroids.

Main educational points/Learning goals:

NIH criteria for diagnosis of cGvHD define signs and symptoms in each affected organ/tissue and recommend scoring of each affected organ/site that describes severity at any given time;

NIH cGvHD global severity staging is derived from combining organ specific scores;

This criteria allow better communication in transplant community and better and individualized therapy planning for cGvHD patients;

They have also replaced the old, mostly descriptive criteria/nomenclature of “limited/ extensive cGvHD” that occurs later than day +100 after transplantation or DLI.

Histopathology of chronic Graft-versus-Host Disease

Ivana Ilić
Department of Pathology and Cytology, University Hospital Center Zagreb, Zagreb, Croatia

Graft-versus-Host Disease (GvHD) is a systemic disease targeting multiple organs; thus the decision which organ to choose for a biopsy depends on which system is giving the symptoms. Even though usually concrete and infallible, histopathological examination is not the gold standard for the diagnosis of chronic GvHD (cGvHD). The changes in organs affected by cGvHD are seldom characteristic and unmistakable. This is why the decision to treat cGvHD should be based on the overall clinical assessment and not histopathology results.

The skin is our largest organ, most frequently affected by cGvHD, easy reachable and therefore the site of the most frequent biopsies when there is doubt of cGvHD. Histopathological changes seen in skin affected by cGvHD can be roughly divided into two categories: lichen planus-like changes and scleroderma-like changes. Each of these two categories has several subtypes such as poikiloderma and lichen sclerosus-like lesions. In lichen planus-like type of cGvHD, one can see hyperkeraois, hypergranulosis, acantosis, dyskeratosis, vacuolar degeneration and colloid bodies in epidermis, as well as lymphocytes and plasma cells around blood vessels and adnexa in the dermis, but often "lichenoid" along dermoepidermal junction. Poikiloderma is characterized by epidermal atrophy, loss of rete rigdes, and few if any lymphocytes and plasma cells in the dermis. The destruction of adnexa can often be seen. In sclerotic type there are deep and thick collagen bundles in the dermis, with or without panniculitis. Destruction and fibrosis of adnexa can be seen through the skin, but usually the most striking are on the hairy part of the head and on the nails. However, one should be aware that, based on histopathology alone, it is impossible to distinguish lichenoid GvHD from idiopathic or drug-induced lichen planus. Moreover, skin changes in sclerotic cGvHD and scleroderma are identical.

Oral and genital mucosa, as well as genital skin, have similar histological appearance as the skin, so the cGvHD changes in these areas are similar to those seen in the skin. In the mouth, there can be mucosal atrophy and destruction of salivary glands with subsequent fibrosis.

Small salivary glands and lacrimal glands may show destruction and fibrosis of acinar structures, but more often lymphocytic infiltration and damage of intralobular ducts with periductal fibroplasias.

Histopathological findings of cGvHD in the gastrointestinal system include chronic inflammation and submucosal fibrosis with a destruction and loss of the glands. Additionally, desquamation and ulceration of mucosa can be found. These changes are not entirely specific for cGvHD.

In the liver, there is small bile duct atypia and damage of ductal epithelium with drop-out necrosis and lymphocytic infiltrate in the portal area, while in the parenchyma there is cholestasis and ballooning of the hepatocytes. These changes are mostly unspecific and can also be seen in infections, drug toxicity, venoocclusive disease and malignant tumors.

Lungs are rarely biopsied because of frequent and severe life threatening complication, even though the different forms of lung cGVHD can have a different outcome and therefore needs different treatment. Histopathological changes seen in the lung include small airway ducts obliterated by granulation tissue or fibrous plugs in the form of obliterative bronchiolitis or obliterative bronchiolitis with organizing pneumonia, but also chronic interstitial inflammatory infiltrate with destruction of acinar tissue and fibrosis.

All of the described histopathological changes are signs of chronic inflammation that lead to tissue destruction and cannot be linked to any specific cause or disease. Therefore, there is no histopathological diagnosis of cGvHD "per se". Clinical information about cGvHD is mandatory. It is only after having insight into all the necessary information about the patient that the pathologist can make one of the following statements: not GvHD, possible GvHD and probable GvHD.

Rabbit Anti-Thymocyte Globulin for Graft-versus-Host Disease prevention in allogeneic hematopoietic stem cell transplantation

Mohamad Mohty
Hopital Saint-Antoine, and University Pierre & Marie Curie, Paris, France

Understanding the mechanisms underlying immunological tolerance is the key to successful transplantation. In the 30 years since the rabbit Anti-Thymocyte Globulin (rATG) was first licensed, its use in solid organ transplantation and hematology has expanded progressively.

In allogeneic hematopoietic stem cell transplantation, rATG has become an important component of both conventional myeloablative and reduced-intensity conditioning regimens, following demonstration of reduced acute and chronic Graft-versus-Host Disease (GvHD) in both retrospective and prospective trials.

The common belief is that rATG efficacy relies on its capacity to deplete T lymphocytes. However, the polyclonal nature of rATG is reflected in its diverse effects on the immune system: (1) T-cell depletion in blood and peripheral lymphoid tissues through complement-dependent lysis and T-cell activation and apoptosis; (2) modulation of key cell surface molecules that mediate leukocyte/endothelium interactions; (3) induction of apoptosis in B-cell lineages; (4) interference with dendritic cell functional properties; and (5) induction of regulatory T and natural killer T cells. As a consequence, ATG provides multifaceted immunomodulation paving the way for several applications to help reduce the incidence of organ rejection and GvHD.

Despite its long history, rATG remains a key component of the immunosuppressive armamentarium for GvHD prevention. The polyclonal nature of rATG preparations provides the basis for multifaceted immunomodulation that is worth to be continuously investigated, especially with the increasing use of mismatched stem cell allografts in high risk patients, the discovery of different new mechanisms of action and a better knowledge of dosages, safety and tolerance.

Front line systemic therapy for chronic Graft-versus-Host Disease

Radovan Vrhovac
Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

Systemic therapy in patients with chronic Graft-versus-Host Disease (cGvHD) should be initiated when there is a high risk of cGvHD-related morbidity and mortality. Selecting patients who need systemic treatment depends upon the number of organs involved, severity of symptoms, characteristics of the underlying hematologic disease, blood counts, organ impairment, etc.

Most treatments are based on the immunosuppression of donor T cells that are responsible for GvHD but also for the immunological Graft-versus-Tumor (GvT) effect. Therefore, the benefit of reducing GvHD always needs to be weighed against the potential harm of decreasing a GvT effect.

The decision regarding treatment is further complicated by the fact that not all patients are the same. Patients with cGvHD have not received the same prophylaxis, they have different clinical presentations, may or may not have had prior acute GvHD, etc. Some patients who develop cGvHD may already be on immunosuppressive therapy. In these cases, optimization of drug dose in order to ensure therapeutic levels might be the only intervention that is needed.

Glucocorticoids are the most commonly used front line systemic treatment. Prednisone at a dose of 1 mg/kg/ day, or methylprednisolone at a dose of 0.8 mg/kg/day are usually given as initial therapy. In case of stable or improving symptoms in a patient after 2 weeks of therapy, glucocorticoid dose is usually tapered by 25% per week to a target dose of 1 mg/kg every other day. Once the symptoms are fully resolved the dose is further tapered with the ultimate goal to either discontinue, or use the lowest acceptable dose of corticosteroids to control symptoms. Some patients require treatment lasting several years, and in some instances even lifelong.

If symptoms progress at 2 weeks or no clinical improvement is apparent by 4-6 weeks, therapy is usually escalated. Patients who develop cGvHD while already receiving systemic glucocorticoids (for acute GVHD, for example) are treated with additional immunosuppressive agents in the first line. It is important to emphasize that patients remarkably benefit from specific supportive care targeted at organs affected by cGvHD. This requires high level of coordination of different specialists, and can only be achieved within a multidisciplinary team. These aspects of cGvHD treatment are discussed in more detail elsewhere.

Whenever possible, patients requiring systemic therapy should be enrolled in clinical trials. A few randomized trials have investigated the addition of other immunosuppressive and immunomodulatory agents (cyclosporine, azathioprine, thalidomide, mycophenolate mofetil) to prednisone in the initial treatment of cGvHD. Only the addition of cyclosporine demonstrated a potential clinical benefit by decreasing prednisone exposure. The prednisone-cyclosporine combination reduced the risk of avascular necrosis connected with glucocorticoids. However, no significant differences between the treatment arms in terms of overall- and transplant-related mortality, relapse rate, or discontinuation of all immunosuppressive therapy were observed.

Main educational points/Learning goals:

- Systemic therapy is indicated in patients with high risk of chronic GvHD-related morbidity and mortality (e.g. patients with involvement of 3 or more organs, an organ with severity score >2, persistent thrombocytopenia, cGvHD evolving from acute GvHD);
- All patients with cGvHD requiring systemic therapy should be encouraged to enroll on clinical trials;
- Systemic glucocorticoids are most commonly used as front line systemic therapy. Prednisone at a dose of 1 mg/kg/day, or methylprednisolone at a dose of 0.8 mg/kg/day are given. The goal is to use the minimum amount of corticosteroids necessary to control symptoms;
- In addition to systemic therapy, patients remarkably benefit from specific ancillary therapy and supportive care targeted at organs affected by cGvHD;
- Additional therapy is needed for patients with progression of cGvHD after 2 weeks of glucocorticoids or lack of response by 4-6 weeks.

Salvage systemic therapy for chronic Graft-versus-Host Disease

Daniel Wolff
Department of Internal Medicine III Hematology/ Oncology, University Medical Center Regensburg, Regensburg, Germany

Approximately two thirds of patients developing moderate or severe chronic Graft-versus-Host Disease (cGvHD) do not respond to primary treatment with steroids and require salvage treatment. Criteria for the application of salvage treatment are: a) progression on prednisone at 1 mg/kg/d for 2 weeks, b) stable disease on more or equal to 0.5 mg/kg/d of prednisone for 4 - 8 weeks and c) inability to taper prednisone below 0.5 mg/kg/d. An additional rarely applicable indication is the intolerance to steroids. Although different treatment options are available for salvage therapy of steroid refractory cGvHD the "trial and error system" remains to date the only way to identify the drug or drug combination effective in an individual patient, with patient's history and side effect profile being the most important variables triggering the choice of treatment. In principle, initial secondary treatment should include agents with an adequate safety profile and well documented activity like CNI, extracorporeal photopheresis (ECP), mTOR inhibitors, or MMF, while agents with significant side effects or limited evidence should be reserved to third- or fourth-line treatment. In addition, steroid sparing should be an important goal of salvage therapy of cGvHD, but steroids remain an important backbone of salvage treatment. Since no predictors of response are yet available, neither for single immunosuppressive agents, nor combination therapies, most patients receive empirical treatment in daily clinical practice and changes of therapeutic components in case of lack of response are performed at the individual clinician's discretion. Nevertheless, at time of initiation of secondary or any further treatment, it is suggested not to change more than one drug at once, since adding several drugs at once may interfere with identification of the active component and might lead to prolonged use of inactive components. This does not apply to patients showing rapid progression of cGvHD indicating complete failure of treatment or the need of withdrawal of agents due to toxicity. In the presence of lack of response, continuation of at least one drug during the change period is suggested, since there is a risk to end up with a new combination without individual efficacy, which would leave the patient without effective immunosuppression. Treatment modalities are the use of steroids and calcineurin inhibitors as well as immunomodulating modalities (photopheresis, mTOR-inhibitors, IL-2, thalidomide, hydroxychloroquine, vitamin A analogues, clofazimine, tocilizumab), and cytostatic agents (MMF, MTX, cyclophosphamide, pentostatin). Recent reports showed efficacy of rituximab on the expense of a long lasting B cell depletion in selected patients. Moreover, tyrosine kinase inhibitors such as imatinib came into the field due to their ability to interfere with the PDGF-R pathway involved in fibrosis. An additional treatment option is low dose thoraco-abdominal irradiation especially active in patients with mucocutaneous GvHD or/and fasciitis. Currently, new treatment options enter clinical practice including the JAK-2 inhibitor ruxolitinib and the proteasome inhibitor bortezomib. In addition, BTK-inhibitor ibrutinib and SYK-inhibitors focusing on B cells are currently explored within phase II on trials in treatment of cGvHD.

Emerging drugs for chronic Graft-versus-Host Disease

Steven Z. Pavletic
National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Although there is much more that will need to be elucidated in the pathophysiology of chronic Graft-versus-Host Disease (cGvHD), better understanding of some these contributing factors has led to development of novel targets in this clinically challenging disease. Some of the proposed targets in the treatment of cGvHD include targeting T cell signaling pathways through novel mechanisms, T cell homing, T cell costimulatory pathways, B cell signaling pathways, and non-lymphocyte targets, as well as expanding thymopoiesis or T regulatory cells.

Today's standard of care in the treatment of cGvHD has been unchanged over the past several decades. An additional consideration aside from efficacy in the study of cGvHD treatments is tolerability. Patients require ongoing immunosuppressive therapy for a median of 2-3 years, and a small percentage of patients will require treatment for over 7 years. Thus, the treatment goals in chronic GvHD include improvement or stabilization of organ manifestations, limitation of long term treatment-related toxicities, improvement in functional capacity or quality of life, and ultimately an improvement in overall survival. The unmet need for cGvHD therapies is underscored by the fact that there are no FDA or EMA approved agents in the treatment of GvHD. Therefore, clinical trials are essential for the discovery of novel targets that are both safe and effective, and all patients with cGvHD should be enrolled in a clinical trial when possible.

There are several obstacles that exist in the drug development in cGvHD. First, this is a rare disease with heterogeneous clinical manifestations, and thus standard drug development pathways for other diseases may not be feasible in cGvHD. Absence of more non-FDA approved agents being tested for cGvHD and the unmet clinical need create pressures for off-label use of promising agents what syphons patients from enrollment in clinical trials. In addition, although a number of developed animal models for cGvHD exist, there is a lack of standardly accepted murine models to capture all of the protean manifestations, or which we know will be predictive of clinical efficacy. Although some recent advances in cGvHD therapy were initially translated from murine models, experts have suggested that the emphasis now also has to be on performing thoughtful, well-designed, and efficient clinical studies. Hope in this field has been restored by the NIH Consensus Conferences in 2005 and 2014. These consensus statements have helped to overcome some of the obstacles in the clinical study of cGvHD by unifying the terminology and approach to clinical assessments, and proposing new drug development pathways for regulatory approvals.

Criteria for new drug development in cGvHD has been proposed: 1) there should exist a plausible mechanism of action based on known immunologic mechanisms of the agent, 2) there should be known activity of the agent in other inflammatory diseases, and 3) there should be a known safety profile in humans, especially as cGvHD patients can be a challenging population in whom to perform clinical trials given the baseline complications that can occur. Finally, the agent of interest has to be available for use in clinical trials.

Cutaneous manifestations of chronic Graft-versus-Host Disease

Robert Knobler
Department of Dermatology, Medical University of Vienna, Vienna, Austria

Chronic Graft-versus-Host Disease (cGvHD), which may occur in patients who have or have not had an episode of acute GvHD, is a very significant cause for both morbidity and mortality in patients who are long-term survivors of allogeneic hematopoietic cell transplantation (HCT). Even though most organs can be affected by this immunological reaction, one key organ where this process most frequently and visibly makes itself noted in the patient is the skin. Changes in the skin in cGvHD can reflect changes in the epidermis, others in the dermis and others reflect an effect on both. Based on the US NIH Consensus Project on GvHD, the following are clinical features of cGvHD, which are usually diagnostic and generally do not require a biopsy for clarification in differential diagnostic questions:

1) **Lichen-planus like lesions** (very similar to true lichen planus) are characterized by erythematous to violacious papules or plaques. In selected cases it may have a follicular distribution similar to keratosis pilaris. Usual location: both dorsum of hands and feet, forearms and trunk front and back. Common symptom: itching.
2) **Sclerotic skin manifestations** can present in any location, either in previously affected cutaneous lesions or not. Morphology in general depends on what layers (depth) of the skin are affected: **a)** Morpheaform: with the typical characteristics of this presentation in adults and children, **b)** Lichen-sclerosus like: affecting the superficial dermis (similar to lichen sclerosus) with characteristic epidermal atrophy and superficial fibrosis; usual location: upper back, **c)**Deep sclerosis/eosinophilic fasciitis-like: here deeper layers of the skin are affected involving subcutaneous fibrosis; characteristic cellulite-like features can be observed; usual location: medial arms and thighs with associated pain and edema when deep sclerosis develops. When it occurs in late chronic GvHD, it is identified by prominent contractures that constrain movement and by typical linear demarcations often referred to as the “groove sign” (reminiscent of the typical signs of eosinophilic fasciitis, Shulmann syndrome). Complications include tension blistering and erosions that are difficult to treat.
3) **Poikiloderma** refers to a composite picture presenting with varying pigmentary changes in association with atrophy and teleangiectasia (mottled skin appearance).

In addition to these changes, a variety of other presentations have been described and documented; among these are maculopapupar presentations and widespread areas of pigmentary changes (hypo-, hyper- and depigmented areas). Frequently, one can also find eczematous regions, facial changes that simulate lupus erythematosus lesions, typical areas of ichthyosis associated with hypohidrosis, and others.

Hair changes: Though not diagnostic in themselves, one can often observe that in cGvHD cutaneous changes in areas with hair can have a profound effect on hair presence and function. Typically, one can identify areas of scarring or non-scarring alopecia, as well as scaly papules. Scalp hair in particular can show signs of thinning, loss, coarseness and premature graying.

Therapy: To minimize long-term damage to the skin and its appendages, recommended measures include control of skin-associated side effects due to chronic use of local and systemic steroids, regular skin cancer check-up, pruritus management, care of open lesions and associated infectious complications. As also recommended by the NIH Consensus Development Project, the following measures should be implemented whenever feasible:

1) Photoprotection: In the long term, there is a higher reported incidence of squamous cell carcinoma in patients with cGvHD. This is closely related to the use of required immunosuppressive therapy.
2) Regular use of emollients on intact skin.
3) Scarce use of topical corticosteroids to limit complications of skin atrophy.
4) As required for symptomatic control, utilization of topical or systemic antipruritics.

Management of affected skin:

As is the case in other transplantation settings (heart, lung, kidney), there is an increased incidence in the development of squamous cell carcinomas SCCs) of the skin in cGvHD. The

combination of immunosuppression, classical PUVA therapy and other forms of phototherapy and non-healing ulcers are all contributing factors that require regular monitoring for early detection and treatment. Within this context and in order to reduce additional cumulative side-effects of UV radiation, it is recommended that patients practice intense photoprotection, which, along with active avoidance of sun exposure, includes the use of broad spectrum sunscreens (SPF 50), appropriate sun protective clothes and hats.

Among the additional routine skin protective measures, the following are recommended:

Regular use of emollients to help manage pruritus and maintain undamaged skin intact as much as possible.

In order to help manage pruritus and to reduce the effect on atrophy of the skin, use of topicals including hydrocortisone in combination with pramoxine or menthol-containing lotions or creams has been suggested.

As symptoms increase or cannot be managed with local measures, systemic drugs can also be considered, and these include: oral antipruritics such as diphenhydramine, hydroxyzine, or doxepin.

Ulcers may develop in affected skin, and these need to be attended to with all possibilities in mind, including infection and neoplasia, the latter at times requiring diagnostic biopsies to rule out malignancy.

Tools for evaluating affected skin have been developed (NIH, Vienna Skin Score), covering range of involvement, as well as motion, and should be utilized to follow these patients whenever possible. Patient education on how best to treat their affected skin and what is to be expected can also ultimately contribute to better outcome of therapy in cGvHD.

Oral chronic Graft-versus-Host Disease

Marinka Mravak-Stipetić
University Dental Clinic, Clinical Department of Oral Medicine, University Hospital Center Zagreb, and University of Zagreb, School of Dental Medicine

Chronic Graft-versus-Host Disease (cGvHD) is a major late complication of allogeneic hematopoietic stem cell transplantation (HSCT) that affects many tissues and organs and manifests with polymorphic clinical features of varying severity and clinical course. Oral cavity is among the most commonly involved sites with a reported prevalence of 45–83%. In some cases oral lesions may be the first sign of the development of cGvHD and oral cavity the only site of involvement.

Clinical presentations of cGvHD in the mouth are highly variable, including oral mucosa lesions, salivary gland dysfunction and reduced mouth opening. Oral mucosal lesions differ by type of lesion, severity and topography, thus making clinical presentation even more complex. Additionally, many of oral clinical manifestations closely resemble other immunologic diseases such as scleroderma, lichen planus and Sjoegren syndrome.

Implementation of the National Institutes of Health (NIH) consensus recommendations for diagnosing and scoring the severity of cGvHD, first proposed in 2005 and refined in 2014, significantly facilitated and improved characterization of cGvHD.

According to the NIH recommendations, oral manifestations in cGvHD are classified in three categories as diagnostic, distinctive or common clinical features. The finding of lichen planus- like lesions on the oral mucosa is considered diagnostic feature sufficient to set up diagnosis of oral cGvHD without further testing. Distinctive features of cGvHD include mucosal atrophy, erythema, ulcers and pseudomembranes, xerostomia and mucoceles, and are not sufficient to establish diagnosis of oral cGvHD without further testing. Manifestations common to both acute and chronic GvHD include gingivitis, mucositis and pain.

Clinical significance of oral cGvHD is in its high frequency, progressive development of debilitating oral symptoms, tendency of recurrence of oral mucosa lesions, prolonged duration and complications due to immunosuppression, which have a negative impact on patient's oral and general health. Oral cGvHD presents significant burden to the patient, leading to impaired oral function and nutrition, increasing the risk of oral infection, drug related oral conditions and occurrence of secondary squamous cell carcinoma, contributing to long-term complications and ultimately decreased quality of life.

Thus, comprehensive oral clinical examination remains an important integral part of a regular protocol during multidisciplinary assessment of patients with cGvHD. Measurements should be made at baseline and at regular intervals every 3-6 months.

This lecture will offer a practical update on oral chronic GvHD:

- presenting the protocol of oral cavity clinical assessment with implementation of NIH scoring measures of oral cGvHD easily applicable for general practitioner, and
- presenting diagnostic tests for the assessment of salivary gland function and oral infection and emphasize their diagnostic and prognostic value.

Also, in the lecture, results of our study will be presented. The aim of the study was to comprehensively characterize a cohort of consented Croatian patients after alloHSCT with cGvHD and assess the prevalence and severity of oral lesions and symptoms according to established NIH consensus criteria.

Ocular chronic Graft-versus-Host Disease - current knowledge

Igor Petriček
Department of Ophthalmology, University Hospital Center Zagreb, Zagreb, Croatia

After skin, the eye is the second most affected organ in chronic Graft-versus-Host Disease (cGvHD). Ocular cGvHD usually presents with various degrees of tissue inflammation, which causes more or less severe dry eye, with tissue cicatrization in the end stages.

Despite its frequency, ocular cGvHD is still rather vaguely defined, usually only as dry eye in general, without any elements of dry eye syndrome that are unique for ocular cGvHD, and not seen in other types of dry eye.

The author presents current published research on ocular cGvHD, as well as several patients with ocular cGvHD followed in the cGvHD study.

Genital chronic Graft-versus-Host Disease – brief overview and case reports

Tajana Klepac Pulanić
Department of Gynecology, Community Health Center Zagreb East, Zagreb, Croatia

Incidence and Risk Factors

Female chronic Graft-versus-Host Disease (cGvHD) affects the vulva and the vagina, and is reported to affect at least one-quarter of female allogeneic hematopoietic stem cell transplant (allo-HSCT) survivors. Prevalence rates of vulvovaginal cGvHD are likely underestimated due to under-diagnosis. Women after HSCT are not typically questioned about gynecologic issues by transplant teams, nor are they routinely examined by gynecologists at regular intervals. Usually, they are referred for gynecologic examination only when they report symptoms. Importantly, patients may be less likely to self-report gynecologic symptoms unless directly asked, leaving many cases of genital cGvHD unrecognized until major complications occur.

The most consistently reported independent risk factor for genital GvHD is stem cell source; peripheral blood stem cell transplantation is associated with a higher risk for development of cGVHD than bone marrow transplantation. Studies have failed to demonstrate an association between the presence of genital tract infections, pre-transplant conditioning regimens or GvHD prophylaxis, donor sex, or age of recipient or donor with the development of genital tract cGvHD.

Clinical evaluation

Initial symptoms of genital cGvHD may include dryness, burning, itching, dysuria, pain to touch and dyspareunia. The most common complaints are vulvar burning and pain, which occur when urine touches the vulva. Corresponding vulvar signs include erythema and eroded or fissured vulvar mucosa. Pelvic and vaginal symptoms may persist a few days after intercourse as the mucosa may be fragile, easily damaged and take several days to heal. It is important to distinguish introital from deep dyspareunia. Introital pain occurs in patients with inflammation of the openings of the vestibular glands (Bartholin's, Skene's), with vulvar erosions or fissures and with labial fusion. Deep dyspareunia occurs in patients with vaginal scarring or shortening, more severe finding in vulvovaginal cGvHD.

When presenting with genital cGvHD, most patients have coexisting cGvHD in other organs, especially skin and oral mucosa. However, genital cGvHD may be an isolated manifestation in rare instances. Although vulvar disease typically precedes vaginal disease, treatment of vulvar disease alone will not prevent development of vaginal disease. Vaginal synechiae can appear as fine cobwebs, arcuate or purse-string narrowing, or dense scars. The dense, sclerotic changes may pull the vaginal walls together to completely obliterate, or shorten and narrow the vaginal canal to about 5 cm long and one finger-breadth wide. Sclerotic vaginal changes have three consequences: 1) inability to perform cervical cytology screening, 2) complaints of amenorrhea or cyclic pain due to hematocolpos or hematometra arising from trapped menstruation or hormonal contraceptive use-associated withdrawal bleeding and 3) sexual dysfunction.

Examination begins with careful inspection of the vulva, perineum and perianal area. The vestibular gland openings and vulvar skin are palpated with a cotton-tipped applicator (q-tip) for tenderness. Vaginal examination starts with a gentle digital examination (using water as a lubricant) to evaluate for the above described synechiae. When the gentle digital exam confirms a patent vagina, a speculum exam can be done, taking care to minimize stretching of any noted tender synechiae.

Treatment and prevention

Treatment is tailored to the clinical features. Erythema, erosions and fissures are treated with topical immunosuppressive medication. Options for treatment include first-line steroids of varying potencies (clobetasol ointment) or calcineurin inhibitors (i.e. cyclosporine, 0.1% tacrolimus ointment). Topical steroid therapy for genital cGvHD is effective in most cases. Usually, within 6 to 8 weeks of starting clobetasol ointment, vulvar erosions and fissures heal, and vulvar pain resolves. If the patient experiences irritation or lack of improvement with the prescribed regimen, other topical agents like cyclosporine or tacrolimus can be used.

Treatment of vaginal scarring addresses the density and extent of architectural changes. Cobweb and other newly formed scars may be gently lysed manually during vaginal examination. Patients with mild and moderate adhesions may be medically managed by using dilators lubricated with a pea-sized amount of clobetasol ointment (or other topical immunosuppressive agent) and one half inch of estrogen cream 2 to 3 times a week, until the vaginal scarring is lysed. Use of dilators of increasing diameters enables an increase in vaginal caliber and depth. Early implementation of mechanical and topical therapy can prevent the need for surgery. Dense fibrotic vaginal scarring or extensive labial fusion may require surgery. After surgery, use of topical immunosuppressive therapy with dilators may prevent development of new scars. For maintenance of vaginal patency, manually inserted dilators or frequent sexual intercourse is recommended.

Successful management of genital GvHD is dependent upon early recognition and early implementation of therapy. Thus, systematic, regular examinations by a gynecologist appear pivotal in reducing morbidity and improving quality of life for these patients.

Three case reports will be presented.

Main educational points/Learning goals:

- Female genital cGvHD is important and frequent late complication after alloHSCT;
- History with emphasis on gynecological symptoms needs to be taken;
- Routine gynecological examination aimed at early recognition and implementation of therapy is the key in successful management of genital cGvHD.

Pericarditis in patients with chronic Graft-versus-Host Disease

Ewa Karakulska-Prystupiuk,
Grzegorz Basak,
Jadwiga Dwilewicz-Trojaczek,
Wiesław Wiktor Jędrzejczak
Department of Hematology, Oncology and Internal Medicine, Warsaw Medical University, Warsaw, Poland

Background

There are only a few cases of pericarditis complicating course following allogeneic bone marrow transplantation described in the literature, and there are no data available on the risk and frequency of this condition.

Aims

The aim of this study was to assess the frequency of exudative pericarditis complicating chronic Graft-versus-Host Disease (cGvHD) in patients transplanted with allogeneic hematopoietic cells (allo-HCT).

Methods

A retrospective analysis involved a group of 105 patients of the Outpatient Transplantation Service of the Department of Hematology, Medical University of Warsaw, transplanted in the years 2010-2016 and evaluated for the years 2014-2016. This group included 38 patients transplanted from HLA-identical siblings and 67 from matched unrelated donors. Seventy-eight patients received myeloablative conditioning, and 27 reduced intensity conditioning.

In this group 50 patients suffered from cGvHD, including 24 with moderate and severe cGVHD.

Cardiology parameters evaluated included ECG, echocardiography, NTproBNP, and systematic clinical follow-up.

Results

Pericarditis was diagnosed in 6 patients (aged 20 to 56 years) within 4 to 23 months post allo-HCT. All patients suffered from severe cGvHD with involvement of at least two other organs, but none had earlier history of heart disease. Four patients underwent CMV reactivation and were successfully treated. One patient had aspergillosis of the heart. All patients demonstrated signs of heart insufficiency grade II or III according to NYHA. Presence of fluid in pericardium was confirmed in all patients with concomitant reduction in ejection fraction of left chamber (EF – minimum 30-58%) and relaxation impairment. Due to incipient heart tamponade, one patient required fenestration and pericardium drainage. All patients had elevated NTproBNP to above 1000 pg/ml (N<1250 pg/ml). There were no major changes in ECG. All patients required intensive immunosuppressive treatment. Only one patient improved following glucocorticosteroids only, while others required complex approaches including TAC/SIR, rituximab, ECP. All patients survived and are in complete remission of their underlying disorders with improved heart function (NYHA 0-II), but still require immunosuppressive treatment of their cGvHD.

Conclusion

Late pericarditis may occur in up to 5% of allo-HCT survivors, primarily affecting patients with moderate and severe cGvHD. It requires escalation of immunosuppressive treatment, but usually has favorable outcome. Early diagnosis may be achieved by systematic NTproBNP testing and periodic ECHO evaluation.

Organ specific manifestations and treatments: chronic Graft-versus-Host Disease of the lung

Jörg Halter
Division of Hematology, University Hospital Basel, Basel, Switzerland

After hematopoietic stem cell transplantation (HSCT), the lung might become affected by a variety of pathologies. Beyond infections, the lung can be affected by a number of other non-infectious immunologic/inflammatory diseases like idiopathic pneumonia syndrome (IPS), cryptogenic organizing pneumonia (COP) or bronchiolitis obliterans. Furthermore, lung function might be impaired due to extra-pulmonary causes.

Bronchiolitis obliterans (BO) and Bronchiolitis obliterans syndrome (BOS) are currently the only accepted manifestations of chronic Graft-versus-Host Disease (cGvHD) of the lung as confirmed in the last NIH cGvHD consensus conference in 2014 with a wide variation of reported incidence rates - mostly between 2-12%. Initial symptoms of BOS are often subtle before more overt dyspnea develops, often together with a dry cough. Definite diagnosis of BO can only be made by lung biopsy, although this is performed rather rarely due to respect from peri-interventional complications. Diagnosis of BOS can be made based on clinical criteria in the presence of other signs of cGvHD (Jagasia MH et al. Biol Blood Marrow Transplant 2015). If BOS is suspected, diagnostic workup includes repeated spirometry or body plethysmography with bronchodilatation. Both absolute values (percentage of expected) and changes over time should be analyzed. Furthermore, a chest CT scan and a broncho-alveolar lavage are recommended to exclude infections, other causes of impaired lung function or dyspnea.

Reported risk factors for development of BOS include an impaired lung function pretransplant, myeloablative conditioning, busulfan conditioning, peripheral blood stem cells, low immunoglobulin-levels and respiratory infections.

Lung cGvHD has an important prognostic impact on long-term survival of patients with cGvHD. Estimated two year overall survival from BOS diagnosis is currently about 72-76%. Diagnosis of BOS early after HSCT (within 6-12 months), low FVC or very low FEV1<30% at diagnosis where found to be poor prognostic factors, whereas the prognostic value of low FEV1 >30% is less clear.

Pathophysiology of BO is still not completely understood. Various triggers may lead to activation of an inflammatory cascade involving donor T- and B-cells, cytokines and in some instances also antibodies. Deposition of intraluminal fibrous tissue with a decrease of the intraluminal diameter, mucus plugging, ongoing chronic inflammation and tissue scarring lead to an irreversible narrowing of bronchioli and development of bronchiectasis.

Treatment in patients with BOS is essentially based on two principles: first on prevention of further airway obstruction and decrease of lung function, and second on pulmonary rehabilitation. The former includes the same therapeutics that are used for other manifestations of cGvHD. Systemic steroids form the basis of the initial treatment, most often in combination with a calcineurin inhibitor. Further options for systemic treatment include mTOR-inhibitors, extracorporal photopheresis or imatinib. Controlled studies will be needed to further define the contribution of these treatments. Combining systemic with topical therapies has been identified as an important therapeutic principle. Budesonid/Formoterol inhalation, as well as the FAM protocol (Fluticason, Azithromycin, Montelukast), both proved to have a positive effect on lung function for the time studied in a majority of patients. Beyond topical and systemic treatment, prevention of respiratory infections by repeated instruction of hygiene (especially hand hygiene) and vaccination are important supportive care measures. Despite all of these measures, lung function tends to become stable or decrease over time in most patients. Further studies will be necessary to define the role of pulmonary rehabilitation in BOS patients to maintain and improve physical performance and quality of life. Early evaluation for lung transplantation should be considered in selected patients.

Main educational points/Learning goals:

- Bronchiolitis obliterans has an important prognostic impact on overall survival;
- Early diagnosis of BO needs a high level of clinical alertness, decreases in lung function are mostly irreversible;
- Treatment of BO is based on systemic and topical treatment, physical training and infection prevention.

Neurologic manifestations of chronic Graft-versus-Host Disease (cGvHD): prevalence and characteristics of neuropathy in patients with moderate to severe cGvHD

Ervina Bilić
Department of Neurology, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

Increasing safety of allogeneic hematopoietic stem cell transplantation (allo-HSCT) has caused the rise of the number of survivors in patients at risk of developing chronic Graft-versus-Host Disease (cGvHD), a leading cause of non-relapse mortality and morbidity after transplantation. Chronic GvHD, a late complication occurring after allogeneic hematopoietic stem cell transplantation (HSCT), is characterized by pleiomorphic clinical manifestations, affecting multiple tissues and organs, including peripheral and central nervous system, with varying severity and clinical course. Neurological manifestations of cGvHD are being increasingly recognized, with peripheral nervous manifestations being more common. They can occur at any level of the peripheral nervous system (PNS), including the peripheral nerve, the neuromuscular junction or the muscle and adjacent fascia. They usually develop several months to years after allo-HSCT and have to be distinguished from different infectious and metabolic complications, as well as from side effects of potentially neurotoxic drugs. The most frequently described neurological manifestations of cGvHD in peripheral nervous system are myositis, immune mediated neuropathy and myasthenia gravis. Myositis is a rare, but typical neuromuscular complication in patients who develop cGvHD. Chronic GvHD-related myositis appears to be similar to idiopathic polymyositis in its clinicopathological presentation. The diagnostic challenge is differentiation of myositis and steroid or other toxic myopathy in patients with cGvHD. These myopathies may sometimes be distinguished only by specific electromyographic changes or pathohistological characteristics. Currently, myasthenia gravis and peripheral neuropathy are considered to be the only complications related to cGvHD and are not sufficient for establishing the diagnosis of the disease. Neuropathies in cGvHD can be acute or chronic, and mostly resemble Guillain-Barrė syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP) or chronic immune-mediated axonal polyneuropathy.
The first described neuropathy in cGvHD was CIDP, and it was a logical step because this demyelinating immune mediated neuropathy affecting large nerve fibers is affected in other autoimmune diseases. However, in the following decades it became clear that CIDP is not the only, nor the most frequently present neuropathy in cGvHD patients. Clinical reality pointed at frequent symptoms (including muscle cramps and paresthesia) in cGvHD patients, indicating possible underlying neuropathy, but the majority of patients haven't met diagnostic criteria for CIDP or any other demyelinative neuropathy. Muscle cramps associated with moderate and severe cGvHD are reported to develop in 16% of patients after allo-HSCT. On the other hand, research by Kraus et al. showed an incidence of muscle cramps to be up to 67% in patients with GvHD, more often in chronic than acute GvHD. For now, only myositis and polymyositis are considered "distinctive" neurological manifestations of cGvHD. Consequently, diagnosis of neurological cGvHD can only be established when additional recognized manifestations of the disease are present. Even though dysfunction of the PNS is often found in cGvHD, neurological manifestations are still not incorporated into diagnostic criteria or the scoring system. One of the reasons for this could be the fact that detailed clinical research targeting neurological symptoms and findings in patients with cGvHD are almost non-existent. This presents a problem because damage of the nervous system in the context of cGvHD can produce severe clinical problems with significant morbidity and mortality. The aim of this study was to show the incidence and characteristics of peripheral nervous system manifestations, with emphasis on small fiber neuropathy (SFN) in patients with cGvHD. Every neuropathy developed in cGvHD is probably multifactorial and may be caused by autoimmune/alloimmune mechanisms, metabolic, drug-related and infective factors or damaged blood-nerve barrier due to immune-mediated multi-systemic inflammation. Current knowledge of drug-induced damage mechanisms affecting peripheral nerves in cGvHD is very limited and likely specific to individual agents. Presence of

small or large fiber neuropathy in cGvHD patients is common and should be monitored and treated whenever possible. Contributing factors possible for development of neuropathy in cGvHD should be vigorously studied, since therapeutic options are mostly symptomatic. It is clear, however, that peripheral nerve damage has an important place in neurological cGvHD presentation. According to our recently published study results, a large proportion of cGvHD patients may have SFN and LFN, which is usually axonal. However, the specific cause of a particular type of neuropathy in cGvHD is currently unknown. Clinical presentation of SFN in cGvHD may be connected to muscle cramps and neuropathic pain, but pruritus may also be a symptom of interest, as it may be a sign of unrecognized SFN. The level or pattern of peripheral nerve damage may not be connected only with clinical presentation, including muscle cramps, but also with the global NIH score and the severity of cGvHD itself. Possible factors contributing the causes of neuropathy in cGvHD should be vigorously studied, since therapeutic options are mostly symptomatic. It is clear, however, that peripheral nerve damage has a central place in neurological cGvHD presentation.

Joint/fascia manifestations and rehabilitation medicine role in chronic Graft-versus-Host Disease

Tamara Vukić
Department of Rehabilitation and Orthopedic Aids, University Hospital Center Zagreb, Zagreb, Croatia

Introduction

Chronic Graft-versus-Host Disease (cGvHD) is a major late complication of allogeneic hematopoietic stem cell transplantation (alloHSCT) and it usually occurs within 3 years after alloHSCT. Chronic GvHD has the potential to cause significant morbidity and mortality, but especially if involving joints, it is a cause of great impairment and disability.

Joint/fascia chronic GvHD characteristics

Chronic GvHD can involve many organ systems, and joint/fascia involvement is relatively common, and can cause significant functional impairment. Caused by inflammation of the fascia, including an eosinophilic component, it may manifest as joint tightness, erythema, oedema, restricted range of motion (ROM), arthralgia, and rarely arthritis or synovitis. Joint/fascia manifestations can be clinically detectable when inflammation and fibrosis arise in deep tissues (deep sclerosis/fasciitis) or skin overlying joints (superficial sclerosis).Widespread sclerosis may result in joint contractures and severe limitation of function. Common sites of involvement include hands/wrists, shoulders, elbows and ankles, with upper joints usually more affected than lower joints.

To access the severity of joint/fascia involvement, the NIH joint/fascia scale that uses a 0-3 point scale to score a composite of tightness, ROM, and activities of daily living (ADL) is used. Joint/fascia manifestations are defined as a NIH joint/fascia score ≥1. The Photographic Range of Motion (P-ROM) scale is also used in assessing joint/fascia manifestations in patients who undergo alloHSCT. P-ROM scale captures ROM separately for shoulders, elbows, wrists/fingers, and ankles in a series of images. P-ROM total score is the sum of scores in all 4 joints for a maximum of 25 points with lower scores indicating more limited ROM. P-ROM scale only considers ROM limitations and does not include tightness or ADLs which is different from the NIH joint/fascia scale. Both scales are recommended by 2005 National Institutes of Health Consensus Criteria.

Timely recognition of these symptoms and careful examination of musculoskeletal involvement in this patient group is of great importance. When examining patients who are suspected of having joint/ fascia chronic GvHD involvement, patients should be asked about joint stiffness, problems with upper and lower body ROM, joint contractures, swollen joints, myalgias, arthralgia, difficulty making a fist, difficulty climbing hills or stairs, and an extensive physical and functional examination should be conducted.

Rehabilitation medicine role in chronic GvHD treatment

Chronic GvHD treatment includes many topical therapy modalities and many forms of systemic immunosuppression therapy. In treating patients with joint and fascial cGvHD, intensive physical and occupational therapy is also an important adjunct to maintaining functional range of motion and reducing joint contractures. Exercises for range of motion and strengthening, balance, and fall prevention should be initiated for patients with joint/fascia manifestations of cGvHD. Specific pulmonary or cardiac rehab exercise program for specific issues in these organ systems can also be indicated. Occupational therapy modalities; targeted splinting for joint contractures, bracing for pain or stability, bracing for motor weakness, wound prevention (proper footwear, frequent skin checks), adaptive equipment (canes, walkers) should also be used if needed. Other physical thermal modalities can be used, such as paraffin baths and/or ultrasound. Simultaneous muscle stretching must be done with each of these modalities, and rehabilitation programs need to be individualized for each patient.

Conclusion

Chronic GvHD can cause devastating effects in terms of causing morbidity, including significant disability, and is the leading cause of non-relapse mortality. Careful monitoring of allogeneic transplant recipients will identify patients at an earliest stage of the disease, ensuring the best chance for successful therapy. A comprehensive and customized rehabilitation plan can improve function and quality of life of these patients and hopefully prevent the consequences of advanced cGvHD.

Infections and chronic Graft-versus-Host Disease

Davorka Dušek
University Hospital for Infectious Diseases "Fran Mihaljević" and University of Zagreb School of Medicine, Zagreb, Croatia

Infections present major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Major factors affecting the risk of infection include type of transplant, pretransplant characteristics and conditioning, donor type, graft type, HLA match, as well as Graft-versus-Host Disease (GvHD). Repertoire of infectious agents causing deleterious complications after allo-HSCT depends on specific risk factors characteristic for each phase after allo-HSCT (pre-engraftment, post-engraftment and late phase). Chronic Graft-versus-Host Disease (cGvHD) is an important factor in late immune deficiency via impaired T-cell mediated immunity, impaired CD4+ function, impaired B-cell function, impaired dendritic cell function, as well as steroids used in treatment of cGvHD that further hamper normal immune response leading to infectious complications.

Advances in transplantation methods, successful early prevention strategies and effective supportive care have led to increased proportion of infectious morbidity and mortality occurring in the late, post-engraftment period. Chronic GvHD is recognized as an important risk factor for infection in this period. Different infectious agents can cause infection in the late phase including bacterial, viral and fungal pathogens. In the setting of impaired antibody responses and opsonization, often caused by cGvHD, infection with encapsulated bacteria, especially *Streptoccous pneumoniae*, can lead to severe pneumonia and meningitis. Other important bacterial pathogens are multidrug resistant bacteria (MDR) and *Clostridium difficile*. Many viruses can cause infections in the late phase, including herpesviruses (especially varicella zoster virus-VZV and cytomegalovirus-CMV), polyomaviruses, and other respiratory pathogens such as influenza and adenovirus. Chronic GvHD is an important risk factor for late VZV reactivation that can be manifested as dermatomal reactivation and disseminated disease. CMV infection is a leading cause of illness and death in patients post allo-HSCT. The use of preemptive therapy and delayed CMV-specific protective immunity leads to CMV disease being a more significant problem after day 100 after allo-HSCT. There is well described association between CMV infection and acute and chronic GvHD, with GvHD being risk factors for CMV reactivation, as well as CMV infection posing risk factor for acute and chronic GvHD. Recent advances in pharmacologic therapy (e.g. letermovir, brincidofovir) and immunotherapy will probably lead to reduction in morbidity. Fungal pathogens, especially filamentous fungi (e.g. *Aspergillus* species and Mucormycoses) and *Pneumocystis jirovecii* cause a significant proportion of morbidity and mortality in the late phase. Implementation of effective prophylaxis regimens, enhanced screening strategies, and vaccination can decrease morbidity and mortality from these pathogens.

However, cGvHD is not only a risk factor for developing infectious complications, but several infectious agents can play a significant role in development of GvHD, such as cytomegalovirus. On the other hand, some infectious agents, usually considered to be pathogens, might even have a protective role in reduction of GvHD (*Helicobacter pylori*, helminths in animal models). Recently, immunomodulatory function of gut microbiota after allo-HSCT and its impact on GvHD has been explored. Several studies have shown that gut colonization by MDR bacteria can decrease the overall survival of patients undergoing allo-HSCT by increasing rate of systemic infection (bacterial translocation from the gut) and acute GvHD. Colonization with *Candida* sp has a significant role in occurrence and pathogenesis of acute GvHD via induction of mucosal innate immunity (Th 17/IL 23 responses). Also, a significant proportion of patients with cGvHD, especially patients with severe global cGvHD, have been found to have oral colonization with *Candida* sp. Human microbiome, especially gut microbiota and its manipulation (e.g. probiotics, prebiotics) could serve as a new therapeutic approach in the future.

Platelets and coagulation in chronic Graft-versus-Host Disease

Dražen Pulanić
Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

Chronic Graft-versus-Host disease (cGvHD) is a multi-organ alloimmune and autoimmune disorder and the most important late complication after allogeneic hematopoietic stem cell transplantation (alloHSCT). Reported incidence rates of cGvHD range from 20-80% of patients after alloHSCT, and are increasing due to lower peritransplant mortality, older recipients, the more frequent use of peripheral blood graft, and more unrelated donors. It is a complex and multisystem disease which affects skin, eyes, mouth, liver, gastrointestinal tract, lungs, muscular-skeletal system, and genital tract, often resembling manifestations of various autoimmune diseases.

Low platelet counts in cGvHD patients at diagnosis of disease are predictors of poor survival across many cGvHD studies even from the earliest works published more than 30 years ago until nowadays; however, such association is still not well understood. Several possible mechanisms of thrombocytopenia in the cGvHD setting were suggested, such as transplant-related thrombocytopenia, malignancy relapse, microangiopatic thrombocytopenia, drug-induced thrombocytopenia, immune-mediated thrombocytopenia, hypersplenism, infection, and cytokine-induced thrombocytopenia.

Moreover, newer studies found increased platelet counts and active thrombopoiesis associated with more active and more severe cGvHD in later course of disease, supporting the hypothesis that ongoing inflammation in cGvHD stimulates increased thrombopoiesis in the most severe patients.

Several acquired disorders of coagulation are described in cGvHD patients as well, such as acquired hemophilia A (acquired factor VIII inhibitor), acquired von Willebrand's disease, and secondary antiphospholipid syndrome. Also, it is described that patients with cGvHD have an increased risk for venous thrombosis in spite of higher bleeding risk.

Main educational points/Learning goals:

- Thrombocytopenia at diagnosis of cGvHD is negative survival predictor across many studies;
- Newer publications showed increased platelet counts and active thrombopoiesis associated with more active and more severe cGvHD in later course of disease;
- Variation of platelet counts and acquired coagulation disorders in the setting of cGvHD have multifactorial etiology with numerous interactions between inflammation and coagulation;
- Improved understanding of these processes may lead to better understanding of pathophysiology of cGvHD and have potential for investigation of biomarkers of this complex and potentially lethal late complication after alloHSCT.

Psychosocial issues and health related quality of life in chronic Graft-versus-Host Disease

Zinaida Perić
Department of Internal Medicine, University of Zagreb School of Medicine, Zagreb, Croatia

There has been a growing interest in quality-of-life (QOL) evaluation following cancer treatment, including hematopoietic stem cell transplantation (HSCT). Information about health-related quality of life provides a broader understanding of the patient's status after treatment beyond simple disease free survival time. Hence, QOL is now considered an index of the effectiveness of treatment and should become an integral component in the assessment of medical outcome. QOL is a broad term that refers to the individual's perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectation, standards, and concern. In 1995, Wilson and Cleary described QOL in terms of biologic and physiologic variables, symptoms status, functional status, general health perceptions, and overall quality of life. This framework continues to be useful today with the broad categories where more specific dimensions of QOL such as physical, social, role, and psychological function are incorporated.

Among patients who survive transplantation, physical functioning rapidly declines immediately after transplantation, reaching a nadir at 30 to 100 days after HSCT. Physical functioning then begins to improve, with some studies showing physical functioning plateaus in the year after transplantation, and others finding continued improvement over following four years.

Emotional functioning for survivors is most compromised before transplantation and immediately after, with significant improvements seen as early as hospital discharge to 100 days. Some data suggest that emotional functioning remains relatively stable after this initial improvement, although other data suggest that it continues to improve in the two to four years after transplantation.

Data are conflicting regarding short-term social functioning in survivors, some finding significant improvement from baseline to 90 days after transplantation, while another suggesting significant deterioration from baseline to 100 days. In all studies, survivors report similar or better levels of social functioning at one year compared with pretransplantation baseline and continued improvements in years 1 through 4.

Role functioning shows an immediate decline after transplantation followed by gradual improvement over time. By 1 year, 59% to 69% of survivors return to work, school, or homemaking.

Survivors also report good overall QOL, particularly as time from transplantation increases. Overall QOL follows a similar pattern to role functioning, with deficits immediately after transplantation and return to baseline levels at day 100. After day 100, overall QOL may stabilize or continue to improve over following years.

Concerning risk factors for impaired QOL, poorer QOL is associated with greater degree of symptoms, lower educational level, older age, shorter period after HSCT, female sex, sexual impotence, advanced disease at time of transplantation, presence of chronic Graft-versus-Host Disease (cGvHD), worse pre-transplantation level of functioning and impairment, greater interpersonal conflict, and reduced level of social support.

The degree of impact on overall QOL and the multiple dimensions varies across the transplant trajectory. The impairments often begin prior to the HSCT, due to the disease itself or previous treatment. During HSCT, physical effects have the greatest impact. As the patient moves through the first year and beyond, the primary effects shift as the impact on social and role function become more evident. Psychological effects are present across all phases of HSCT. Psychological distress and negative mood states are most prevalent before and during HSCT. During the later phases of transplant, patients report improvements in their level of distress along with interpersonal growth. Despite the overall improvements, worry is still commonly reported after allogeneic HSCT, possibly related to continued uncertainty of relapse. Cognitive function has also been recognized as an area of concern, with recent evidence suggesting that cognitive function may not be a significant or long-lasting problem after allogeneic HSCT.

GvHD shows a robust, negative relationship with QOL. Of seven studies investigating the relationship between acute and chronic GvHD

and QOL, six have reported a significant, negative relationship. Only one study has found no relationship between cGvHD and QOL, but it may have been underpowered. Both acute and chronic GvHD have been shown to be associated with worse physical functioning, role functioning, social functioning, mental health, general health, and overall QOL. Only 60% of patients with chronic GvHD are able to work.

Although there is recognition of the importance of standardized assessment of QOL across clinical trials, there is currently no consensus regarding which measure should be used. Instead, HSCT literature encompasses a variety of QOL measures, including broad measures of QOL in healthy and patient populations, measures of cancer-specific QOL, HSCT-specific QOL, and GvHD specific side effects. General measures of QOL have the advantage of applicability for both patients who underwent HSCT and comparison groups, but may be less sensitive to side effects of transplantation, including acute and chronic GvHD, than HSCT specific measures.

There is widespread interest in behavioral interventions to improve quality of life after HCT. Supervised exercise results in better patient-reported physical well-being at discharge and smaller decline in physician-rated performance status and maintenance of muscle strength at 100 days. Exercise is generally well-tolerated during the hospitalization period, even exercise up to five times a week or more while hospitalized. Further, inpatients report several benefits of exercise, including improved strength and energy, alleviation of boredom, increased endurance, maintenance of flexibility, and emotional distraction. Among outpatient survivors of HSCT, supervised treadmill walking and home-based aerobic exercise are associated with decreased fatigue, increased physical well-being and increased aerobic fitness.

Psychosocial interventions in HSCT have been examined in two randomized controlled trials. Neither examined QOL as an outcome, but were instead designed to test the effects of stress management and coping skills training on pain, nausea, and emesis compared with usual care and a time and attention control. Patients in the stress management and coping skills groups reported reduced pain. Behavioral interventions show promise to maintain or improve QOL after allogeneic HCT, consistent with a larger body of evidence regarding the benefits of exercise and stress management in cancer patients.

Finally, a dedicated post-transplantion GvHD clinic, with an active involvement of the multidisciplinary team of specialists with an interest in GvHD, was also shown to significantly improve patients' QOL. In this way, long-term HSCT survivors with GvHD could obtain complex and specialized medical and psychological care, which may not be fully accomplished in standard transplant or late-effects follow-up clinics due to time restraints or lack of resources.

Nutrition in chronic Graft-versus-Host Disease

Dina Ljubas Kelečić
Clinical Unit of Clinical Nutrition, University Hospital Center Zagreb, Zagreb, Croatia

Malnutrition and weight loss are common features of chronic Graft-versus-Host Disease (cGvHD). The first study by Lenssen et al. in allogeneic hematopoietic stem cell transplantation (allo-HSCT) patients, one year after transplantation, reported nutrition-related problems, weight loss, changes in anthropometry measurements and inadequate energy intake to be more common in patients with extensive cGvHD than in patients with limited or without GvHD. According to recent studies that have assessed nutritional status by using BMI (Body Mass Index) and SGA (Subjective Global Assessment), malnutrition is present in 29-43% of cGvHD patients. Interestingly, symptoms as odynophagia and oral sensitivity, contrary to expectations, were not related to weight loss in this population. Other late complications of cGvHD, together with steroid induced diabetes mellitus, osteoporosis metabolic syndrome and hyperlipidemia, can also adversely affect nutritional status in cGvHD patients. Moreover, changes in body composition in adult survivors of allo-HSCT are independent of BMI. Low lean body mass index in cGvHD is significantly associated with the complications of cGvHD, decreased performance status and/or steroid therapy treatment.

Nutritional support has a considerable role in allo-HSCT treatment, especially due to the conditioning regimens that can have a deleterious effect on gastrointestinal integrity and nutritional status. Maintaining nutritional status in patients with intestinal GvHD malabsorption and weight loss as a consequence of severe diarrhea and vomiting is the most challenging for the nutrition support team. Additionally, decreased intestinal bile salt due to cholestatic liver disease, pancreatic insufficiency, bacterial overgrowth and infection can worsen malabsorption. According to the studies on micronutrient deficiency, patients with cGvHD are deficient in vitamin D, which is associated not only with the medications, prohibition of sun exposure, inadequate supplementation, and altered gastrointestinal absorptive capacity, but also with severe and moderate malnutrition. Patients with cGvHD are also at risk of vitamin B12, zinc and magnesium deficiency.

Nutritional counseling is important to ensure adequate nutrition due to the energy, protein and micronutrient needs. In some cases of oral and intestinal cGvHD it is necessary to change diet texture or to introduce and enteral nutrition. Up to date, there are no studies that support the use of immunonutrition (glutamine, arginine, n-3 PUFAs) or prebiotics and probiotics in cGvHD. Nutritional assessment and monitoring consisting of anthropometrics, SGA, symptoms that alter food intake, laboratory parameters and dietary intake should be performed in all patients before and after allo-HSCT. Nutrition support team is an integral part of multidisciplinary approach in cGvHD treatment.

Main educational points/Learning goals:

- Malnutrition is often present in patients with cGvHD independently of BMI;
- Anthropometric measurements and SGA can provide a better insight into the nutritional status of patients than BMI;
- Nutritional status should be assessed and monitored not only in cGvHD, but in all patients undergoing allogeneic HSCT by an expert nutrition support team;
- There is still a lack of data on nutrition issues in cGvHD. Nutrition support should be individually prescribed in each patient.

Chronic Graft-versus-Host Disease: Translating from mouse to man

Kirk R. Schultz
Division of Hematology and Oncology, Department of Pediatrics, British Columbia Children's Hospital, University of British Columbia, Vancouver, BC, Canada

Chronic Graft-versus-Host Disease (GvHD) is a major complication after hematopoietic stem cell transplantation (HSCT) and appears to have a high heterogeneity in its biology. Although murine GvHD models have worked well in evaluating the biology of acute GvHD, mouse GvHD models have been only partially accurate in their mirroring of human cGvHD clinical manifestations. Moreover, the biology seen in humans many times is not a predominate mechanism in current murine GvHD models. This has been recognized and new more representative murine chronic GvHD models are being developed. In spite of these model limitations, what we know of biology of chronic GvHD has been a combination of findings from murine GvHD models and from human biomarker studies. Chronic GVHD is the net result of an imbalance between relatively higher immune effector mechanisms that cause inflammation and disease and lower inhibitory (regulatory) mechanisms, which may maintain tolerance. The National Institutes of Health (NIH) Chronic GvHD Consensus Biology working group has developed a proposed three phase biological model for the initiation and development of chronic GVHD based on current knowledge. The three phases are outlined below:

Phase 1 - Early inflammation and tissue injury

Both human and murine studies have supported the role of inflammation early after HCT from conditioning, activation of donor T-cells, and acute GvHD. Triggering these inflammatory pathways in scavenger macrophages, plasmacytoid and myeloid DCs, B cells and neutrophils results in the production of key mediators, which enhance antigen presentation and direct the commitment of naïve T cells into differentiated Th1/Tc1 and Th17/Tc17 T-cell effector lineages for chronic GvHD. Hyper-responsiveness to TLR9 agonists and BCR agonists has been described in B cells at the onset of chronic GvHD. IFN-inducible chemokines CXCL9, CXCL10, and CXCL11, responsible for CXCR3 expressing Th1 lymphocyte and natural killer (NK) cells recruitment into tissue, have recently been identified as plasma biomarkers for chronic GvHD and are upregulated at diagnosis, remaining elevated in severely affected patients. In animal models, endothelial cell activation and apoptosis in the setting of intense lymphocytic infiltration during acute GvHD has been observed in advance of epithelial injury of the oral mucosa and lungs of mice. Microvascular loss and tissue ischemia may contribute to organ fibrosis as part of cGvHD.

Phase 2 - Chronic inflammation and dysregulated immunity

In vivo T-cell depletion using lymphocyte antibody therapies or a short, early post-transplant course of cyclophosphamide each have been shown to reduce the incidence and severity of chronic GvHD, suggesting that chronic GvHD is dependent, at least in part, upon mature donor T cells and their precursors derived from the HSC graft. Antigen specificity of the T cells in murine GvHD involved in acute GvHD appear to differ from those of chronic GvHD. T cell clones from mice with acute GvHD are specific for restricted histocompatibility antigens of the host, where as the majority of T-cell clones from mice with chronic GvHD were specific or restricted by histocompatibility antigens shared by the donor and recipient strains. Activated, clonally expanded, donor T cells differentiate into distinct functional subsets including Th/Tc1, Th/Tc and Th/Tc17 cells. Elevated Th17 cell numbers have been found in patients with acute and chronic GvHD and are associated with disease status.

The role of $CD4^+$ Tregs in chronic GvHD development appears to be more complex. Increased, normal, and decreased numbers of Tregs have been reported at the onset of chronic GvHD. Following HCT, Treg reconstitution is altered and is dependent on a variety of factors, including: thymic repopulation and recovery, "homeostatic" Treg proliferation, subsequent survival of regenerated activated Tregs, and the choice and use of immune suppressive drugs. Recent studies have examined possible mechanisms that lead to Treg deficiency during chronic GvHD. An imbalance between Treg cells and T-cell effectors has been observed in humans with too few Tregs, resulting in skewed thymic production of naïve T cells compared to conventional T cells. Murine models have shown that IL-2 is the primary homeostatic cytokine that regulates $CD4^+$ Treg development

and maintains the Treg pool *in vivo*. Low dose IL-2 can be administered safely after allogeneic HSCT for prolonged periods resulting in expansion of Treg cells and with clinical response in both mice and patients with chronic GvHD. Other regulatory mechanisms also have an important role in establishing balance with T-cell effector cells. Decreased regulatory functions of B cells may contribute to chronic GvHD in some patients. The $CXCR3^{+}$ subpopulation of $CD56^{bright}$, cytokine producing, NK cells are associated with inhibiting chronic GvHD. Murine models have identified CD4+ invariant NKT cells as regulators of Treg expansion and function in vivo.

Mechanistic links between BCR activation, TLR ligation, and BAFF in disease microenvironments require further study in murine models. Emergence of a population of $CD19^{+}CD21^{low}$ B cells by day 100 correlates with the subsequent development of chronic and specifically those patients who have BOS and hypogammaglobulinemia. One problem with murine studies is that human studies are based on peripheral blood B-cells, while murine studies preferentially use splenic B-cells. Furthermore, the expression of B cell surface antigens differs between mice and humans. In mice, germinal center reactions can be critical for chronic GvHD development and may produce clues to pathogenic mechanisms operative in the development of clinical disease as described below. Patients with chronic GvHD are often characterized by functional hyposplenism, although patients without chronic GvHD can also have splenic dysfunction. Secondary lymphoid organs are difficult to study in patients, and reconstitution of follicular B-cells in lymph nodes is known to be delayed and atypical in chronic GvHD patients, although in several murine models allo-immunity is required for the development of chronic GvHD. The production of auto-antibodies appears to play a key role in patients with chronic GvHD suggesting the loss of B cell tolerance. While a number of autoantibodies including anti-nuclear antibodies, anti-double stranded DNA, and anti-platelet-derived growth factor receptor alpha have been found in association with chronic GvHD, these findings have been variable. Only antibodies directed against Y-chromosome encoded epitopes (H-Y antibodies) in male recipients with female donors have had a consistent association with chronic GvHD. Multiple groups have now shown that chronic GvHD is closely associated with aberrant BAFF levels, an activated B-cell phenotype, and aberrant BAFF/B cell ratios.

Phase 3 - aberrant tissue repair often with fibrosis

Epithelial and vascular endothelial regeneration is critical for normal reparative processes. Dysregulated repair can lead to scaring or fibrosis, defined by the excessive accumulation of components of the extracellular matrix in and around inflamed or damaged tissue. Acute inflammatory responses often initiate the fibrotic cascade. Early endothelial damage activates coagulation pathways and results in the release of chemotactic factors that recruit immune cells to sites of tissue injury. The mechanisms around these processes are lacking and require additional evaluation both in humans and mice.

For the foreseeable future, there remains an unmet need for relevant chronic GvHD murine models, especially those that simulate the multi-organ manifestations and complex immune pathology of chronic GvHD observed in humans. The development of optimal mouse models in the chronic setting should include several factors, including: a clinical pattern and organ involvement by chronic GvHD that recapitulates that seen in human cGVHD and whether interventions used to abrogate chronic GvHD. Establishing a model of chronic GvHD developing in an adult or aged immune system may have significant merit as well. Improvements in current chronic GvHD models that are limited by the general absence of immune suppressive drugs for acute or chronic GvHD prophylaxis and treatment are needed to increase the likelihood of identifying the most clinically relevant pathways of chronic GvHD generation and maintenance. Finally, development of pre-clinical models is needed to optimally address how interventions to abrogate chronic GvHD and its impact on graft-versus-leukemia.

Main educational points/Learning goals:

- Our current understanding of chronic GvHD biology is from both murine GvHD models and biomarker studies;
- The 2014 NIH Chronic GvHD Consensus Biology Working Group has identified three phase of chronic GvHD that can be used to evaluate the biology of cGvHD in future studies;
- Mouse models for chronic GvHD are still limited in their clinical and biological correlation with cGvHD and require further improvements and development.

Biomarkers in chronic Graft-versus-Host Disease

Kirk R. Schultz
Division of Hematology and Oncology, Department of Pediatrics, British Columbia Children's Hospital, University of British Columbia, Vancouver, BC, Canada

Despite higher BMT rates and successes in recent decades, our ability to treat chronic Graft-versus-Host Disease (cGvHD) has improved very little. As such, it is crucial to find new strategies to quickly and definitively assign risk for development of cGvHD and to accurately assess response to cGvHD therapies. One potentially powerful strategy is to identify biomarkers that could be used to categorize patients as low or high risk. If so—that is, if one or more biomarkers had a strong prognostic ability to identify a group at high risk for development of cGvHD—then these high-risk patients could be treated post-BMT using low toxicity preemptive therapies to minimize or prevent cGvHD. The primary intent of a biomarker is to guide clinical practice, not understand the biology of cGvHD. Thus, the design of biomarker studies may be different than studies focused on understanding the biology of cGvHD in humans. In 2004 and again in 2014, the National Institutes of Health (NIH) Chronic GvHD Consensus biomarkers working group met and evaluated the current state of the art in cGvHD biomarkers. In 2014, the consensus group determined the primary types of biomarkers in cGvHD using definitions for the FDA. The types of marker are as follows:

a. Diagnostic - An assay that identifies patients at the onset of clinical disease. Different forms of chronic GvHD may have different markers. Different tissues may have different markers. These markers are useful due to the fact that cGvHD onset is insidious and gradual, and this may lead to an earlier diagnosis and initiation of therapy.

b. Prognostic - An assay that categorizes patients by degree of risk for disease occurrence or progression or resolution. These markers are useful in that they can assign risk to allow for preemptive cGvHD therapies only in high risk populations. They also allow for identification of patient populations for innovative interventions to minimize cGvHD. Lastly, they may allow for selection of donor and recipient combinations.

c. Predictive - An assay that categorizes patients by their likelihood of response or outcome to a particular treatment when measured prior to the treatment. The marker can be used to determine which type of therapy to use and potentially to biologically categorize cGvHD into categories most amenable to different immune suppressive interventions.

d. Response to treatment - An assay measured after initiation of therapy that is intended to substitute for a clinical efficacy endpoint (note: a pre therapy sample for comparison is required). These markers will allow for early discontinuation of cGvHD therapy and could be used as clinical trial endpoints.

In spite of an increasing number of markers being described, various studies have resulted in inconsistent descriptions of various biomarkers. The NIH chronic GvHD Consensus Biomarker working group also determined that biomarker validation is being limited by the relative rarity of cGvHD with smaller patient numbers, as well as the high heterogeneity of immune and clinical factor that appear to impact in cGvHD. ***They identified a number of clinical factors affecting biomarkers directly including:***

a) Tissues involved and NIH chronic GvHD score
b) Concomitant acute GvHD
c) Previous acute GvHD and treatment/prophylaxis of acute GvHD
d) Current infection
e) Sample processing and storage.

There are a number of covariates and potential confounding factors, including:

a) Recipient characteristics
b) Donor characteristics
c) Preparative conditioning regimen.

Other factors that impact on interpretation of biomarkers include a) immune reconstitution after HSCT, b) diminished innate responses at least 3 month after BMT, c) splenic dysfunction in both patienst that do not have cGvHD, d) that both T and B cells function are decreased ≥ 2 years after BMT in patients without cGvHD. The last factor results in the fact that a "normal" control varies depending on the time post BMT when determining the significance of a cGvHD biomarker.

Progress in biomarkers: Multiple tissues have been evaluated as cGvHD biomarkers. These

include: a) peripheral blood cells, b) plasma/serum, c) urine, d) tissues (biopsies), and e) BAL. Currently there is an increasing number of cGvHD biomarker studies particularly focused on diagnostic and prognostic biomarkers. A majority still include discovery of new markers with very few that can be considered as validation or replication studies. Even the "large" biomarker studies performed on different cohorts have included no more than 400 patients in any single study. The NIH consensus biomarker work group determined that the minimum requirement for validation of a marker is that the marker is considered significant in at least two cohorts by at least 2 groups. There are a handful of diagnostic plasma or serum markers that meet these criteria. Diagnostic markers that have been described by at least 3 groups in 3 cohorts include CXCL9, CXCL10, and soluble BAFF. Markers seen in at least 2 cohorts by two groups include anti-H-Y antibodies, IL-2Rα, and aminopeptidase N (CD13). For each of these markers, other groups have evaluated them and could not find a confirmation. To date, none of these markers are in clinical use. Diagnostic cellular markers are even more problematic. Each group that evaluates cellular markers performs cell analyses in a different way. Thus, autoreactive and activated B cell populations are recurring populations but the studies have been relatively small. The same can be said for memory B cells, B_{regs}, $CD56^{bright}$ NK_{regs}, and in particularly Tregs. Tregs have had the highest variability. Thus, although there are a number of exciting cellular biomarkers, none can be considered validated. The same can be said of a very large number of plasma and serum biomarkers each seen in single center relatively small studies.

Immune tolerance: Another issue is the definition of immune tolerance. The standard definition would be a patient off all immune suppression, no clinical manifestations of cGvHD, and a normal response to exogenous antigens such as a vaccine. The question of whether immune tolerance is identical for a patient that never developed either aGvHD or cGvHD versus a patient who has resolved aGvHD and/or cGVHD is not know. Whether the group should be lumped as controls is uncertain.

What biomarkers can teach us about the biology of cGvHD: Biomarker work has identified areas not well recognized in murine GvHD models in particular that lead to inflammation is an important part of cGvHD. Recently, biomarkers have supported the presence of vascular endothelial inflammation leading to migration of $CXCR3^{+}$ donor immune cells into target organs. The current hypothesis is that cGvHD occurs in three phases: 1) initiating injury, 2) inflammation and repair, and 3) propagation of tissue injury and fibrosis.

Challenges to biomarker studies: All cGvHD biomarkers can be impacted by a number of clinical covariates (e.g. donor source, TBI, recipient age) that may determine in which clinical setting they can be applied. One additional challenge for biomarker studies is the clinical validation of diagnosis. Based on our experience in cGvHD biomarker studies, we found that only 16 (70%) of those identified by the center as having cGvHD met NIH consensus criteria. The reason for this discrepancy was misclassification of late-onset aGvHD as cGvHD. This emphasizes the ongoing challenges in cGvHD assessment and the importance of rigorous chronic GvHD clinical data capture in biomarker studies.

Future directions: A 2014 NIH consensus conference on cGvHD concluded that there are no validated cGvHD markers. While candidate biomarkers (for either diagnosis and/or prognosis) are being tested with adults, little is being done with children. This is especially important as children have a functional thymus that has not yet involuted and they are more likely than adults to receive cord blood, bone marrow, and haploidentical donor transplants.

Main educational points/Learning goals:

- The 2014 Chronic GvHD Consensus Biomarker working group has determined the types of biomarkers for application in cGvHD: diagnostic, prognostic, predictive, and response to treatment;
- Biomarkers can be used both to clinically manage patients, better understand the biology of human cGvHD, and potentially develop targeted therapies for cGvHD;
- Currently there are no validated cGvHD biomarkers due to the heterogeneous nature of cGvHD and large multicenter studies using standardized approaches are required.

High-throughput glycomics for patient stratification: What did we learn from the first 50,000 analyses?

Gordan Lauc
University of Zagreb & Genos Glycoscience Research Laboratory, Zagreb, Croatia

The majority of proteins are glycosylated and their glycan parts have numerous structural and functional roles. This essential posttranslational modification is generated by a complex biosynthetic pathway comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, ion channels and other proteins. Since glycans are created without the genetic template, alternative glycosylation creates an additional layer of protein complexity by combining genetic variability with past and present environmental factors. Individual variability in glycome composition is very large, but glycosylation of an individual protein seems to be under strong genetic influence, with heritability being up to 80% for some glycans and age being the strongest environmental confounder. Structural details of the attached glycans are of great physiological significance, and many pathological conditions are associated with various types of glycan changes. For example, glycans attached to the Fc part of immunoglobulin G are important modulators of IgG effector functions. Slight modifications in the composition of the IgG glycome significantly tune IgG towards binding to different Fc receptors and can convert IgG from a pro-inflammatory effector into an anti-inflammatory agent.

Since the onset of genome wide association studies, thousands of genetic loci have been associated with different diseases and traits. However, in the last few years it is becoming increasingly clear that variations in DNA sequence are only the beginning of the understanding of complex human diseases. Genetic polymorphisms have to be put in the context of complex biology of life and a more elaborate approach that combines different 'omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex epiproteomic modification, has an immense potential in this respect, which is only starting to be investigated.

Main educational points/Learning goals:

- Nearly all membrane and circulatory proteins are glycosylated;
- Glycans are an important structural component of proteins that significantly affect their structure and function;
- Inter-individual variability of glycosylation is large, with significant impact on predisposition and course of many diseases;
- Variations in composition of the IgG glycome affects IgG effector functions.

Extracorporeal photopheresis mechanisms and effects in chronic Graft-versus-Host Disease

Ines Bojanić
Department of Transfusion Medicine and Transplantation Biology, University Hospital Center Zagreb, Zagreb, Croatia

Extracorporeal photopheresis (ECP) is an immunomodulating cell therapy, which was initially developed for use in cutaneous T-cell lymphoma, but over time has also shown to be beneficial in the treatment of other severe and refractory conditions, such as acute or chronic Graft-versus-Host Disease (GvHD) after allogenic stem cell transplantation, solid organ transplant rejection, and various autoimmune diseases. During ECP, the patient's blood is processed outside the body and the autologous peripheral blood mononuclear cells (MNC) are separated from the red blood cells and plasma by centrifugation. Separated cells are treated extracorporeally with a photoactive agent psoralen, exposed to ultraviolet A (UVA) light irradiation and subsequently reinfused to the patient during the same procedure.

ECP methods: ECP can be performed as inline method in closed system, offline method in open systems, or mini-ECP technique using manual MNC preparation from only 100 to 200 mL of whole blood. Several closed and open ECP systems are now available for clinical use. In a closed ECP system (i.e. a 'one-step' method), the cell separation, drug photoactivation and reinfusion stages are fully integrated and automated. Open ECP systems use separate leukapheresis device for cell separation and illumination device for drug photoactivation ('two-step' methods). Classic apheresis ECP procedures involve processing of a large volume of whole blood ranging from 3 to 6 L per treatment, depending on ECP technique and patient's body weight. Although the dose of UVA-irradiated MNC in mini-ECP is significantly lower compared to the classic apheresis procedure, mini-ECP offers technical and procedural advantages and facilitates treatment of patients with low body weight, contraindications for apheresis, or those who are critically ill.

Treatment schedule: Chronic GvHD has been treated with one cycle of two ECP procedures on consecutive days every 1–2 weeks for three months. Subsequently, treatment intervals could possibly be increased by 1 week every 3 months, depending on the type of lesions, extent of cGvHD and clinical response. Tapering is influenced by the ability to reduce concurrent immunosuppressive therapy. Response should be assessed according to NIH guidelines. If cGvHD progresses, a change in treatment strategy should be considered. Recurrence of cGvHD during tapering or after discontinuation of ECP procedures may be controlled by restarting ECP or intensification of treatment schedule. The length of therapy required for individual patients is difficult to predict, but it usually takes at least six months.

Mechanism of action: Even though ECP has been widely used for a variety of clinical entities, the mechanism of action is not fully understood. UVA psoralen photoacivation induces psoralen-mediated DNA crosslinks, and causes apoptosis in treated lymphocytes, particularly alloreactive T cells, which actively proliferate during GvHD, and have higher susceptibility to apoptosis than resting lymphocytes. Monocytes treated in the same way appear to be more resistant than lymphocytes to apoptosis, undergoing a differentiation process within 2 days and expressing surface markers that are characteristic of immature dendritic cells. This differentiation appears to be independent of psoralen-induced photoactivation, and is mostly driven by contact of cells with plastic and other synthetic materials during passage through the photopheresis system. ECP induces tolerogenic dendritic cells that stimulate a Th2 rather than Th1 response, and, after phagocytosis of apoptotic T cells, gives rise to a clonotypic immune reaction and leads to tolerance. Recent studies demonstrated that ECP treatment also causes downregulation of autoreactive B cells, alterations in T helper subset populations and lymphocyte homing antigen display, switch from pro-inflammatory to anti-inflammatory cytokine production, and generation of regulatory T cells.

Clinical results: Most of the evidence on the use of ECP in cGvHD comes from patients with steroid-refractory disease, and there are very few data currently available for the use of ECP as first-line therapy of cGvHD. ECP is usually performed in specialized centers as second-line therapy for patients with steroid-refractory, dependent or intolerant cGvHD in need of

systemic therapy. Efficacy has been evaluated on multiple small cohort or case–control studies, with overall response rates of 50% and higher. Best responses were observed in skin (both lichenoid and sclerodermoid), mucous membrane and liver manifestations of cGvHD. Importantly, steroid sparing effect occurs, even in absence of organ improvement, and therefore quality of life is increased. Maximal responses for cGvHD require 2–6 months of treatment, and longer treatment duration may also be necessary to obtain best responses to ECP in patients with sclerodermatous manifestations.

Regardless of the system used, treatment with ECP is usually well tolerated and no severe side-effects have been reported. The main limiting factors are vascular access and patient's compliance with lengthy repeated procedures. In patients treated long term with ECP, neither increased risk of opportunistic infections, nor disease relapse were observed.

Many clinical practice guidelines and consensus statements addressing the use of ECP for cGvHD have been published (German/Austrian/Swiss consensus conference on second line treatment of cGVHD 2011, British Committee for Standards in Haematology and the British Society for Blood and Marrow Transplantation 2012, American Society for Apheresis 2016). ECP has been considered an established second line therapy option for steroid refractory cGvHD, which allows tapering of immunosuppressive therapy and ameliorates the quality of life for responder patients.

Further prospective studies are needed to determine variables predicting response to ECP treatment and identify patients who will benefit from this complex, demanding and costly treatment.

Issues in the design of chronic Graft-versus-Host Disease clinical studies

Stephanie J. Lee
Clinical Research Division, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA, USA

Introduction

Clinical trials in the chronic Graft-versus-Host Disease (GvHD) population pose a number of design challenges. Failure to conduct a robust study could result in a false positive (moving a study intervention forward in development when it is not effective) or a false negative (abandoning a treatment when it does in fact improve chronic GvHD). Both incorrect results prevent progress in chronic GvHD and are ultimately harmful to patients.

Study population

The most homogeneous population in which to study chronic GvHD is the newly diagnosed patient population because they are unexposed to prior treatments. However, they are heterogeneous overall because of their diverse clinical manifestations, varied time since transplant, and prior exposure to immunosuppressive agents for acute GvHD prophylaxis and treatment.

Newly diagnosed chronic GvHD is the most responsive to treatment and has the highest likelihood of achieving a complete response. Thus, it can be difficult to convince providers to offer and patients to enroll on trials of initial therapies because both may prefer the standard approach of steroids. Because initial response rates are high in newly diagnosed chronic GvHD, it is difficult to show an improvement in this endpoint with an experimental approach. Trials may need to consider additional endpoint components such as the ability to taper steroids or avoiding the need for secondary treatment.

Once patients are beyond initial treatment, they are called "steroid-refractory" under the assumption that all patients receive an appropriate trial of steroids. However, this assumption is not always true in practice and an operational definition of "steroid-refractory" can be difficult. Patients are more heterogeneous in terms of their clinical manifestations and prior therapies as they move to second line, third line and beyond. Even the definition of a "line" of therapy is controversial since this could refer to a group of agents all given at approximately the same time or the number of agents used for treatment whether or not administered together.

Inclusion and exclusion criteria that are too broad will result in greater heterogeneity of the population potentially masking an efficacy signal. Criteria that are too narrow may fail to accrue patients sufficiently fast to complete the trial and are not reflective of the general chronic GvHD population.

Comparator

For the newly diagnosed population, prednisone with or without calcineurin inhibitors is recognized as the comparator. For the steroid-refractory population, the choice of comparator is challenging. If using a historical control, few clear benchmarks are available. If a concurrent control, many steroid-refractory agents could be used. Patients beyond first line therapy who are participating in clinical trials generally need timely therapy for chronic GvHD; clinicians are usually not willing to wait to start therapy, and they are not willing to risk assignment to a placebo.

Design

Single arm studies are the most popular design for steroid-refractory chronic GvHD because most trials are just looking for an efficacy signal in a relatively small number of subjects. However, results can be misleading because the historical benchmark is not established. Randomized designs are superior, especially if the treatment can be blinded, but require more subjects. Although skewed randomization e.g., 2:1 or 3:1 is often considered, in practice it is rare.

Logistics

I have found conducting chronic GvHD studies to be very challenging. In the USA, the dominant delay is no longer IRB approval, although that still takes 2-4 months. The major delay tends to be contract negotiation around both the per subject amount (if a pharma sponsored trial) and the specific terms. Legal teams on behalf of both parties send redlined contracts back and forth until consensus is reached. Oftentimes the specific issues do not seem as important to investigators as they are to institutions. Each company's and

institution's contracts are unique: no standard contract language has been widely endorsed.

The local site research team needs to be very strong and well-integrated to successfully enroll patients, conduct the trial and provide accurate and meaningful data. I have found that the critical team members are the study staff, less so the site principal investigator. It is the study coordinator along with the research nurse and data coordinator who actually screen and track the patients ensuring that appropriate research tasks are completed. Sites vary tremendously in the strength of their clinical trial teams.

Patients with chronic GvHD, especially those who are heavily pre-treated, are medically fragile and have frequent adverse events. They are on numerous other medications related to chronic GvHD treatment, infectious prophylaxis and comorbidity management. Managing toxicities due to unfamiliar agents can be very challenging.

Main educational points/Learning goals:

- Design of chronic GvHD clinical trials is challenging but critical to achieve meaningful results;
- Balancing population and outcome homogeneity with generalizability is difficult given the underlying heterogeneity of the chronic GvHD population.

Evaluating therapeutic response in chronic Graft-versus-Host Disease by NIH criteria

Attilio Olivieri
Clinica di Ematologia Ancona, Università Politecnica delle Marche, Ancona, Italy

Chronic Graft-versus-Host Disease (cGvHD) is a rare disease, occurring as a late complication of allogeneic hematopoietic stem cell transplantation (HSCT), representing the first cause of transplant-related mortality (NRM). cGvHD is still an unmet challenge: steroids are usually given as first-line treatment, but therapy of steroid-refractory cGvHD (SR-cGvHD), which occurs in nearly half of cGvHD patients, is unsatisfactory. Although more than 50 interventions have been tested in the past, no drug has been approved for SR-cGvHD by the FDA or the European Medicines Agency; furthermore, the issued guidelines do not clearly advise against use of any of the drugs studied, confirming that clinical results have been inconclusive in this setting.

A more objective response assessment might help to reduce false expectations about new interventions tested, avoiding further patients being at risk of adverse effects from ineffective treatments. To bridge this gap, in 2006 the NIH cGvHD Consensus Project provided recommendations for design of future clinical trials. This document proposed measures and criteria for assessing outcomes in clinical trials in cGvHD; the Working Group proposed provisional definitions of complete response, partial response, and disease progression for each organ and for overall response. The proposed cGvHD-specific core measures include: a) Clinician- or patient-assessed signs and symptoms, b) The chronic GvHD Lee symptom scale, and c) The clinician- or patient-reported global rating scales (1). The uptake of these recommendations in the recent literature has not been yet extensively evaluated and their impact on the quality of clinical research of SR-cGVHD is unknown.

We have recently investigated whether methodological deviations from NIH recommendations affected the reported effect size in SR-cGvHD. To measure adherence to NIH recommendations, we applied a 61 item checklist derived from the NIH consensus document and included 82 studies related to nine interventions. Conformity to NIH recommendations was evenly low across the analyzed timeframe (1998–2013), and did not change significantly after publication of NIH recommendations. We performed a meta-analysis to measure pooled effect size for overall response rate (ORR) and meta-regression analyses to measure the effect of deviations from NIH recommendations on pooled effect size. The pooled effect size for ORR for systemic treatment of SR-cGVHD was 0.66 (95% CI 0.62–0.70). Increased adherence to NIH recommendations in a score of items defining correct response assessment was associated with a significant reduction in ORR (4.2%, 95% CI 6.6 to 1.9; p=0.001); these findings suggest a bias in the reported efficacy of treatment of SR-cGvHD, while NIH recommendations seem to improve the assessment of response, possibly reducing the overestimation bias (2).

The new consensus criteria for response evaluation (3) in cGvHD offers a shared framework to study this rare disease. However, a learning period is needed to train the practicing physicians and to highlight and correct possible criticalities emerging from the application of new criteria. Moreover, as treatment of cGvHD is clearly intended to achieve a sustained clinical benefit (rather than cosmetic improvement), the Working Group focused on endpoints to be included in the next clinical trials: 1) failure-free survival (FFS), 2) survival (OS) without progressive impairment, 3) complete or partial response, 4) patient-reported outcomes, and 5) an aggregate scale incorporating several different types of measures, similar to scales used for regulatory review of autoimmune diseases.

We have recently conducted a prospective study of Imatinib in SR-cGvHD (4). Response at 6 months was evaluated using Center Response, and NIH response criteria (2006). Outcome according to response and NIH global score improvement at 6 months was also evaluated. Treatment failure was defined as cGvHD progression or death because of cGvHD, relapse of the underlying disease, addition (or increase) of immunosuppressive drug/procedure, or severe toxicity. A landmark analysis for OS at 6 months according to response status revealed that achieving ≥PR at this time point strongly predicted the outcome, suggesting that adopting a centralized NIH response criteria is a

reliable tool for predicting outcome after second line treatment. The 3-year OS was 94% for patients responding at 6 months and 58% for non-responders according to NIH response. Another larger study in a similar setting showed that FFS can effectively serve as a meaningful end point for clinical trials (5).

A recent observational study in 575 patients validated the NIH updated criteria: both the 6 month clinician-reported response and the NIH-calculated response correlated with subsequent FFS. However, FFS, OS, and NRM were primarily predicted by changes in patient-reported measures (e.g. Lee scale and FACT). These data suggest that patient-reported symptoms and quality of life may be more sensitive to overall health than clinician reported cGvHD measures (6).

Finally, cGvHD often has an unpredictable trajectory, and unclear definition of an a-priori time-point for response determination could be interpreted as "waiting for the best response to happen" The NIH response criteria do not account for the prior trajectory of abnormalities. For example, "stable" or "unchanged" disease might be considered a meaningful response when the prior trajectory was clear progression, as indicated, for example, by serial pulmonary function tests or rapidly progressive sclerosis, whereas "stable disease" after prior improvement or stability should not be considered a "response". The advantage of incorporating a continuous disease activity score in the response evaluation is that there is no loss of power due to categorization. Also, when no "success or failure" cut-off point is used, more precision is available to assess the benefits of very effective treatments. Especially when the response criteria are relatively "easy" to meet, the effectiveness of the treatment may be underestimated. When, on the other hand, the response criteria are "too difficult" to fulfill, none of the patients in the treatment arms may be responders, and an actual difference between two treatments cannot be shown.

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Modern media technology in chronic Graft-versus-Host Disease evaluation

Helene Schoemans
University Hospitals of Leuven and Catholic University of Leuven, Leuven, Belgium

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative and established treatment approach for patients with hematological malignancies. However, Graft-versus-Host Disease (GvHD), where the transplanted stem cells react against the body of the patient, is a major complication after HSCT. It is the second most important cause of morbidity and death after relapse, and also often results in reduction of quality of life. The accurate evaluation of this disease after transplantation is thus of paramount importance to correctly evaluate transplantation outcome.

Yet, this pleiotropic disease's assessment is challenging. The transplantation community has made major efforts to develop international guidelines to diagnose and score GvHD accurately: the National Institutes of Health (NIH) criteria have been described by Filipovich et al (Biol Blood Marrow Transplant. 2005) and recently updated by Jagasia et al (Biol Blood Marrow Transplant. 2015). However, it still remains a challenge to effectively implement them in daily clinical practice (Duarte et al, Bone Marrow Transplantation 2014), as clinicians tend to consider the guidelines to be relatively complex and time consuming. Even experienced professionals will tend to misdiagnose patients: up to 10% of patients entered by GvHD consortium centers in a recent GvHD interventional trial actually needed to be excluded from study analysis post hoc due to inadequate diagnosis of GvHD at inclusion (Carpenter et al abstract 42, ASBTM-IBMTR 2016). The EBMT Complications and Quality of life (CQoL) working party therefore set out to develop a tool to capture GvHD in a reliable manner.

This tool was developed as a computer-based algorithm, the "eGvHD App", using a user centered design process. This process includes several rounds of user feedback in the development to ensure user friendliness of the tool. In a pilot test, accuracy of the eGvHD App was tested using a quasi-experimental cross-over design with four expert-reviewed case-vignettes in a convenience sample of twenty-eight clinical professionals from a single institution (Schoemans et al, BMT 2016). Perceived usefulness was evaluated by the technology acceptance model (TAM) and User satisfaction by the Post-Study System Usability Questionnaire (PSSUQ). User experience was positive and the "eGvHD App" significantly increased diagnostic and scoring accuracy of the selected clinical vignettes, by about 30%. This pilot test led to further development of the app: refinement of details in the algorithm, improvement of term description, addition of a user's manual and the option of generating patient reports.

Further steps are currently being taken to refine accuracy testing. Implementation is also currently being planned in diverse settings to further evaluate effectiveness and scalability of using this electronic tool in daily practice.

Main educational points/Learning goals:

- The accurate evaluation of GvHD after stem cell transplantation is of paramount importance to correctly evaluate transplantation outcome;
- The "eGvHD App" offers clinicians support to diagnose and score the severity of GvHD;
- Pilot testing of the "eGvHD App" was promising as it showed high user satisfaction and increased accuracy of GVHD assessment.

Use of composite endpoints in clinical trials and observational studies

Ted A. Gooley
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Composite endpoints have gained in popularity in both clinical trials and observational studies. Caution must be exercised when proposing such an endpoint, however. On the other hand, if one uses as an endpoint a component of a composite, care must also be taken in interpreting results if such an endpoint has competing risks. A competing risk is defined as an event whose occurrence precludes the event of interest from occurring, or whose occurrence fundamentally alters the probability of the event of interest from occurring. Chronic GvHD is such an endpoint, with competing risks of death without cGvHD and, sometimes, relapse before cGvHD (as relapse may fundamentally alter the probability of cGvHD, particularly if relapse is treated with donor lymphocyte infusions). If comparing the risk of cGvHD between two groups or looking for risk factors for cGvHD, how one conducts such analyses dictates assumptions made and how one interprets results. If interest lies in comparing the probabilities of cGvHD between groups, then an appropriate approach is to use Gray's test, or in the setting of regression, Fine-Gray regression to compare cumulative incidence functions. Competing-risk failures impact this function so that more such failures lead to a reduced cumulative incidence (or probability). On the other hand, one may instead desire to compare the so-called cause-specific hazards of cGvHD. An appropriate test for these purposes is the log-rank test or in the setting of regression, Cox regression. In this approach, failures from a competing risk do not impact the cause-specific hazard. Both approaches are perfectly reasonable, but one must understand each in order to properly interpret results. Given these options, which could lead to qualitatively different conclusions, researchers often try to avoid endpoints with competing risks. Composite endpoints are one approach towards this goal. However, composite endpoints are also fraught with potential difficulties in interpretation, particularly if one component of the composite occurs much more frequently in one group relative to the other group, but the composite failure is roughly the same in each group. Because of this possibility, one must also ensure that the severity of each component in a composite endpoint be of roughly the same magnitude. In this talk we will discuss all these issues and provide examples where interpretation is straightforward as well as not so straightforward. Examples will come from simulations, where the true differences between groups are known for both the composite endpoint as well as the individual components, as well as examples from real data taken from the field of hematopoietic cell transplant.

Establishment of multidisciplinary team for chronic Graft-versus-Host Disease in Zagreb, Croatia

Damir Nemet
Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

The Division of Hematology of the University Hospital Center Zagreb (UHC Zagreb) and University of Zagreb School of Medicine (UZSM), Zagreb, Croatia, have had long experience with allogeneic stem cell transplantation (alloHSCT), since 1983, performing in recent years up to 80 procedures annually, with more than 900 patients treated with alloHSCT in this center to date. Chronic Graft-versus-Host Disease (cGvHD) is the leading cause of non-relapse morbidity and mortality after alloHSCT, and is significantly associated with impairments in quality of life and poor functional status in patients after alloHSCT. It is a multisystemic alloimmune and autoimmune disease, affecting skin, lungs, mouth, liver, eyes, joints, gastrointestinal and genital tract. Recognizing the increasing problem in cGvHD, and with the goal to implement the newest National Institutes of Health (NIH) standards for cGvHD in Croatia, a multidisciplinary clinical and laboratory infrastructure for cGvHD was established at the UHC Zagreb in mid-2013. This initiative was supported by the Unity Through Knowledge Fund (UKF) international project funded by the World Bank and the Croatian Ministry of Science, Education and Sports. The project, entitled "Clinical and biological factors determining severity and activity of cGVHD after alloHSCT" was led by project leaders Prof. Dr. Damir Nemet (from UHC Zagreb and UZSM, Zagreb, Croatia) and Prof. Dr. Steven Z. Pavletic (from National Cancer Institute, NIH, USA).

Through this UKF project, a multidisciplinary team for cGvHD was formed and has been maintained in Zagreb since 2013. It consists of around 30 different clinical and laboratory specialists, who evaluate cGvHD patients and also assess patients after alloHSCT who did not develop cGvHD. Such a multidisciplinary approach has improved the consistency of assessment and treatment of cGvHD patients, and has also promoted interdisciplinary and international collaboration, development of different scientific subprojects and databases, with scientific publications and active participation at scientific meetings.

Main educational points/Learning goals:

- Assessment and evaluation of cGvHD as a multisystemic disease require a multidisciplinary approach with different specialists, improving treatment of cGvHD, but also establishing a platform for further scientific research of this often devastating chronic disease.

Pediatric aspects of chronic Graft–versus–Host Disease

Anita Lawitschka
St. Anna Children's Hospital, Medical University Vienna, Vienna, Austria

Chronic Graft-versus-Host Disease (cGvHD) is recognized as the most important long-term complication of allogeneic hematopoietic stem cell transplantation (HSCT) with a 20-fold increase in functional impairment and 10-15% mortality. Long-term medical neurocognitive and psychosocial effects of cGvHD may be magnified in children and adolescents, given their developmental stage and longer life expectancy compared to adults. Both cGvHD and systemic immunosuppressive treatment (especially in combination with prolonged corticosteroid treatment) have a profound deleterious impact on organ and psychomotoric development of the growing child. This makes pediatric cGvHD a relevant transplant complication deserving of further interest and research.

In general, pathophysiology and manifestations of cGvHD in children and adolescents show many similarities when compared to the adult disease. Most of what is known about pediatric GvHD has arisen from adult data and trials (with or without children) complemented by numerous expert opinions and recommendations. Therefore this review is designed to help young oncologists to understand pediatric principles with regard to both HSCT and cGvHD.

Indications for HSCT: Regarding the indications for HSCT, pediatric HSCT is more often performed to cure non-malignant diseases than in adults. For these non-malignant diseases like hematological, immune- and metabolic disorders there is no need for the graft-versus-malignancy effect, which may be a beneficial effect of GvHD. This aspect influences strongly dosage, route of administration and duration of GvHD prophylaxis and treatment with the aim to avoid acute and chronic GvHD. Knowledge of the underlying disease may also improve diagnosis of co-morbidities potentially overlapping GvHD symptoms subsequently.

The immune-reconstitution after HSCT in children is different compared to adults particularly characterized by an increased thymic function. Less pediatric data are available about the B-cell reconstitution. Immunological specifics pertaining to underlying diseases and conditioning regimens are also highlighted.

Published data about the incidence, risk factors and outcome of pediatric cGvHD are reviewed and supplemented by unicentric results. Regarding infectious complications prospective and comparable data between adults and children are scarce but relevant pediatric details will be briefly described.

A further focus is to guide the adult clinician in the various clinical presentations of pediatric cGvHD by organ system.

General principles governing diagnostic specifics of children and adolescents are also offered when related to the diagnosis of GvHD. Close serial monitoring of many organ systems is crucial in order to ensure prompt detection and recognition especially in patients where communication is at a stage of development. Recommendations are reviewed for follow-up and management of children with cGvHD and suggestions are offered recognizing that there is no standard approach.

In the absence of advances in treatment of cGvHD, multidisciplinary approaches and international co-operations should enhance diagnosis, supportive care and preventive strategies.

Late effects of chronic Graft-versus-Host Disease in children

Ernest Bilić, Nevena Krnić
Department of Pediatric Hematology and Oncology, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

An increasing number of pediatric patients are treated with hematopoietic stem cell transplantation (HSCT) due to high-risk malignant disease. With improvement in care more patients survive, requiring long-term follow-up for late adverse effects. The incidence of chronic Graft-versus-Host Disease (cGvHD) is lower in children (20-50%) than adults, but tends to increase due to growing use of peripheral blood stem cells and unrelated donors. cGvHD causes significant morbidity and mortality and can lead to serious chronic conditions affecting almost all organ systems.

Endocrine late effects are among the most common chronic conditions following HSCT. Growth impairment affects 50-85% of children. Besides growth hormone deficiency occurring after total body irradiation (TBI), other hormone deficiencies, malnutrition and chemotherapy affect growth. With prolonged use of corticosteroids and malabsorption, cGvHD in particular can adversely alter growth rate. Therefore, annual follow-up of height, weight, body mass index and pubertal development is mandatory in all children following HSCT.

Primary hypothyroidism, thyroid nodules, cancer development and rarely hyperthyroidism can develop following HSCT. Statistically significant risk factors for secondary thyroid carcinoma are younger age, female sex, TBI and cGvHD, with median interval between HSCT and diagnosis 8.5 years. All patients should be followed by annual thyroid palpation, measurement of fT4 and TSH and thyroid ultrasound throughout life.

Gonadal damage can occur due to TBI or alkylating agents, and can lead to a range of symptoms, from delayed puberty to infertility. In males, spermatogenesis is more sensitive to damage than Leydig cells function and testosterone production. However, low testosterone levels were found in cGvHD patients, probably due to glucocorticoid inhibitory effect on GnRH secretion and secretion of adrenal androgens, although cytotoxic effect of cyclosporine on Leydig cells cannot be excluded. Low sperm count is particularly frequent among patients with cGvHD. In females, older age and puberty stage increase the risk of ovarian damage with both hormonal dysfunction and adverse effect on oocyte production. cGvHD can lead to vaginal/vulvar stenosis and fibrosis, predisposition for recurrent vaginal infection and inflammation, or intrauterine adhesions. Immunosuppression in cGvHD with subsequent adrenal suppression and ovarian damage also reduces levels of circulating androgens. Since gonadal failure can develop years after exposure to gonadotoxic agents, follow-up of pubertal development in both sexes and menstrual regularity in females is mandatory in all HSCT patients. In male patients, FSH, LH and testosterone should be measured at the beginning and end of puberty, and later as indicated. In female patients, FSH, LH and E2 should be measured at the beginning and end of puberty, and between as indicated. Female patients with cGvHD should be referred for gynecologic examination. Semen analysis in males should be suggested in adulthood. In patients with gonadal failure, appropriate hormonal replacement therapy (HRT) should be started. However, chronic liver GvHD might disallow HRT and skin or gastrointestinal cGvHD might interfere with drug absorption. Recovery of gonadal function, both hormonal and reproductive, has been reported in both sexes, so hormonal replacement therapy should be periodically discontinued to evaluate gonadal function.

HSCT patients are at increased risk of obesity, dyslipidemia, insulin resistance, impaired glucose tolerance, diabetes mellitus and metabolic syndrome. Furthermore, cGvHD can lead to pancreatic atrophy and insufficiency. Therefore, patients should be screened for fasting blood glucose or HbA1c and lipid profile every two years, and further evaluation is needed as indicated. Healthy life-style modifications are strongly encouraged.

Low bone mineral density (BMD) is common among HSCT patients, due to effect of cancer therapy, irradiation, suboptimal nutrition, decreased physical activity, less exposure to sunshine and secondary endocrinopathies. cGvHD treatment further increases the risk (dexamethasone, cumulative exposure to >9 g/m2 prednisone equivalent, methotrexate with

direct cytotoxic effect on osteoblasts, calcineurin inhibitors, e.g. cyclosporine, tacrolimus). Most cancer survivors recover bone mass with increasing time off therapy. All HSCT patients should undergo DXA scan at baseline, 1-2 years following HSCT and after completion of pubertal development, and between as indicated. Z-scores based on age and gender matched normal values should be used in evaluation of children with low BMD. Children should be screened for vitamin D deficiency and should receive appropriate calcium and vitamin D intake, along with regular weight-bearing physical activity.

Follow-up of patients treated with HSCT, particularly those who develop cGvHD, requires a multidisciplinary approach, since virtually any organ system can be affected. Endocrinological assessment should be essential during follow-up, since early recognition of endocrinological complications can significantly improve quality of life of cancer survivors. As endocrinopathies in HSCT patients can develop years after completion of treatment, endocrinological follow-up should be lifelong.

Late effects and chronic Graft-versus-Host Disease – and are they connected?

Nadira Duraković
Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

In recent years, we have been witnessing a surge in the number of hematopoietic stem cell transplantation procedures worldwide. The use of unrelated donors, as well as reduced intensity conditioning that enabled us to transplant older patients and patients with comorbidities, have certainly contributed to the rise in number of procedures done. On the other hand, the betterment of supportive care has resulted in more patients surviving the procedure. So we are seeing an increasing number of patients becoming long-term survivors. And living long enough to have long-term effects. Long-term effects are defined as all complications occurring 3 months after transplantation. They are divided into delayed (3 months to 2 years), late (2-10 years), and very late events (>10 years). Long-term survivors report more medical problems in comparison with non-transplanted patients, varying from musculoskeletal issues to the use of psychotropic medication. Long-term complications are extremely heterogeneous in appearance, duration, and severity, but all lead to increased physical, emotional, financial, social and sexual stress, resulting in decreased overall quality of life. And even though the most prominent cause of death post transplant is still recurrence of the disease and chronic Graft-versus-Host Disease (cGvHD) (combined with infection due to prolonged immunosuppression), long-term effects have recently gained focus. Risk factors for late effects can be attributed to the patient (disease, patient age, comorbidities), transplant procedure (conditioning, irradiation) and other complications, namely cGvHD. And indeed, cGvHD and late effects are connected. They often emerge in the same time frame; they are both more frequent in older patients, they are sometimes intertwined due to the deleterious effect of immunosuppression, causing not only infection, but diabetes, muscle weakness, avascular joint necrosis, to name just a few. But we need to stress the fact that the patients who have no recurrence of disease, and have no cGvHD, can have a whole spectrum of late effects of their prior treatment and are also in need of very intense and comprehensive health care approach. The World Health Organization defines health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity". The focus nowadays is moving from immediate post-transplant patient care (that has been fairly perfected) to long-term maintenance of health. Currently, there are efforts set in that direction (intensified activity within EBMT Working Party on Late Effects as well as recently held NIH Blood and Marrow Transplant Late Effects Consensus Conference) aimed to help the transplant community with establishing procedures for follow-up, guidelines and steer future research. Transplanted patients are in need of very close follow-up, with involvement of multiple specialists informed and trained in addressing issues that transplantation might cause in such patients. Also, a secondary malignant disease is considered an important cause of death in long-term survivors. More stringent screening procedures should be employed in all long-term survivors. Probably the most efficient way to achieve better health of long-term survivors is establishing long-term follow up clinics that would enable transplant physician to seek counsel from various other specialist involved in care for such patients. Also, collaboration with primary care physicians is of utmost importance, along with providing them with training, support, and sometimes much needed advice when dealing with health issues in transplant patients.

Graft-versus-Leukemia effect of allogeneic hematopoietic stem cell transplantation

Grzegorz W. Basak
Department of Hematology, Oncology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland

The antitumor potency of allogeneic hematopoietic stem cell transplantation (alloHSCT) relies on 2 basic mechanisms: direct intensity of conditioning and alloimmune reaction of lymphocytes transplanted with or derived from hematopoietic stem cells obtained from stem cell donor. There is currently broad clinical evidence for Graft-versus- Leukemia (GvL) reaction supported by initial observations of: 1) more frequent relapses in case of transplantation from syngeneic compared with sibling donors, through 2) association between existence of graft versus host disease (GvHD, especially chronic) and lower relapse incidence, 3) association between intensification of transplant-associated immune suppression (e.g. ATG) and increased rate of relapses, 4) clinical efficacy of donor lymphocyte infusions (DLI) to treat relapse after alloHSCT and especially 5) durable remissions induced by transplantations after nonmyeloablative conditioning. One of the challenges for today's and future transplantatology is elaboration of methods to separate GvL from GvHD in controlled way in order to further increase efficacy of alloHSCT, while protecting the patient from immune side effects. The mechanisms of GvL in HLA-matched transplants are believed to be based primarily on the action of T lymphocytes (both CD4+ and CD8+) against the tumor. The target for donor's lymphocytes is either characterized by recipient's HLA molecules or new antigens (not known to immune system of the donor), presented by common HLA molecules. In case of HLA-matched transplants, the targets are most frequently derived from minor histocompatibility antigens (miHAs) encoded by polymorphic genetic loci located outside MHC, presenented by HLA molecules. They are most frequently result of single nucleotide polymorphisms in sequence of antigens, between individuals. Around 60 miHAs have been identified to date, with potential to find many more. Of those, six are located in Y chromosome, and more frequent GvHD reaction after transplantation from female to male is an evidence of their importance as antigens recognized by donor's cells. The most optimal targets for GvL are expressed only in hematopoietic cell line, however, more frequent is the expression of miHAs not only by tumor cells, but also nonhematopoietic cells, which may contribute to concurrent GvHD. The other targets for GvL are nonpolymorphic – malignant cells may be distinguished from nonhematopoietic tissues of the recipient by the aberrant or overexpression of antigens found also in healthy hematopoietic tissue. GvL may be also directed towards unique idiotypes or TCRs, which result from rearranged immunoglobulin or TCR genes, respectively, and this may occur in some B and T cell malignancies. Foreign proteins encoded by the viral genome in malignancies that occur in association with viral infections, such as EBV-associated B cell lymphomas, may also serve as targets for GvL reaction. In turn, there is very little evidence that products of specific oncogenes naturally serve as GvL targets.

Another cell population implied in GvL are NK cells and their role seems to be a mainstay of GvL reaction in case of T-cell depleted haploidentical cell transplantations. Their action is thought to be based on "missing self" hypothesis (attacking cells missing known HLA molecules), but also specific recognition of certain antigens (e.g. MICA). There are also anegdotal data regarding possible implication of donor's B lymphocytes producing cytotoxic antibodies.

Several strategies for enhancing GVL and preventing GvHD are under development:

1) Improved donor selection. It was proven that HLA-DP mismatches may contribute to lowering relapses, however it seems to occur at a price of GvHD. In turn, choosing donors with selected NK cell KIR haplotypes was shown to improve GvL, while not inducing GvHD.

2) Lowering the risk of GvHD through optimization of transplant conditioning. Nonmyeloablative conditioning is clearly associated with lower risk and intensity of GvHD, while GvL is preserved. Unfortunately, the dose intensity of drugs also plays an important role in tumor eradication and when reduced, GvL reaction frequently also fails.

3) Engineering stem cell product or DLI for improved antileukemic activity and less toxicity. Engineering the stem cell product by pretreatment of donors with certain immunomodulatory agents or vaccination against potential tumor-associated antigens is currently investigated.
4) Posttransplant immunomodulation. The use of immune checkpoint inhibitors in the posttransplant setting is currently under intensive investigations as a sole treatment and in combination with DLI. Another approach is to increase tissue resistance towards GvHD reaction, while not inhibiting GvL. It is based on targeting key elements (such as cytokines or pattern recognition receptors) important for GvHD and not GvL. There are also trials to modulate lymphocyte trafficing to secondary lymphoid organs or to target tissues of GvHD reaction by interferring with certain chemokines or their ligands. Another important element of these reactions are T reg cells, since they may potentially quench both GvHD and GvL. Some experimental approaches try to use e.g. T regs specific for GvHD targets, which may inhibit GvHD and not GvL or modify T reg cells to direct them selectively to the sites being targets of GvHD.
5) Tumor-specific immunotherapy with vaccination or adoptive cell transfer. Methodology for genetically modifying T cells to redirect their specificity – are collectively stimulating the development of a new generation of adoptive transfer strategies for enhancing both antiviral and antileukemic immunity after alloHCT. There is enormous progress in generation of chimeric antigen receptor (CAR) T or NK cells by modification of patient's or donor's lymphocytes with artificial receptors based on antigen-binding fragments of antibody associated with intracellular domains augmenting the cell activation upon antigen binding. They are usually designed to target antigens with broad expression, but limited to certain cellular lineage, e.g. CD19 thus eliminate the e.g. ALL clone, but also healthy B lymphocytes, without causing GvHD. As this strategy develops, new generations of CARs arrive (such as 4th generation including genes encoding immunomodulatory cytokines). The potential problem of this treatment is observed immune escape of the tumor. The traditional TCR receptors have the advantage over CARs by recognizing also intracellular antigens, potentially derived from proteins mutated in neoplastic clone and characteristic for the tumor. Therefore, there are ongoing trials with T lymphocytes modified with engineered TCRs, specific for certain tumor antigen. In order to avoid competition of endogenous TCRs, gene editing tools are implied allowing disruption of endogenous TCRs. Using same methods, it is possible to remove expression of HLA molecules thus allowing production of modified T cells, which are universal and not patient-specific.

Graft versus leukemia reaction is a tool, which is certainly genuine, but we still do not know how to exploit its huge potential. However, when the problem of selective augmentation of GvL while preserving patients from GvHD is finally solved, alloHSCT will become even more efficient and safe.

Models of care delivery in the United States

Iskra Pusic
Division of Oncology, Washington University School of Medicine, St. Louis, MO, USA

Hematopoietic stem cell transplantation (HSCT) is a complex and specialized treatment procedure limited to selected tertiary care centers with necessary expertise and resources. Its utilization has increased globally over time with improvement in survival, and it is projected that the number of HSCT survivors will exceed half a million by 2030 in the United States alone. With the growing number of HSCT survivors and the recognition of their unique treatment exposures and risk for late transplant-related morbidity, there is an increasing awareness of the complexity and specialized nature of their healthcare needs. Those needs are best addressed through patient-centered health delivery approach, which is adaptable and effectively coordinates care between transplant institutions, local oncologist and/or health-care provider, payers and public health systems. The American Society for Clinical Oncology and Institute of Medicine have emphasized the urgent need for developing comprehensive longitudinal healthcare delivery models ("survivorship models") for cancer survivors. However, the applicability of these survivorship models to HSCT recipients has not been fully assessed, mostly because transplant survivors develop a unique set of complications and late-effects depending on the interplay between their original diagnosis, prior chemotherapy, immunotherapy, conditioning regimen, and Graft-versus-Host disease.

The main components and considerations in the conceptual framework of survivorship care for HSCT patients are: (1) individualized patient health-care needs and preferences, (2) presence of active transplant-related complications, (3) distance from the transplant center and/or availability of local providers willing to care for HSCT survivors in partnership with the transplant team, (4) availability of resources and personnel available at transplant center, and (5) need for care model to be dynamic and adaptable as patient status and healthcare needs change over time. Congruently, barriers to providing coordinated care may be related to: (1) patient/caregiver (knowledge, distance, access, literacy, preferences, disparity), (2) provider (knowledge, time, competing priorities), (3) resources (personnel, space), (4) research (lack of medical evidence or clinical trials), and (5) financial barriers (lack of optimal payment models).

Transition from acute peri-transplant care to long-term follow up (LTFU) is a process, rather than an event. There are several different models of LTFU of HSCT survivors, all of them with distinct advantages and disadvantages. Consideration for the choice of a LTFU model depends on the available resources, commitment, size of the transplant center, geographic area, and the national system of insurance. In the *integrated care model* transplant center provides LTFU and survivorship care is incorporated within the routine post-transplant follow-up. Continuity of care is assured but particular knowledge of late effects might be lacking and needs of long-term survivors left behind. Some programs have *LTFU clinic* for HSCT survivors. Such clinics may be independent or integrated within the transplant centers. Integrated, dedicated LTFU clinic allows for continuity of care but requires multiple resources, specialized personnel and work space. In a *community-based consultative model* patient care is transitioned to non-transplant provider at established time-point; transplant center may provide occasional visits dedicated to survivorship care in addition to any ongoing community-based oncologic or primary care. A *combined or collaborative care model* includes coordinated care between transplant center and other providers, based on patient circumstances. Such a model, with predefined roles and good communication, appears to be the most ideal. However, there is not one best universal model for all transplant centers, and for a given center the model may change over time. Ultimately, survivorship care should be high-quality, individualized, technology-integrated, research-based, accessible, adaptable, affordable, coordinated, dynamic and equitable.

There are different reimbursement mechanisms for HSCT survivorship care: Fee for Service, Pay for Performance, Bundled Payments, Accountable Care Organization and Patient Centered Medial Home, each with its own pros and cons. Considering the unique characteristics of HSCT survivors it is unlikely that one reimbursement model will comprehensively address all of it.

Instead, integrated hybrid models should be considered, depending on the patient status and location. Recently, the Healthcare Delivery Working Group was established as one of the 6 working groups of the National Institutes of Health (NIH) Blood and Marrow Transplant Late Effects Initiative, with a task of identifying research gaps pertaining to healthcare delivery and understanding the long-term value and costs of care for HSCT survivors. The Working Group has representatives from transplant center medical directors, patients, payers, NIH, Health Resources and Services Administration, and allied health practitioners from the USA, Canada and Europe.

Main educational points/Learning goals:

- Transplant centers are at the forefront of routinely incorporating high quality LTFC for HSCT recipients;
- The choice of a specific model for LTFU program does not need to be definite and can evolve with changes affecting the transplant center, survivorship needs and resources;
- Primary health providers and local oncologists that will take care of patients after HSCT need to be educated on the unique issues of HSCT long-term survivors;
- LTFU requires multidisciplinary approach.

Models of care delivery in Europe

Helene Schoemans
University Hospitals of Leuven and Catholic University of Leuven, Leuven, Belgium

Long term care of allogeneic stem cell transplantation (HSCT) recipients is a relevant subject, considering that the majority of these patients have an expected survival of 50% at one year and will enjoy a more than 70% chance of remaining alive long term if they reach the two years post transplantation milestone (Bathia, Blood 2005). However, although allogeneic stem cell transplantation has the potential to cure the original disease, patients will carry on the burden of specific co-morbidities induced by the treatment, and their life expectancy will remain about 30% lower than that expected for their age (Martin, JCO 2010). Specific HSCT survivorship issues are therefore becoming a true concern of transplantation healthcare teams (Clark, BMT 2016), as they hold a unique position to predict complications in the long term. This is obviously even more so when patients are faced with chronic complications due to Graft-versus-Host Disease (GvHD) caused by the transplantation itself.

The Institute of Medicine refers to survivorship care in cancer patients as a process aiming at: (1) **preventing** of new (primary) and recurrent cancers and other late effects, (2) an active **surveillance** for recurrence or new cancers, (3) **interventions** for illnesses secondary to cancer and cancer treatment in the context of (4) **coordination** between specialists and primary care providers. This model of care is applicable to HSCT recipients, regardless of whether their original disease was malignant or not, as it encompasses the major themes along which patients need to be evaluated in the long term after receiving a conditioning regimen (including chemotherapy and or radiotherapy).

Furthermore, HSCT survivorship models need to encompass aspects of chronic illness management, as already implemented in other chronically ill populations (such as diabetes or heart failure). Again, this is of particular importance for patients faced with chronic GvHD. Such aspects of chronic illness care are for instance the implementation of continuity of care (within a system designed to optimize care processes), the availability of decision making tools and support for patient self-management. The postulate is that if chronically ill patients are not supported to be in driving seat of their care in daily life during contact with health care providers, their outcomes will be less favorable, considering the majority of the care really happens outside of healthcare facilities (i.e. at home).

The literature on this subject is still very scarce but several groups, such as the National Institutes of Health (NIH) Blood and Marrow Transplant Late Effects Initiative in June 2016, are currently coordinating efforts to offer some structure to long term care models after HSCT. Such models will undoubtedly vary according to local specificities and macro-economic factors such as social security system, access to work reinsertion programs and financial support during medical incapacity. Finally, including patient perspective and self-management in these models will further increase their relevance by placing the recovery process in the broader context of returning to a normal role in the society.

Main educational points/Learning goals:

- Survivorship care should be offered to HSCT recipients according to models relevant to cancer survivors considering that they have been exposed to chemotherapy and/or radiotherapy before stem cell infusion;
- Chronic care models are also relevant considering the large number of patients with long term complications after HSCT, particularly when they are affected with chronic GvHD;
- Including patient perspective and self-management in these models is of paramount importance.

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